

# GROWTH

## Genetics & Hormones

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## Growth Hormone Physiology and Pathophysiology: A Review

The complex system that encompasses the release and action of growth hormone (GH) includes many neurotransmitters, hormones, and organs. Among these are biogenic amines such as dopamine and serotonin in the brain; somatotropin-releasing hormone (SRH) and somatostatin or somatotropin-release-inhibiting hormone (SRIH) in the hypothalamus; somatotropin or GH in the pituitary; and insulin-like growth factors I (IGF-I) and II (IGF-II) in the liver and possibly in other organs. The mechanisms by which this complex system generates growth as a result of GH production and release from the pituitary are rapidly being elucidated.

One purpose of this article is to review the current concepts regarding these mechanisms, thus facilitating interpretation of the abstracts that are highlighted in this newsletter. The second goal is to briefly emphasize that more is known about the mechanisms involved in the secretion of GH and IGF than about the indications for treatment with GH.

A number of phasic changes in GH secretion are mediated by brain centers under the stimulus of bioamines. For example, dopamine is a stimulus to GH secretion. The arcuate nucleus, in particular, and possibly the ventromedial nucleus as well, respond by releasing SRH and SRIH. Both are transported from the hypothalamus via the portal system to the pituitary, where they attach to their respective receptors on the somatotrophs. Interestingly, SRIH, also referred to as somatotropin-release-inhibiting factor (SRIF) or somatostatin, is present in organ systems other than the

brain (ie, the pancreas and gut). However, SRH has not been shown to be present normally in structures other than the hypothalamus. Three forms of SRH have been identified—one with 44, one with 40, and one with 37 amino acids. These forms are approximately equipotent. The first two have been identified in the hypothalamus.

The synthesis and release of GH in the somatotrophs are under the control of the cAMP system. Both synthesis and release are sensitive to calcium ion fluxes and diacylglycerol. Protein kinase-C, the putative phorbol ester receptor, also plays an important role in the stimulated secretory pathway for GH, as indicated by marked increases in GH release by anterior pituitary cells of rats following stimulation with the phorbol ester, phorbol-12-myristate-13-acetate. SRH, cholera toxin, and forskolin lead to cAMP accumulation in somatotrophs and stimulate growth hormone release. SRIF inhibits both actions of these secretagogues, and thus its action is also closely related to the cAMP system. As the pituitary portal blood concentrations of SRH and SRIF change, the serum levels of GH rise

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# Growth Hormone Physiology and Pathophysiology: A Review

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and fall in an intermittent pulsatile fashion.

The feedback mechanisms to control GH release are multiple and complex. For example, SRH can diminish its own secretion in the rat, as shown by Tannenbaum, who injected SRH in graded doses into the cerebral ventricles of rats. Increasing doses given in this manner led to a dose-dependent inhibition of GH secretion. This profound effect was not due to SRIF secretion, as shown by the inability of the antiserum to SRIF to reverse the suppression of GH release. Thus, SRH can affect its own secretion by means of an ultra-short negative feedback loop mechanism.

In addition, SRH produces negative feedback at the somatotroph when there is lengthy exposure to the peptide. Pretreatment of anterior pituitary cell cultures from rats with SRH resulted in decreased cAMP and GH concentrations in these cells when the cells were reexposed to SRH.

Insulin-like growth factors also are involved in the feedback control of GH secretion. When placed in the cerebral ventricles, IGF-I causes a profound decrease in the spontaneous intermittent secretion of GH in rats. This action may occur through the release of SRIF. Berelowitz et al demonstrated that IGF-I directly stimulates the acute release of SRIF from rat hypothalamic fragments in culture.

Growth hormone also plays a feedback role in GH secretion. Berelowitz et al noted that GH acts at the hypothalamus to stimulate both the synthesis and release of SRIF. Abrams, Grumbach, and Kaplan demonstrated in humans that GH injections given every six hours for six days diminished GH release by the pituitary when insulin was given eight hours after the last GH injection. It was not ascertained whether this was a direct effect of GH or an indirect effect through somatomedin generation. In summary, there is a complex series of negative feedback loops that control the tonic and phasic secretion of GH.

After GH is released into the circulation, it travels to the liver and other

tissues, including chondrocytes in growing cartilage. In the liver, and possibly in cells of other tissues, GH stimulates the production of IGF-I and IGF-II. These growth factors, homologues of the proinsulin molecule, have biologic effects that are qualitatively similar to those of insulin. Although IGF-II possesses more insulin-like activity than IGF-I, neither factor reacts with anti-insulin antibodies. The molecular weight of each is about 7,500 daltons, and the factors resemble proinsulin in that about 50% of the amino acid residues in the A and B chains are identical with the corresponding sequences in human proinsulin. Radioimmunoassays specific for each of these have been developed, and a radio-receptor assay for IGF-II is performed in several laboratories.

Both IGF-I and IGF-II are under GH control, since concentrations of both have been reported to fall with GH deficiency. There is no question that IGF-I uniformly falls with GH deficiency; however, Bucher et al reported that IGF-II levels were normal in most patients with GH deficiency. Only IGF-I rises above adult values with GH excess. Moreover, the concentration of IGF-I rises slowly throughout childhood and peaks during adolescence at values that are two to three times higher than preadolescent and postadolescent values. IGF-II increases sharply after birth and normally remains constant throughout life. The insulin-like growth factors also differ in their growth-promoting activity: IGF-I is a potent sulfation factor, but IGF-II is weak in this regard.

IGF-I itself is probably essential to growth, although the possibility that GH may act directly on chondrocytes has not been totally excluded. Recent studies have suggested a direct effect on the longitudinal bone growth process by GH to the epiphyseal cartilage growth plate of hypophysectomized rats. Even the generation of IGF-I, however, does not guarantee normal growth. In certain humans with a GH-deficient-like phenotype, GH and IGF-I concentrations are normal or elevated, but growth does not occur normally. Therefore, the cell must be

able to accept IGF-I and translate its presence into action with synthesis of DNA, leading to cell multiplication (see page 12, Bierich et al: *Eur J Pediatr* 1984;142:186).

It is apparent that many steps are required for the synthesis and secretion of GH, and in the synthesis and action of insulin-like growth factors. Consequently, the physician evaluating a child with short stature, delayed bone age, and the clinical appearance of GH deficiency may be perplexed by the results of tests for GH secretion: Growth hormone deficiency may be complete, partial, or even transient as in children with psychosocial short stature. The child being evaluated may even secrete normal or increased amounts of GH but not generate IGF-I normally.

Therefore, IGF-I (somatomedin-C) determinations become important adjuncts in the evaluation of such patients. However, as mentioned previously, there are some patients who secrete GH and IGF-I normally, but who are unable to translate the presence of IGF-I into action on cell growth and multiplication. Consequently, consultation and sharing of knowledge among physicians interested in growth problems is an essential component of appropriate diagnosis and treatment.

Treatment of patients with obvious GH deficiency is straightforward. However, treatment of patients who have a GH-deficient-like phenotype—but who generate GH in at least certain testing situations—is not straightforward. Growth hormone may be effective in some such children, but not in others. Appropriate controlled studies need to be done to determine which children with a GH-deficient-like phenotype will increase their growth rates and, possibly, their ultimate heights.

Until such time as these studies have been done and we know as much about the therapeutic aspects of GH as we know about the physiologic aspects as outlined above, cautious prescribing of GH is judicious.

Alan D. Rogol, M.D., Ph.D.  
Robert M. Blizzard, M.D.

References supplied upon request to authors.

# A Letter to Our Readers

Dear Colleague:

The Editorial Board is pleased to introduce the inaugural issue of *Growth, Genetics, and Hormones*, a publication for academicians and practicing physicians who are interested in these important areas of medical practice. We are pleased to welcome you as a reader and invite you to participate as a reader and as a correspondent.

Normal and abnormal growth, genetically determined conditions, and the overall development of children are important aspects of pediatric practice. Hormonal production is essential in growth and development. It is probable that these areas will assume even greater prominence because pediatricians are showing greater interest in growth and development as immunizations and antibiotics diminish the incidence of infectious disease, as greater numbers of children with leukemia and other cancers enter sustained remissions, and as growing numbers of handicapped infants with congenital anomalies survive.

It is also well recognized that the literature concerning these topics is voluminous. Therefore, *Growth, Genetics, and Hormones* was developed, primarily, to provide a close look at current—and often controversial—topics in endocrinology, genetics, and metabolism and their potential clinical applications. To ensure that this goal is met now and in the future, several nationally and internationally respected authorities in genetics, endocrinology, anthropometrics, pediatrics, pharmacology, and metabolism have agreed to serve on the Editorial Board.

The eminent investigators who have agreed to serve as Associate Editors are: Dr. Jürgen Bierich of the University of Tübingen, West Germany; Dr. Judith Hall of the University of British Columbia Medical School; Dr. Fima Lifshitz of Cornell University School of Medicine; Dr. David Rimon of the University of California at Los Angeles; Dr. Alan Rogol of the University of Virginia School of Medicine; and myself. You will meet each of the Board members in the early issues of *Growth, Genetics, and Hormones* (see page 5 of this issue).

The editorial content of this quarterly publication was chosen with your interests in mind. This issue, for example, features an article about the incidence of growth hormone deficiency, a review of growth hormone physiology and pathophysiology, and a summary of a recent conference concerning the psychosocial aspects of growth delay. These are scientific, timely, and representative of the topics that *Growth, Genetics, and Hormones* will address.

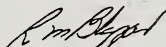
Abstracts of pertinent articles and reports will appear in each issue. In the future, the abstracts will serve as "mini reviews" and integrate multiple reports on a particular topic.

An Editor's Column will be a regular feature of future issues. You are invited to correspond with the Editorial Board. Such correspondence will be included whenever possible and will receive an open reply when indicated.

To help you keep up with major developments in pediatrics, genetics, and endocrinology, a calendar of meetings and postgraduate courses will appear in each issue. You are invited to advise us of meetings that you think pertinent for publication.

We are pleased to present this inaugural issue to you. We welcome your readership and look forward to hearing from you about *Growth, Genetics, and Hormones*. We would also appreciate your filling out the enclosed reply card to let us know of your initial interest.

On behalf of the Editorial Board:  
Sincerely,



Robert M. Blizzard, M.D.  
Professor and Chairman  
Department of Pediatrics  
University of Virginia School of Medicine  
Charlottesville, Virginia

# The Incidence of Growth Hormone Deficiency: Does Anyone Know?

Much attention is being directed by clinical investigators, pharmaceutical firms, geneticists, and pediatric endocrinologists to the incidence of growth hormone (GH) deficiency. Investigators are interested in this because the incidence dictates the number and type of studies that can be performed. Pharmaceutical firms are interested because the incidence will determine the market for the sale of native or DNA-recombinant hormone. Geneticists are interested because of the multiple biochemical or anatomic lesions that might be associated with GH deficiency. Pediatric endocrinologists are interested because they are the physicians primarily responsible for the diagnosis and treatment of GH deficiency.

No one knows for certain what the actual incidence of GH deficiency is. The reason for this is related to our inability to define GH deficiency itself. According to a study by Vimpani et al (*Brit Med J* 1977;2:247), GH deficiency is present if the GH concentration is  $<9$  ng/ml to two stimuli; the height is  $>2.5$  SD below the mean height for age; and the height velocity falls at or below the 25th percentile for chronological age. The prevalence of idiopathic GH deficiency in Scotland, according to Vimpani's study, is approximately 1:5,000 (4,000 to 6,500) births. Extrapolating from this ratio, there would be 14,500 such patients under 21 years of age in the United States.

This incidence, however, does not take into account those patients with organic hypopituitarism resulting from tumors of the hypothalamus or pituitary, or from other lesions, such as histiocytosis X, that produce GH deficiency. A review of several articles suggests that one case of organic hypopituitarism occurs for every four cases of obvious idiopathic GH deficiency. During the past ten years in our own clinic at the University of Virginia Medical Center, 84 patients with idiopathic GH deficiency and 36 patients with craniopharyngiomas or other causes of organic hypopituitarism have re-

ceived GH. If our figures are representative, approximately 20,000 cases of hypopituitarism exist nationwide in children less than 21 years of age.

However, there are other questions that should be considered before we accept this figure even as an approximation. What about patients with partial GH deficiency? What about patients who have what could be an immunologically active but biologically inactive hormone? What about other children who have the phenotype of GH deficiency and low somatomedin-C determinations, but who have significant levels of GH when tested? What about the patients who have severe constitutionally delayed growth and adolescent development?

There are many patients who might be considered GH deficient if one takes these patient groups into account. Patients with partial GH deficiency include those like the seven patients described by Spiliotis (see page 8 of this issue), who are believed to have GH neurosecretory dysfunction. The criteria consistent with the clinical picture of GH deficiency were present in these patients, although they had GH concentrations greater than 10 ng/ml when tested with pharmacological agents. Compared with normal-sized children, these patients had decreased integrated concentrations of GH over a 24-hour period and a decreased number of GH secretory episodes during the 24 hours. They responded to GH injections with growth rates comparable to those patients who were classified as GH deficient. Rudman et al (*N Engl J Med* 1981;305:123), Hayek et al (*J Pediatr* 1981;99:868), and others have described similar patients, although integrated concentrations of GH have not always been determined. The patients of Rudman et al and Hayek et al also have responded to GH with growth comparable to that observed in patients who are unequivocally GH deficient.

The problem of determining the incidence of GH deficiency may even be more complex than cited

above. Patients with constitutional delay of growth may have a relative GH deficiency (Bierich and Polthoff, *Monatsschr Kinderheilkd* 1979;127:561), as determined by low nocturnal GH levels, when compared with children without constitutional growth delay. If these patients have GH deficiency, it may be transient and is often reversed when adolescence begins. Of interest is the observation that 10% to 20% of patients believed to have GH deficiency prepubertally, and who have been treated with GH, do not have GH deficiency as adults. This percentage range was determined from our studies of more than 60 adults who were treated with GH as children because they were found to be GH deficient by pharmacological testing. Gournemelen et al have also drawn attention to transient partial GH deficiency in prepubertal children with growth delay (*Pediatr Res* 1979;13:221).

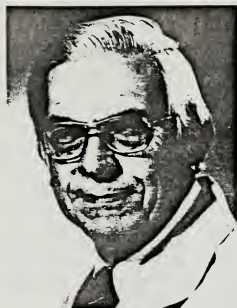
Thus, it bears repeating that no one really knows the actual incidence of GH deficiency. In the United States, the incidence is undoubtedly much greater than the 14,500 cases estimated from the data of Vimpani et al. Even discounting patients with constitutional growth delay, there are probably 20,000 or more American children with complete or relative GH deficiency. Unfortunately, the tedious nature and expense of determining integrated concentrations of GH over a 24-hour period will prevent the diagnosis of GH deficiency in many patients who have the phenotype of GH deficiency, but who respond normally to provocative stimuli with GH release.

At this time, it should be emphasized that methods other than those currently available, including therapeutic trials, must be designed to determine the incidence of GH deficiency as a cause of short stature. Therefore, it is essential that we devote special attention in the next few years to research concerning the incidence, diagnosis, and treatment of GH deficiency.

Robert M. Blizzard, M.D.



# Meet the Editorial Board Chairman:



Robert M. Blizzard, M.D.

Dr. Blizzard is Professor and Chairman of the Department of Pediatrics at the University of Virginia School of Medicine in Charlottesville, Va. He also serves as Associate Director of the Clinical Research Center there. Before coming to Virginia, Dr. Blizzard was Chief of the Division of

Pediatric Endocrinology at Johns Hopkins Hospital in Baltimore. During this time he was also Associate Professor of Pediatrics and, later, Professor of Pediatrics at The Johns Hopkins University School of Medicine.

Educated at Northwestern University in Evanston, Ill., and Northwestern University Medical School in Chicago, Dr. Blizzard has been involved in pediatrics and pediatric endocrinology since his graduation more than 30 years ago. After his internship and residency at the Raymond Blank Memorial Hospital for Children in Des Moines, he became a fellow in pediatric endocrinology at the Harriet Lane Home and Johns Hopkins Hospital. He then joined the medical faculty at Ohio State University in Columbus, serving at the same time as Chief of the Endocrine and Metabolic Unit at The Children's Hospital of Columbus.

Dr. Blizzard was President of the Human Growth Foundation in 1969-

1970 and President of the Lawson Wilkins Pediatric Endocrine Society for 1974-1975. A former Director of the National Pituitary Agency of the National Institutes of Health (NIH) in Bethesda, Md., he still serves on its medical advisory board and as a special consultant to the NIH.

Dr. Blizzard has extensively studied the role of growth hormone in patients with normal and abnormal growth. He has authored, or co-authored 160 articles and co-authored, edited, or contributed to 19 textbooks on this and related subjects.

## In Future Issues

Introduction of the other members of the Editorial Board

Assessing the Efficacy of Growth-Promoting Substances in Children: Methods and Problems

by James Tanner, M.D.

Nutrition, Growth, and Growth Failure  
by Fima Lifshitz, M.D.

## Associate Editor:



Fima Lifshitz, M.D.

At present, Dr. Lifshitz is Professor of Pediatrics at Cornell University Medical College in New York. He is also Associate Director of the De-

partment of Pediatrics; Chief of the Division of Pediatric Endocrinology, Metabolism, and Nutrition; and Chief of Pediatric Research at North Shore University Hospital in Manhasset, NY.

A native of Mexico City, Dr. Lifshitz graduated from that city's Yavne College and the National University of Mexico School of Medicine. After graduation, he served an internship at Children's Mercy Hospital in Kansas City, Mo., and a residency in pediatrics at the University of Kansas Medical Center in Kansas City, Kan. He then became a fellow in endocrinology and nutrition in the pediatric research training program at the Children's Medical and Surgical Center at Johns Hopkins Hospital in Baltimore. Returning to Mexico City, he served for two years as physician-investigator at the Hospital de Pediatría.

Since 1970, Dr. Lifshitz has been a visiting professor at numerous institutions in the United States, Israel, Egypt, South America, and China. A prolific author, he has written extensively about metabolic disease and gastrointestinal disturbances and how these conditions produce altered growth patterns. He has authored or coauthored 82 journal articles, 42 textbook chapters and review articles, and 107 abstracts and other short communications. He has also edited eight textbooks.

His writings and research efforts have contributed significantly to the understanding of carbohydrate intolerance in diarrheal disease, vitamin D-dependency rickets in children on long-term anticonvulsant therapy, experimental magnesium deficiency, and intestinal transport of vitamins and minerals in experimental malnutrition and diarrhea.

### Address for Correspondence

Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

# The Psychosocial Aspects of Growth Delay

During the past century, studies of the effects of short stature on personality development and social function have been plagued by anecdotal reporting and lack of objective, reliable testing procedures. This has led to inconsistent and often conflicting results. However, with the possibility of an unlimited supply of growth hormone (GH) for therapeutic use in the near future, there is an urgent need for sound scientific data on which to base future therapeutic decisions.

To address this problem, approximately 150 psychologists, sociologists, social workers, psychiatrists, endocrinologists, parents, and patients gathered in Washington, DC, this past October to attend a symposium on the psychosocial aspects of growth delay. Brian Stabler, Ph.D., and Louis Underwood, M.D., both of the University of North Carolina at Chapel Hill, served as moderators of 13 formal presentations and discussion periods that dealt with the behavior patterns, cognitive functioning, psychological status, and social integration of persons with GH deficiency, constitutional short stature, Turner's syndrome, chondrodysplasias, and deprivation dwarfism.

The issue of whether height affects academic achievement was addressed by Drs. C. Holmes (Des Moines), R.A. Richman (Syracuse, NY), P.T. Siegel (Ann Arbor, MI), and D. Young-Hyman (Baltimore). They all reported that actual school performance levels are discouraging, despite a normal range of intelligence quotients (IQs) in children with hypopituitarism and constitutional short stature. Dr. Holmes suggested that this may be related, in part, to decreased social competence in the mid-teen years. Dr. Young-Hyman pointed out that perhaps not enough attention had been paid to differentiating measures of personality adjustment from measures of social competence—eg, peer relationships and participation in extracurricular activities.

Dr. Richman found no severe psychological problems (as determined by a variety of psychological

tests) in these children. However, he suggested that subtle personality traits, such as shyness, low self-esteem, and increased internalization of complaints, as well as specific parental attitudes, such as increased permissiveness and decreased communication, may contribute to the children's poor school performance. Dr. Siegel reported that the pattern of poor academic performance was due, in part, to specific unrecognized cognitive defects, ie, learning disabilities. She noted, however, that in her group of GH-deficient subjects, 50% had at least one high-risk perinatal factor such as asphyxia or breech delivery, which could also have contributed to the cognitive defects.

In contrast to these four studies, R. Rosenfeld and D. Wilson of Stanford University reported a positive relationship between height and IQ. Their finding was based on a very large sample of normal children from the National Health Examination Survey. Rosenfeld and Wilson hypothesized that about 4% of the variance of IQ in the general population could be accounted for by height.

The unrecognized cognitive defects described by Dr. Siegel in the group of children with GH deficiency were underscored by Drs. H.C. Steinhausen (Berlin) and J. Downey (Columbia University) in their discussions of the perceptual defects in women with Turner's syndrome. The minor psychiatric problems experienced by the majority of these women were similar to those in an age- and size-matched group of females with constitutional short stature, suggesting that these problems are directly related to stature.

To assess the psychosocial benefit of increasing final adult height, Drs. R. Clopper (Buffalo), A. Johanson (Charlottesville, VA), and H. Dean (Winnipeg, Manitoba) described the long-term social outcome of GH-deficient adults treated with GH during childhood. In the three populations studied, the educational records were average but the rates of employment and marriage were low. The reasons for this

overall social maladjustment remain speculative. A significant proportion of these subjects expressed greater concern over their immature physical appearance than their short stature.

Dr. D. Rotnem (Yale University) discussed the pivotal role of family interaction in the ultimate psychosocial outcome of children with all forms of growth delay. She addressed the problems of parenting a short child, specifically in terms of the ambiguity of the child's size v age. She outlined various ways in which parents have learned to cope with the problem and identified risk factors associated with poor capability: lack of consistency, conflict between parents, ambivalence, and guilt.

Drs. D. Drotar (Chicago) and C. Annetillo (Baltimore) described poor psychosocial adaptation as not only a result, but also a major cause, of growth delay in infants.

One of the recurring themes of the meeting was the continued inconsistency in results obtained from the currently available battery of psychological tests. These tests do not appear to be sufficiently sensitive to identify subtle social problems. There was general agreement among the conferees that there are serious psychosocial problems associated with growth delay, and concern was expressed that these problems remain ill-defined. In his after-dinner speech, Dr. L.P. Sawisch noted that the limited attention paid to growth-related psychosocial problems is deeply rooted in society's preoccupation with height. It is therefore difficult to study these problems in isolation. The frustration expressed by the investigators regarding inadequate research tools, study design, and testable hypotheses was echoed by the parents and patients. Children and parents also perceived a lack of sensitivity on the part of the health-care and education systems. Parents felt that school personnel in particular were ill equipped to deal with the psychological needs of children with delayed growth and suggested that health educators be actively involved in programs to increase public awareness of growth-related problems.

Among the questions posed by

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## The Psychosocial Aspects of Growth Delay

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the participants at the end of the conference were the following: In the study of psychosocial issues should all persons with short stature be considered as a group or divided into separate diagnostic categories? Should they be viewed as a group with a chronic disease and compared with other groups of children with chronic medical disorders? Is it relevant to study groups to compare early v late adolescence and late adolescence v early adulthood? What is the best way to explore and control for varying degrees of family functioning? What is the most appropriate way to develop more reliable, sensitive, and standardized test procedures? It seems likely that the rapid pace of development in biotechnology and the future commercial availability of GH will provide the impetus for a new era in scientific endeavor to answer these important social questions.

Heather J. Dean, M.D.  
Associate Professor of Pediatrics  
Faculty of Medicine  
University of Manitoba  
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*Dr. Dean is a guest contributor for this issue. A highly respected pediatric endocrinologist with a special interest in the psychological aspects of growth disorders, Dr. Dean was among the speakers at the symposium reported above.*

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## Use of a Two-Site IRMA for GH in Identifying Children With GH-Dependent Growth Failure

Blethen and Chasalow compare the circulating growth hormone (GH) concentrations in adults of normal stature, endocrinologically normal short children with normal growth rates, and children with GH-dependent growth failure.

They used both the standard double antibody radioimmunoassay (RIA) method with a polyclonal guinea pig antiserum and a new immunoradiometric assay (IRMA). The former employs the standard National Hormone and Pituitary Program reagents and the latter uses two different monoclonal antibodies prepared against human growth hormone (hGH) (Hybritech, San Diego, CA). One antibody is covalently linked to a sepharose bead and the second is labeled with  $^{125}$ I. These monoclonal antibodies were selected for IRMA on the basis of antibody competition for GH binding with the  $^{125}$ I-labeled antibody. This assay is successful because each antibody binds to the GH molecule at a different epitope. The IRMA procedure is simpler and less time-consuming than the RIA technique.

The theoretic advantages of the IRMA method are: (1) linearity; (2) a relatively stable coefficient of variation over a greater range of antigen concentrations than in the classical RIA techniques; and (3) improved sensitivity and precision. With the use of monoclonal rather than polyclonal antibodies, several additional benefits are derived: (1) the amount of antibody is unlimited so that one can generate a very high capacity solid phase antibody system; and (2) since only a single type of antibody (selected for high affinity) is attached to the solid phase support, higher antigen concentrations can be tested.

The investigators sought to compare the results of circulating GH levels in normal adult volunteers, normal short children, and children with GH-dependent growth failure to determine if children in the last group (whose pharmacologic stimulation tests for GH secretion were normal) had an immunologically distinguishable circulating GH spe-

cies. Since samples that had no measurable GH by RIA were always unmeasurable with IRMA, samples for the IRMA were selected from samples with GH detectable by RIA.

When purified hGH was added either to human serum or the kit "zero calibrator," there was a strong correlation between the values found by RIA and IRMA (slope of the regression line = 0.86). In both normal individuals and children with GH-dependent growth failure, the ratio of IRMA-GH to RIA-GH was not affected by the time of sampling relative to the peak. The mean IRMA-GH to RIA-GH ratios were  $0.48 \pm 0.02$  for normal subjects (slope = 0.62) v  $0.35 \pm 0.001$  (slope = 0.39) for subjects with GH-dependent growth failure. These values are significantly different at  $P < 0.001$ .

These results indicate that both assays measured the authentic material with approximately equal effectiveness. For the normal group the slope of the regression line was less, indicating that there were differences in the folded structure of pituitary and circulating GH. However, the slope of the assay for those children with GH-dependent growth failure was even lower, indicating that their circulating forms of GH differed from those of normal subjects. The latter group of children is precisely the group that responded to exogenous replacement of GH.

Blethen SL, Chasalow FI: *JCE&M* 1983;57:1031.

*Editor's comment*—These data are exciting and, if confirmed, could materially aid physicians in deciding which children might respond to exogenous GH therapy. At present, some of these patients are considered to be at variance from normal, since they have normal levels of GH following physiologic or pharmacologic stimuli to GH secretion. The simple expedient of assaying their circulating levels of GH in two separate assays may enhance our knowledge of the syndrome of GH-dependent growth failure and target a group for a therapeutic trial with GH.



## Neurosecretory Dysfunction: A Treatable Cause of Short Stature

Studies presented in this article indicate that there is a group of short children who, although not growth hormone (GH) deficient by classic definition, do not secrete an adequate amount of GH during a 24-hour period to grow normally. Seven children (7.4 to 15.5 years of age) so classified met criteria consistent with GH deficiency: height less than first percentile, growth velocity  $<4$  cm/yr, bone age at least 2 years behind chronological age, and low somatomedin-C concentrations for age, except that there was a GH peak  $>10$  ng/ml to provocative testing. These children are classified as children with neurosecretory dysfunction (NSD). Twenty-four-hour integrated concentrations of GH (ICGH) (samples withdrawn every 20 minutes) were compared with concentrations from 16 GH-deficient children and 22 controls.

All children with NSD had nocturnal GH peaks of 10 ng/ml or greater. Six of the 16 GH-deficient patients also had nocturnal peaks of 10 ng/ml or greater. These data indicate the poor correlation between pharmacologic testing and nocturnal peaks of GH in GH-deficient children and the poor correlation between pharmacologic testing, nocturnal peaks, and ICGH in children with NSD.

|              | N  | ICGH                | No. of peaks 24 h | Area under curve | Mean per amplitude |
|--------------|----|---------------------|-------------------|------------------|--------------------|
| Controls     | 7  | $5.4 \pm 0.5$ ng/ml | $6.4 \pm 0.3$     | $129 \pm 14$ U   | $17.0 \pm 1.4$     |
| GH deficient | 16 | $1.6 \pm 0.2$ ng/ml | $1.9 \pm 0.5$     | $26 \pm 6$ U     | $9.0 \pm 2.2$      |
| NSD          | 22 | $2.1 \pm 0.3$ ng/ml | $3.9 \pm 0.6$     | $42 \pm 5$ U     | $9.3 \pm 1.2$      |

Six of the seven patients with NSD responded to GH treatment (0.07 U/kg body weight three times weekly) nearly as well as the GH-deficient patients (a mean change in growth rate of  $4.1$  v  $5.4$  cm/yr).

In addition, the authors observed that nocturnal GH peaks in many of the children who manifested these peaks occurred in all stages of sleep except stage 4. In fact, the nocturnal GH peak may occur during another stage of sleep or in a subsequent period of stage 3 or 4. Interestingly, these investigators also found no differences in ICGH or patterns of GH secretion in children of various Tanner stages of sexual development. This is in accord with previous studies of some investigators (Thompson et al: *JCE&M* 1972;35:334), but not in accord with studies by Howse et al (*Clin Endocrinol* 1977;6:347), who suggested a pubertal increase in GH secretion based on five-hour nocturnal sampling in several short children.

As a result of these observations, the authors suggest that there is a spectrum of GH neurosecretory abnormalities ranging from absolute deficiency to a problem in GH regu-

lation not readily identified with provocative testing. They also suggest that these abnormalities are manifested by reduced number and/or amplitude of pulses, not readily identifiable with GH-stimulation tests, and that a majority of these patients respond to GH therapy with significant and sustained growth.

Spiliotis BE, August GP, Hung WJ et al: *JAMA* 1984;251:2223

**Editor's comment**—Spiliotis et al have demonstrated convincingly the points made in their report. It is apparent that not all patients with GH deficiency can be demonstrated by utilizing pharmacologic testing for GH release. The dilemma regarding the criteria for diagnosis of GH deficiency is emphasized from the data presented. Although the ideal method of diagnosis is to perform integrated concentrations of GH over 24 hours, this is impractical except in the research setting. These data emphasize the fact that it is difficult to determine the incidence of GH deficiency because it depends upon the criteria used to make the diagnosis.

## Precocious Puberty After Hypothalamic and Pituitary Irradiation in Young Children

R. Brauner and co-workers at the Hôpital des Enfants-Malades in Paris report that six of 29 children treated with irradiation before seven years of age for medulloblastoma or other head and neck tumors, or for acute lymphoblastic leukemia, developed precocious puberty. Most developed precocious puberty within 30 months of irradiation therapy. Five had associated growth hormone (GH) deficiency. This combination of sexual precocity and GH deficiency produces short stature (136.7 cm, 143.5 cm, and 145 cm in the three patients whose heights were reported) in adult-

hood. It is important to consider that such children are at high risk for having very short adult stature, and require specific treatment of precocious puberty combined with GH therapy when a deficiency of this hormone is demonstrated.

Brauner R, Czernichow P, Rappaport R: *N Eng J Med* 1984;311:920.

**Editor's comment**—More and more children with tumors are surviving following irradiation therapy. This will increase the incidence of organic hypopituitarism and increase

the use of GH as a therapeutic agent. Studies using luteinizing hormone-releasing hormone analogues in conjunction with human growth hormone are being conducted at the University of Virginia, Boston Children's Hospital, Massachusetts General Hospital, and the University of California, San Francisco, by R.M. Blizzard, J. Crigler, J. Crawford, and S. Kaplan respectively. Physicians who encounter patients with GH deficiency accompanied by normal adolescent sexual development, and who are going to be unacceptably short, are urged to contact these investigators.

## Laron Type Dwarfism (Hereditary Somatomedin Deficiency): A Review

Laron type dwarfism is a syndrome of familial dwarfism that is indistinguishable from isolated growth hormone (GH) deficiency except that patients have normal or elevated GH concentrations. The syndrome was described by Laron et al in 1966.

In the current review, Laron tabulates 72 cases. Many are non-Jewish. The birth weight, known in 21 cases, was  $>2,500$  g in 18, and the birth length was more than 2 SD below the mean in ten of 16. Pregnancies and deliveries were unremarkable. Approximately 50% had skeletal or mesenchymal anomalies, none of which was life threatening.

Development in children with Laron type dwarfism is generally slow, many sit only after the age of 1 year and walk after 18 months. Fontanel closure occurs between 3 and 7 years. Symptoms of hypoglycemia and high-pitched voice are also characteristic. With the passage of time, the acromicria and disproportion between the face, with its saddle-nose, and the cranium become more apparent. The teeth are discolored, defective, and crowded. Growth is slow, with males reaching ultimate heights of 119 to 142 cm and females, 108 to 136 cm. Surprisingly, the upper/lower ratios are more than 2 SD above the mean, indicating that the limbs are short in comparison to the trunk. After puberty, the skin assumes a prematurely aged appearance. The genitalia in affected children and adults are very small, and pubertal development is slow. Menarche occurs between 13 and 18 years of age and ejaculation between 17 and 21 (compared to a normal mean of 13½ years).

Skeletal age is delayed. By x-ray analysis, the long bones are small and delicate, the sella is of normal size, and the facial bones are small in comparison to the cranium. The head consequently appears enlarged, but it is not (on the basis of standard measurement). Glucose intolerance is present even when hypoglycemia and hypoinsulinemia

occur. Growth hormone levels often are elevated, but are suppressed normally with glucose. Serum somatomedin-C (Sm-C) concentrations are low, and do not increase after GH injections, although 50% of patients have an increase in free fatty acids. Nitrogen retention and hypercalcuria are minimal following GH administration.

No neurologic deficits were observed in these patients, and pneumoencephalograms were normal. IQ scores were strongly skewed toward the lower part of the curve (mean IQ = 82.1). Visual-motor coordination was uniformly poor. The parents regarded their own and their children's lives as ruined, since no remedial treatment exists for Laron type dwarfism. School was a

negative experience for these children.

The etiology is believed to be related to the hGH receptors, since liver cell microsomes from these patients do not bind hGH normally, although insulin binds normally. Consequently, Sm-C is not generated.

Laron Z. *Advances in Internal Medicine and Pediatrics*. Heidelberg, Springer-Verlag, 1984, p 118.

*Editor's comment*—Laron et al have clarified the etiology and provided additional information about the syndrome. Treatment with Sm-C (IGF-I) might be effective. Unfortunately, adequate quantities are not currently available to test this hypothesis.

## Height and Weight Status of Indo-Chinese Refugee Children

Pediatricians and other health practitioners who deal with children have no guidelines with which to evaluate the growth or growth potential of refugee children. The absence of such guidelines becomes problematic when one tries to determine if growth retardation exists in a particular child (which, in itself, would indicate that a search should be made to determine a cause). This report attempts to supply the needed information, and succeeds partially.

Height and weight measurements were obtained from 1,650 children residing in Laotian and Cambodian refugee camps and in areas surrounding these camps. Reference tables that are available from China, Thailand, and the United States were also used. The mean weights and mean heights for age of the groups studied are approximately 2 SD below US means, but there is variation.

These studies are inadequate because they are not randomized, as readily stated by the authors. To what extent catch-up growth may occur in refugee children remains to be determined. Evidence that nutrition plays a role was presented in one study in which the heights and weights of children from upper-class and professional backgrounds were compared to American standards. The mean heights and weights more nearly approached the US standards than the heights and weights found in refugee children.

Olness K, Yip R, Indritz A, et al: *AJDC* 1984;138:544

*Editor's comment*—While these studies are limited, they are of value. We practitioners can assume with some justification that the normal growth curves for refugee Oriental children are approximately 2 SD below US curves.

|                | Height (SD) | Weight (SD) |
|----------------|-------------|-------------|
| US reference   | 0           | 0           |
| Chinese urban  | -0.8        | -0.8        |
| Chinese rural  | -1.4        | -1.3        |
| Thai reference | -1.3        | -1.8        |
| Khmer refugee  | -1.8        | -1.8        |
| Lao refugee    | -2.1        | -2.2        |
| Thai village   | -2.3        | -1.9        |

## Report of the Conference on Uses and Possible Uses of Biosynthetic hGH

In a society that values tallness, enormous pressure will be put on physicians to prescribe human growth hormone (hGH). The pressure will come from parents whose children are not fulfilling parental expectations in sports, social interactions, and academic achievement. Physicians will determine whether to prescribe hGH to children who are short because of normal genetic variation. They will be forced to decide whether to tamper with normal children in the hope of making them "better." Is it ethical to administer hGH to short children who are probably not growth hormone (GH) deficient according to current criteria? Will such treatment produce taller or better adults? What are the possible adverse side effects? How will misuse be prevented?

These considerations were addressed by 50 experts at a conference in late 1983 on the uses and possible uses of DNA-hGH. The conferees were asked to address the full spectrum of concerns about uses and abuses of hGH. Underwood summarized the conference findings in an editorial for the *New England Journal of Medicine*.

At the conference, one group addressed the question of how to distinguish partial GH deficiency. Provocative tests are not always reliable in determining whether insufficient GH is the cause of limited growth, since some patients with partial GH deficiency release significant amounts of GH when tested with pharmacologic agents. Participants discussed the increasing interest in measuring serum GH levels under physiologic conditions. Data are insufficient at present to permit judgment of optimal times, duration, and methods of measurement. The participants agreed that somatomedin-C concentrations are sometimes helpful in diagnosis if used in conjunction with other tests. Low values must be confirmed by GH testing before the diagnosis of GH deficiency is made, and low values in young children must be interpreted cautiously.

The terms used to describe short stature were also discussed: normal variant short stature, GH-dependent growth failure, and the syndrome of immunoreactive-bioinactive GH are poor terms.

The potential complications of

glucose intolerance, hyperlipidemia, and possible acceleration of the atherosclerotic process with GH administration were considered. The conferees recommended that an epidemiologic survey of possible late-appearing side effects be undertaken in patients who have been or are being treated with hGH.

The consensus of the conferees was that there is an urgent need for therapeutic trials to determine the effect of GH in short children who do not have GH deficiency. It was deemed ethical to administer GH to such children under a controlled research study. Because no mechanism for direct regulation of prescribing hGH is available, it was agreed that the most effective way to avoid abuses is through the education of physicians and the public.

Underwood L. *N Eng J Med* 1984; 311:606.

*Editor's comment*—The above abstract is brief, and the interested reader is encouraged to review the entire report. Consideration of this well-reasoned editorial by all physicians who will be prescribing hGH for any cause is imperative.

## Comparison of Physiologic and Pharmacologic Assessment of GH Secretion

Siegel et al evaluated and compared growth hormone (GH) release to arginine (ATT), insulin (ITT), and sleep. Samples were drawn every 30 minutes between 11:00 PM or midnight and 6:00 AM via an indwelling catheter. Sixty-two short children (53 males and nine females) were evaluated. Twenty (32%) failed to respond significantly to either test (maximal GH,  $<3.5$  ng/ml). Surprisingly, only 14 of these 20 were classified by the authors as truly and permanently GH deficient. The other six were patients with constitutional growth delay, psychosocial dwarfism, and hypoadrenal hypogonadism. Five

subsequently had normal peaks during sleep.

Thirty-three (53%) of the 62 responded normally to both the pharmacologic and physiologic tests. Eight (13%) had abnormal responses to pharmacologic testing but normal responses to physiologic testing (mean peak GH =  $19.0 \pm 2.0$  ng/ml). Seven of these eight were growing 5.0 cm/yr or more and were believed to have constitutional growth delay. Only one patient ( $<2\%$ ) failed to respond to physiologic stimuli but responded to pharmacologic stimuli.

These results confirm previous studies that show there is often a discordance in the GH response in normal individuals who are tested with arginine- and insulin-induced hypoglycemia. The authors state that the responses to the two tests were concordant in 43 of 62 patients (69%). However, if the 28 patients

who responded to neither ATT nor ITT are removed, the authors found that only 23 of 42 patients (55%) who responded did so to both stimuli.

These studies verified previous reports that more than one pharmacologic test must be used to diagnose GH deficiency and that physiologic testing (nocturnal frequent sampling) is preferable to pharmacologic testing. The data also reaffirm the impressions of many that even with both tests, erroneous diagnoses are still frequently possible.

Siegel SF, Becker DJ, Lee PA et al. *AJDC* 1984;138:540.

*Editor's comment*—These data further emphasize how difficult it may be to diagnose all patients with GH deficiency, and therefore how difficult it is to determine precisely the incidence of GH deficiency.



## The Effect of Small But Sustained Elevations in Circulating Growth Hormone on Fuel Metabolism in GH Deficiency

This study was designed to examine the effects of maintaining modest but constant levels of circulating growth hormone (GH). To test the hypothesis that some of the metabolic consequences of acromegaly might be attributable to the loss of the normal pulsatile pattern of GH release, Tamborlane and co-workers examined the effects of continuous subcutaneous infusions of GH (CSIGH) on glucose tolerance and apparent insulin sensitivity.

To eliminate the variability introduced by endogenous GH secretion, eight children and adolescents with GH deficiency and 12 normal controls were tested. An oral glucose tolerance test was performed. A 90-hour subcutaneous infusion of GH (corresponding to 0.05 U/kg/24 h) then was started in the GH-deficient patients. On the morning of the fourth day, a second oral glucose tolerance test was done. The results of the glucose tolerance tests were compared with those in seven nonobese children and adolescents.

CSIGH produced small but sustained elevations in GH concentrations (mean, 5.9 ng/ml with a coefficient of variation [CV] of 21%). The normal controls (no infusion) had a mean of 10.1 ng/ml, but a CV of 105%. CSIGH had no significant effects on fasting plasma glucose or insulin levels, but sharply altered oral glucose tolerance (plasma glucose was 30 to 40 mg/dl above preinfusion values). This occurred despite a virtual doubling of insulin secretion during the test.

Only transient changes in fasting free fatty acid concentrations were found, and no significant changes were noted in the fasting concentrations of alanine or branched-chain amino acids. After CSIGH, somatomedin-C (Sm-C) levels increased sharply in two subjects, but remained virtually unchanged in five.

taining fuel homeostasis as the intermittent secretion of gonadotropin-releasing hormone for the gonadal axis. The sustained nature of constant GH levels, even at a relatively low concentration, is sufficient to induce marked derangements in oral glucose tolerance and insulin action. In five of seven children, these actions occurred in the absence of elevated Sm-C concentrations.

trations. Thus, the actions of GH on intermediary metabolism may be the direct effects of GH. For our colleagues who take care of adult patients, these data suggest that severe metabolic alterations and their long-term consequences may accompany so-called mild acromegaly. Since these moderately elevated concentrations of GH can cause metabolic derangements, it may be that anyone whose GH levels are not suppressed into the unmeasurable range is at risk for the continuing metabolic complications of GH excess.

## Meeting Calendar

**April 13-18** American Academy of Pediatrics Spring Session. Atlanta, Georgia. Contact: American Academy of Pediatrics, 1801 Hinman Avenue, Evanston, IL 60204

**May 7-10** American Pediatric Society, Society for Pediatric Research, and Ambulatory Pediatric Association Annual Meeting. Sheraton Washington Hotel, Washington, DC. Contact: Charles B. Slack, Inc., 6900 Grove Road, Thorofare, NJ 08086

**May 22-25** 4th International Clinical Genetics Seminar. Endocrine Genetics and the Genetics of Growth. Athens, Greece. Contact: Dr. Christof Vartsocas, 47 Vasilissis Sofias Avenue, Athens 140, Greece

**June 15-18** American Society for Bone and Mineral Research

Meeting. Washington, DC. Contact: Shirley Hohl (707) 279-1344

**June 16-18** 45th Annual Meeting and Scientific Sessions of the American Diabetes Association. Baltimore Convention Center, Baltimore, Maryland. Contact: Carolyn Sciortino, ADA, 2 Park Avenue, New York, NY 10016

**June 19-21** 67th Annual Meeting of The Endocrine Society. Baltimore Convention Center, Baltimore, Maryland. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814

**June 22-25** Second Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology. The Hyatt Regency, Baltimore, MD

Tamborlane WV: *Pediatr Res* 1984; 18:212.

*Editor's comment*—It appears that the intermittent pulsatile signal of GH release is as important for main-

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Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

## Pseudopituitary Dwarfism Due to Resistance to Somatomedin: A New Syndrome

Bierich et al report a patient with elevated circulating growth hormone (GH) and somatomedin-C (Sm-C) concentrations. Although birth length and weight were normal (48 cm and 3 kg), all parameters of growth fell behind quickly. At 12 months of age, the infant's length was 58 cm and the weight 5.6 kg. The bone age was 6 months. Dental eruption occurred at 13 months. Hypoglycemia occurred during the second year. Circulating concentrations of Sm-C were increased for age when measured by bioassay at 10 months (1.99 and 2.03 U/ml). Sm-C by specific radioimmunoassay was elevated for age (1.28 U/ml). Administration of 4 IU of GH daily for four days did not increase the levels.

Fibroblasts from a skin biopsy taken when the patient was 21 months old were incubated with <sup>125</sup>I Sm-C. Compared with multiple controls, binding to the patient's fibroblasts was diminished by 50%. The

authors attribute the abnormality to defective Sm-C receptors.

This syndrome differs from Laron type dwarfism and the dwarfism described by Hayek et al (*J Pediatr* 1981;99:868) and Kowarski et al (*JCE&M* 1978;47:461). Sm-C concentrations are low in patients with Laron type dwarfism and do not increase after human growth hormone (hGH) administration. Sm-C levels were low in the patients described by Hayek et al and Kowarski et al, but they did respond to GH injections with increased Sm-C levels. In the patient currently presented, the Sm-C concentration was elevated. The authors term all of these types of dwarfism pseudopituitary dwarfism.

Two different actions of Sm-C are discussed. First are the acute effects upon skeletal muscle, heart muscle, and adipocytes. The second are the long-term metabolic effects that act through fibroblasts and chondrocytes. The authors be-

lieve that Sm-C works through the insulin receptors and affects the classic insulin-dependent tissues in the acute processes. The long-term or later effects influence fibroblast and chondrocytes, which are induced to proliferate. In the long term, Sm-C is postulated to act primarily through the specific IGF-I receptors.

Bierich JR, Moeller H, Panke MB, et al: *Eur J Pediatr* 1984;142:186

**Editor's comment**—This new syndrome is another in the ever-increasing list of syndromes in which the patients have GH-deficient-like phenotypes. It is probably one of the least common of such syndromes, but it obviously exists. The authors refer in their bibliography to other patients who may have the same syndrome. We have observed one patient at the University of Virginia who unequivocally has this syndrome. We prefer to use the term "short stature with GH-deficient-like phenotype" for all of these patients who do not have GH deficiency.

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# GROWTH

## Genetics & Hormones

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### Nutrition, Growth, and Growth Failure

Nutritional causes of short stature and/or poor growth often remain unrecognized by pediatricians and pediatric endocrinologists even though the need for adequate weight gain and body fat to sustain growth during puberty is well described. Although undernutrition resulting from the unavailability of food or psychosocial deprivation accounts for most cases of growth retardation throughout the world, it is a rare cause of short stature in the United States.

When a nutritional deficiency is suspected as a possible cause of short stature, the physician should first ascertain whether the deficit is due to decreased intake resulting from increased energy metabolism, or to increased caloric loss of protein or fat via the stool. A dietary history and/or a brief period of observation in a hospital usually reveals whether there is a decreased intake of calories and/or substrates, hyperactivity, or abnormalities of the gastrointestinal (GI) tract. Anorexia, which can occur as a nonspecific phenomenon secondary to disease or as a primary psychological disorder, is a classic example of poor intake. Nonspecific causes of anorexia—iron deficiency, for example—are seen during infancy and childhood.

**Iron deficiency** is the end result of an imbalance between the sum of the patient's iron endowment, intake, and absorption, and the sum of his iron needs for growth and replacement of losses. The peak incidence of iron deficiency in childhood is between 6 months and 1 year of age; another such peak is seen during early adolescence. The average American diet provides only 15 to 18 mg of iron per day, of

which only an average of 10% is absorbed. The normal daily requirement of elemental iron is 15 mg for an adolescent. It is therefore not surprising that as many as 10% of children have been found to have iron-deficiency anemia. Iron deficiency can also account for anorexia in some high school students.

In addition to looking for evidence of anemia, physicians should also determine serum iron levels, total iron binding capacity, and ferritin levels in infants with failure to thrive

and in older children with anorexia. If iron deficiency is found, one should try to determine whether it is the initiating cause of the anorexia or the result. Iron replacement over a period of two to three months may improve growth and appetite regardless of the etiology of the iron deficiency.

**Gluten sensitivity (celiac disease)** is also associated with low caloric intake and may be associated with continued on page 2

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### Problems in Assessing the Efficacy of Growth-Promoting Substances: The Role of Height Prediction

There are two objectives in treating children with growth-promoting substances such as growth hormone (GH) or anabolic steroids. The primary objective is to promote an increase in the child's ultimate height so that he will be taller than he otherwise would have been. The secondary objective is to accelerate the rate of growth, and thus permit the child to achieve an adult height sooner, even if the ultimate height

remains unchanged. To attain the second objective, the child must grow at an increased rate over a sustained period, but the increased rate must not diminish the ultimate adult height.

Growth hormone made by recombinant DNA techniques will soon be available, and the opportunity—or temptation—to use it on children who are short, but not GH deficient (GHD), will present itself. The problem is to know whether trials assessing GH in children have been successful and whether there is evidence, at the end of a single year, that GH administration is likely to achieve one or both of the aforementioned objectives.

The first difficulty with these questions relates to the compensatory deceleration of growth observed in GHD patients who are taken off GH therapy. In the year after the first year of therapy, the growth rate is

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# Nutrition, Growth, and Growth Failure

continued from page 1

anemia as well. Onset most often occurs during infancy. In Europe, much attention has been given to the association between celiac disease without significant GI symptoms and short stature. It is therefore reasonable to suspect celiac disease in children who are short without an adequate explanation. After the child has been challenged with a high-gluten diet for four to six weeks, an intestinal biopsy to confirm the diagnosis of celiac disease can be considered (see page 10 of this issue).

Adolescent girls with **anorexia nervosa** of the characteristic type are well recognized by most pediatricians and pediatric endocrinologists. The associated endocrine abnormalities found in these patients are related primarily to undernutrition and are similar to those seen in people with severe caloric deficiency in third world countries. Gonadotropins and somatomedin-C determinations are usually low. Growth hormone levels are usually normal. Since Crohn's disease can masquerade as anorexia nervosa, it should be considered in the differential diagnosis.

Similar to, but certainly not identical with, typical anorexia nervosa is "**fear of obesity**," so termed because it leads to self-imposed malnutrition (see page 9 of this issue). Numerous lay and scientific journals have published articles describing poor growth and delayed development in adolescents who have severely restricted their food intake or gone on fad diets. We recently recognized a group of 14 patients who failed to grow because of self-imposed malnutrition that was rooted in a fear of becoming obese. Their diets were deficient in calories, minerals, and vitamin D. After at least one year of inadequate weight gain, these patients showed signs of deteriorating linear growth and failed to attain puberty, the latter a characteristic also of anorexia nervosa. No organic causes were identified. Once the fear of obesity was recognized, the patients were given nutritional and psychological counseling. They resumed an adequate

caloric intake for their age and recovered, as demonstrated by improved linear growth and progression of adolescent sexual development. Only one patient had a permanent alteration of height potential, probably because of delayed diagnosis and treatment. Menarche occurred in this patient soon after adequate weight gain had been established, but her height increased only minimally.

These patients appear to differ from those with other bariprophic syndromes. They had not lost significant amounts of weight, but rather had ceased to gain weight as they progressed along previously defined height percentiles. They also did not have a distorted body image; they realized they were slim and wanted to stay that way. In contrast, patients with true anorexia nervosa lose weight rapidly over a short period and usually see themselves as heavy even though they are markedly undernourished.

Unlike classic anorexics, these patients fearing obesity had no self-induced vomiting, did not abuse laxatives or diuretics, did not exercise compulsively, and did not hoard food. We believe that fear of obesity as manifested in these patients represents an exaggeration of our social concerns with achieving and maintaining an "ideal" trim figure. The incidence of this syndrome is unknown, especially since patients with mild forms of the disorder may not even attract medical attention. Interestingly, we have recently identified infants with failure to thrive because of inadequate nutrition (calories were inappropriately withheld) stemming from parental concern about obesity in their children.

**Chronic inflammatory bowel disease (CIBD)** is another condition that retards linear growth. Growth failure and sexual infantilism (prevalence 30% to 85%) are major complaints in many adolescents with CIBD. Children, however, may be asymptomatic and present primarily for short stature and delayed development. Digital clubbing, aphthous stomatitis, arthritis, or pyoderma gangrenosum are clues to the un-

derlying GI pathology in short-statured patients with CIBD. Growth may slow down or cease without any other sign or symptom, sometimes for more than three years before GI complaints appear. Therefore, CIBD should be considered as a cause of inadequate growth even in the absence of GI complaints. Gut motility studies are helpful in confirming the diagnosis, as is an abnormal sedimentation rate, although not all patients with CIBD have abnormal rates.

Children with **Crohn's disease** or **ulcerative colitis** may not grow normally because of impaired nutrient absorption, decreased nutrient intake, specific nutrient deficiencies, or increased protein losses through the GI tract. Glucocorticoid excess during treatment with steroids is another cause. While some children with CIBD have intestinal malabsorption, the majority do not have significant steatorrhea and are able to absorb xylose normally. Thus, malabsorption of nutrients does not fully explain the poor growth in most of these patients. Anorexia, however, plays a significant role in patients who have abdominal pain following meals and who may also be losing protein through the GI tract.

Nutritional rehabilitation often promotes growth in growth-retarded children with CIBD. Short-term parenteral nutrition in a hospital, as well as long-term total parenteral nutrition at home, can produce marked increases in height and catch-up growth. Oral feedings may also promote catch-up growth if enough nutrients are ingested. On occasion, nutritional rehabilitation has induced remission of the disease, suggesting that adequate nutrition is needed to control it. Appropriate nutrition may also be necessary for medications such as sulfasalazine or steroids to exert their therapeutic effects, which in turn may permit the resumption of normal growth.

**Zinc metabolism and deficiency** are associated with a number of clinical syndromes. Moreover, many recent articles have implicated zinc deficiency as a cause of growth retardation.

Zinc is an essential nutrient. Adolescents and adults require 15

mg/d, infants and children require 3 to 5 mg/d in their first year and 10 mg/d until early adolescence. Zinc deficiency may result from malabsorption states, or it may develop during total parenteral nutrition or along with cirrhosis of the liver and renal disease. Symptoms of mild to moderate zinc deficiency include diminished taste sensitivity, anorexia, and growth retardation. Acrodermatitis enteropathica, diminished cellular immunity, and poor wound healing may also indicate zinc deficiency. High concentrations of dietary phytate (as seen in the typical Iranian or Egyptian diet) can diminish the availability of zinc and precipitate zinc deficiency syndromes. The clinical diagnosis of zinc deficiency can be confirmed by a low concentration of plasma zinc. Zinc levels in hair are unreliable indicators of deficiency.

In summary, physicians must recognize the important role that nutrition plays in normal and abnormal growth. Indeed, nutritional causes of growth disturbances may be as obscure and subtle as endocrine causes such as partial growth hormone or thyroid deficiency.

Fima Lifshitz, M.D.

References will be supplied upon request to Dr. Blizzard.

### Address for Correspondence

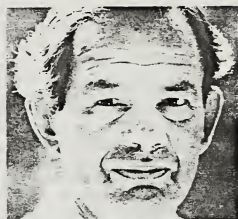
Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

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## Meet the Editorial Board Associate Editor:



Jürgen R. Bierich, M.D.

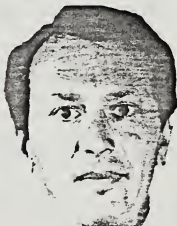
A native of Hamburg, West Germany, Dr. Bierich has been Professor and Chairman of Pediatrics at the University of Tübingen since 1968. After graduating from the Medical School of Hamburg University in 1946, he served his residency in pediatrics under Drs. Degkwitz, Eckstein, and Schäfer in Hamburg. He also served a residency in internal medicine under Dr. Jores in the same city.

During his residencies and for several years thereafter, Dr. Bierich's major scientific interest was pediatric endocrinology. Much of his work dealt with the physiology and pathology of the pituitary and adrenal glands, as well as with sexual maturation.

Dr. Bierich received the title Privat-Dozent in 1956 and was appointed Professor in 1962. During 1963 and 1964, he served as President of the European Pediatric Endocrinology Club, which was later renamed the European Society of Pediatric Endocrinology. A decade later, in 1973 and 1974, he served as President of the German Society of Endocrinology. In 1979, he was elected to membership in the German Academy of Natural Scientists Leopoldina.

An author or coauthor of numerous articles, Dr. Bierich is currently involved in work concerning problems of growth and development and pediatric aid in the third world.

## Associate Editor:



David L. Rimoin, M.D., Ph.D.

Born in Montreal, Quebec, Canada, Dr. Rimoin has lived in California since 1970, when he joined the staff of Harbor-UCLA Medical Center in Torrance as Chief of the Division of Medical Genetics. He has also been Professor of Pediatrics and Medicine at UCLA School of Medicine since 1973. In addition, he is a consultant physician to several hospitals in the Los Angeles area, including Cedars-Sinai Medical Center, Orthopedic Hospital, and Shriners' Hospital for Crippled Children.

Dr. Rimoin graduated from McGill University in Montreal (with first class honors in genetics) in 1957. He received both his medical degree and a Master of Science degree in genetics from McGill University in 1961. He then served a rotating internship at Royal Victoria Hospital and Montreal Children's Hospital, and a residency in medicine at Royal Victoria. He continued his residency in medicine at Johns Hopkins Hospital in Baltimore. Between 1964 and 1967, he was a Fellow in Medicine (medical genetics) at The Johns Hopkins University School of Medicine. He received his doctorate in human genetics from that institution in 1967.

Between 1979 and 1983, Dr. Rimoin was President of the American Board of Genetics. In 1984, he was President of the American Society of Human Genetics. As author of more than 200 articles, 8 textbooks, 17 chapters in books and atlases of genetic defects, and 141 abstracts, Dr. Rimoin has written extensively on genetics, diabetes, endocrine disorders, and growth disorders.

# Problems in Assessing the Efficacy of Growth-Promoting Substances: The Role of Height Prediction

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even slower than the rate before treatment. In 14 prepubescent patients whom we studied, growth velocity was 3.4 cm/yr before treatment, 8.1 cm/yr during treatment, and 2.2 cm/yr posttreatment. In addition, 13 children with the Silver-Russell syndrome had growth velocities of 5.4 cm/yr, 6.8 cm/yr, and 4.6 cm/yr in their pretreatment, treatment, and posttreatment years, respectively. The deceleration was greater in the first six months of the posttreatment year than in the second six months in both groups of patients.

Some slowing of the growth rate in the posttreatment year, compared with the pretreatment year, is expected, since growth velocity normally declines during prepubescent years. Corrections can be made for this slowing by using the standard velocity curves of Tanner, Whitehouse, and Takaishi. The estimated net gain during the year of treatment is given by a formula:

$$V_T + V_2 - 2(V_1 - D_T) + D_2$$

$V_T$  represents velocity.  $D_T$ , the expected normal diminution of velocity from pretreatment to treatment year, and  $D_2$ , the normal diminution from treatment to posttreatment year. As calculated by this method, the net gain in the Silver-Russell children was only 0.5 cm/yr. Some heterogeneity of response is concealed, however, as two of the 13 children had net gains of over 3 cm, and the remaining 11 had no indication of any net gain.

Such compensatory deceleration may be irrelevant with continuous treatment over many years, but this is difficult to judge without a clinical trial that continues for the entire period of observation. In considering the results of a one-year trial, one must take the posttreatment year into account.

There is difficulty also in evaluating short-term v long-term effects of GH treatment. Nitrogen balance studies, which were used when treatment with GH was a new therapy, demonstrated that there was little correlation between short-term nitrogen retention and long-term growth response in GHD patients. This was not surprising since short-

term response can only predict long-term response if all patients have similar time courses of response to treatment.

Worse still, there is considerable doubt whether even the response in height velocity during the entire first year is predictive of the final outcome. In treated GHD patients, the strongest correlation of final adult height is with parental height, just as in children of normal stature. So, in examining the relation between first-year velocity and final height, parental height must be taken into account, either by using partial correlations or by considering final height as standard deviations (SD's) of the parental height target.

Burns et al found a correlation of only 0.22 between first-year velocity and final height in 39 idiopathic GHD patients treated until growth ceased. Joss et al found a correlation of 0.65 between final height and the increase in velocity in 18 GHD patients. Since Joss et al found no relation between pretreatment velocity and final height, the coefficient of 0.65 is derived largely from the first-year velocity; this is in contrast to the findings of Burns et al. All that can safely be said at present is that the first-year response is certainly *not* a good guide

to the final result, and may be no guide at all.

Short-term testing of bone growth response to treatment over periods of one to three months was recently proposed, but it is probably not relevant when assessing whether objectives are achieved. Even the observation of growth velocity or acceleration in the first year of treatment may be of limited value in relation to our prime objective.

There may be a third difficulty: the effect of the psychophysiologic changes accompanying the increased attention paid to subjects—the famous “Hawthorn effect.” Although there are no solid data at present, every clinician dealing with growth disorders knows how sensitive the patient's growth rate is to subtle differences in psychosocial factors. Occasionally, we have seen an increase in growth rate during the year *before* treatment begins, a year filled with tests, measurements, and anticipation.

Thus, any trial assessing a growth-promoting agent should include a placebo. In a current trial of GH in short-statured non-GHD children, we have had no difficulty in securing parents' agreement to a double-blind design in which an inert substance would be injected for

**Table** Increment in Prediction During Treatment (cm)

|                          | 1st Year | 2nd Year | 3rd Year | First 2 years | First 3 years |
|--------------------------|----------|----------|----------|---------------|---------------|
| Growth hormone deficient |          |          |          |               |               |
| Males (51)               |          |          |          |               |               |
| Mean                     | 5.2      | 2.7      | 2.1      | 7.9           | 10.0          |
| Range                    | 1 to 12  | -1 to 9  | -2 to 9  | 2 to 21       | 3 to 24       |
| Females (15)             |          |          |          |               |               |
| Mean                     | 4.1      | 2.0      | 0.0      | 6.1           | 6.1           |
| Range                    | 1 to 9   | -1 to 7  | -5 to 3  | 0 to 15       | 1 to 18       |
| Small/delay              |          |          |          |               |               |
| Males (19)               |          |          |          |               |               |
| Mean                     | -0.3     | 0.2      |          | 0.0           |               |
| Range                    | -2 to 2  | -3 to 4  |          | -5 to 6       |               |
| Silver-Russell (11)      |          |          |          |               |               |
| Mean                     | 2.2      |          |          |               |               |
| Range                    | 1 to 4   |          |          |               |               |



six of the 12 months. Parents were assured that treatment would continue if the comparison showed a significant effect of GH in their own children.

### Height Prediction

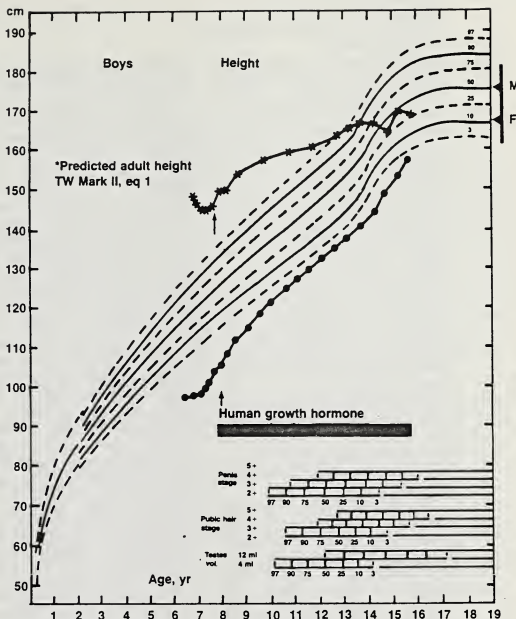
Having discussed the difficulties, we can turn to a possible method of assessing the effect of growth-promoting agents. A change in the prediction of adult height while the patient is receiving treatment could be a very positive indicator, although such predictions need to be evaluated with caution.

Predicted adult heights for children with idiopathic GHD whose treatment begins between ages 5 and 11 are, according to the Tanner Whitehouse (TW) system of equations, an average of 150 cm in males (4 SD below the mean) and 145 cm in females. This probably represents, at least approximately, the height these children would have attained without treatment. Some might say that this is an overestimation since Rimoin et al have reported that males and females with hereditary-isolated GHD averaged 132 and 128 cm, respectively.

However, the majority of GHD patients are less severely affected than those with hereditary GHD. When GH is given, the prediction rises, reflecting the greater advance in height velocity than increase in bone age. Probably this increase in prediction is the best indication, at present, of whether we are achieving an increase in final adult height when we give a growth-stimulating agent.

It seems that this increase does not take place in GHD patients if Bayley-Pinneau predictions are used. These are too inflexible to allow for the significant retardation in bone age, and the predictions made at the beginning of treatment are excessive. The same applies to boys with constitutional delay of growth. It is unknown whether the prediction equations of Roche and co-workers (based on bone-specific Gruelich-Pyle ratings of the hand and wrist or knee) would show the increase with treatment; like the TW system, these equations are based on regression equations, and there seems no reason why they should not behave in the same way.

My colleagues and I recently



Increments of Predicted Adult Height

studied the change in prediction in three groups of subjects. The predictions were made using the TW RUS Mark II system, equation 1. We used the records of all current patients with idiopathic GHD, who had been treated for three years or more starting before age 11. There were 43 males and 15 females. Eight males whose treatment had been completed were added. Puberty had not developed during the first three years of treatment. The average ages at the beginning of treatment were 7.4 and 7.6 years in boys and girls, respectively, with ranges of 4.6 to 10.9 and 4.7 to 10.4. The respective mean SD scores (SDS) of height for chronological age at the beginning of treatment were -3.9 and -4.0. Those patients who had thyroid-stimulating hormone and cortisol deficiency were given thyroxine and cortisol as necessary.

Treatment with GH was either 10 U/bw, 5 U/bw, or 4 U/bw. The Table

shows the increments of predicted height obtained in the first, second, and third years of treatment. The Figure shows a typical example. In the first year, the prediction rose on an average of 5.2 cm in boys and 4.1 cm in girls. The increment in the second year was approximately half as much as in the first, and in the third year it diminished further. Over the first two years combined, the prediction rose by  $7.9 \pm 0.5$  cm, and over the first three years it rose  $10.0 \pm 0.7$  cm. The ranges, however, are considerable: from 1 to 12 cm in the first year, 2 to 21 cm in the second year, and collectively, 3 to 24 cm in the first two years.

As one might expect, there was some tendency for patients with the lowest initial SDS for height to improve their predictions most, but the tendency was not strong ( $r = -0.3$ ). In eight of these boys whose final

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heights are available, the difference between their initially predicted adult heights and their actual attained heights averaged 16 cm, with a range of 5 to 26 cm. An additional five mature boys, whose treatment started between ages 11 and 13, gained the same amount over the initial prediction. Those starting treatment later than age 13 gained less. There was no difference between the groups that had isolated GHD and GH plus other growth-related hormone deficiencies. Ten of the boys with multiple deficiencies, who were treated with testosterone at the pubertal age and who had reached their final height, gained an average of 18 cm over the initial prediction.

A small number of patients with GHD due to craniopharyngiomas and other anatomic lesions were studied. They appeared to increase their predictions less than patients with isolated GHD.

The second group of patients consisted of 19 males who were followed for growth delay and seen before age 11. Their average age at first examination was 7.3 years (range, 4.1 to 10.5) and their average SDS of height for chronological age was  $-2.6$ . The results for these patients are included in the Table. No treatment was given. The mean change in prediction was almost exactly zero. The range in the first year was small,  $-2$  to  $+2$  cm.

The third group consisted of 11 patients of both sexes with Silver-Russell syndrome. GH treatment with 10 U/bi/w was begun before age 11 (average age was 6.4 years, with a range of 3.9 to 9.3). Their average SDS of height for chronological age was  $-3.9$ . In age and SDS they were thus comparable to the GHD patients. The results for the Silver-Russell patients fell between those of the other two groups. Data from only one year of treatment were available. In this year, there was an average increment in prediction of  $2.2 \pm 0.4$  cm. The range was 0 to 4 cm.

The increments of prediction in the GHD patients we evaluated fall between those reported by Ranke et al and by Joss et al. Ranke used TW RUS Mark I, CABA-(chronological

age-bone age) based equations. He obtained a mean prediction increment of about 6.5 cm during an average of four years of treatment. Vicens-Calvet et al, using the same equations, obtained a 7.2-cm increment over three years in four children receiving 12 U/wk of GH and 13.0 cm increment in five children on 24 U/wk over three years.

Joss obtained an increment of 10 cm in the first year alone and a further increase of 10 cm in the subsequent three years. His patients were older, with an average age of about 12.0. In addition, he used the TW RUS Mark I system of equations based on bone age alone, a system originally intended for use only on certain occasions when the CABA system was not applicable. His beginning-of-treatment figures, however, indicate that he would have obtained lower increments if the CABA system had been used.

With continuous treatment, the predicted height gradually approaches the height actually to be attained in GHD patients. But looking at individual long-term records reveals that in middle or late puberty, the predictions usually seem to be too high by an average of about 4 cm (range 2 to 7 cm). This may be due to a defect in the predictions when applied to this time period. Use of Mark II, equation 3, which allows for height velocity in the previous year, reduces this excessive prediction to an average value of 2 cm. However, the excessive prediction may also mean that the treatment during puberty is less than optimal. It now seems clear that the total 24-hour secretion rate of GH may increase during puberty, so perhaps we should be giving a greater amount of human growth hormone (hGH) at that time. If we did so, we might obtain an extra 2 to 4 cm in response.

In the early days of hGH treatment, we employed a protocol of one pretreatment year, followed by one treatment year, followed by one posttreatment year. This was followed by continuous treatment, if justified. We have observed 12 patients during this posttreatment year. As expected, the predictions dropped, on average, during this

year. The mean drop was 2 cm (with ten of the 12 decreasing between 0 and 3 cm). In all other respects, these 12 patients resembled those discussed above.

In summary, difficulties in assessing the efficacy of hGH or other growth-promoting substances related to (a) compensatory deceleration, (b) short-term v long-term effects, and (c) placebo effects. Change in the adult height prediction during treatment offers a possibility for testing the outcome of treatment with growth-promoting agents. In 66 idiopathic GHD patients whose treatment started before the age of 11.0, the prediction rose by an average of 5 cm in the first treatment year, 8 cm in the first two years, 10 cm in the first three years, and 16 cm (in eight subjects only) in all the years until growth ceased. In 19 patients with growth delay who were not treated, no average change of prediction occurred over a control year, and in 11 Silver-Russell syndrome patients there was an average increase over the first year of treatment of  $2.2 \pm 0.4$  cm.

The difficulty in evaluating the effect of a growth-promoting agent is evident from the discussion of the data presented. Continuous GH therapy in patients with GHD undoubtedly permits both of the treatment goals to be achieved in most instances. However, to ascertain whether either or both of the goals necessary to evaluate the value of a growth-promoting agent are met, it is quite evident that many studies need to be done in patients with short stature that is not associated with GHD.

References will be sent on request to Dr. Robert Blizzard.

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*Dr. Tanner is a guest contributor for this issue. He is a world-renowned authority on growth, development, and anthropometrics.*

# Malnutrition: Definition, Incidence, and Effect on Growth

With reports from famine-stricken regions appearing almost daily on radio and television, any discussion of energy-protein malnutrition (EPM), its definition, its incidence, and how it affects growth, is particularly timely. An estimated 200 million to 1 billion people in the world suffer from EPM. However, EPM is an ambiguous concept, and it is therefore difficult to determine what, if anything, these prevalence estimates mean.

The basic problem in understanding EPM is that it is defined by three different criteria: (1) *dietary deficiency*, where the intake and/or utilization of energy and protein nutrients are lower than the recommended daily allowances; (2) *substandard anthropometry*, where a person is below the international or local measurement standards of height and weight for age, weight for height, or skinfold thickness; and (3) *functional impairments*, where dietary deficiency results in physical, mental, or emotional changes. High rates of morbidity are linked to functional impairment through decreased immunocompetence, impaired efficiency of physical work performance, impaired mental performance, and emotional stress caused by sensations of hunger.

By utilizing a Venn diagram (Figure), one can identify seven sets of EPM. Dietary deficiency, substandard anthropometry, or functional impairment can occur alone (set 1, 3, or 7) or in various combinations (set 2, 4, 5, or 6). A closer look at these sets reveals the imprecise nature of the definitions given for the three major categories. Many people in set 7 may have functional impairments from disease, emotional stress, social or environmental deprivation, exposure, or other dysfunctional factors of poverty rather than from dietary deficiency. Also of great interest are individuals in set 3: They may have substandard anthropometry of genetic origin and not be functionally impaired. Heredity may be the etiology for the majority of people with substandard anthropometry, who are nevertheless considered to suffer from EPM by

the present definitions. If these energy-protein standards and statistical procedures used to estimate dietary deficiency in developing countries were applied to the United States, 67% of males and 80% of females would be considered EPM victims. Do we seriously believe that such a large proportion of the US population is undernourished?

Reexamining the diagram, one sees that only those individuals who fall in set 5 have malnutrition characterized by diminished intake, substandard anthropometry, and functional impairment. This set represents a smaller number of EPM victims than would be identified when a broader definition of EPM—for instance, only one of the three standards—is applied.

To reduce the confusion surrounding the definitions of malnutrition, one must consider two alternative theories of the growth process and be acquainted with the "small-but-healthy" hypothesis.

The average person usually thinks of malnutrition in terms of thin, wasted people who have substandard weights for height—in other words, acute EPM. Chronic EPM occurs often when the individual is below standard height-for-age levels. While it may be thought that most people with chronic EPM also have acute EPM, this is not necessarily true. In a study that assessed the nutritional status of children

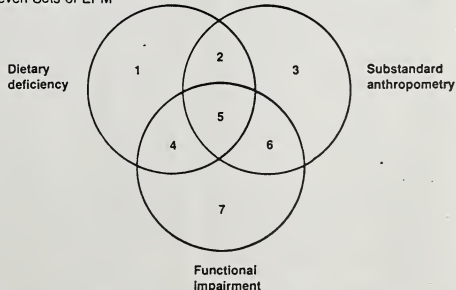
aged 6 to 18 months in 14 developing countries, it was found, on the average, that 90% of the EPM encountered was chronic rather than acute. These children had low height for age, but normal weight for their short stature. Further, only 17% of those with low weight for age had low weight for height according to the classification system used (Gomez). The children who are short but have normal weight typify the small-but-healthy hypothesis. Even though they are not underweight for height, and are thus likely to be considered healthy, they may be nutritionally dwarfed.

Most published reports assume these children are not healthy, but without independent evidence of functional impairment, the meaning of this kind of malnutrition is ambiguous. If, on the other hand, these children are healthy, one must wonder why they are short even though they have appropriate weight for height and other body proportions, body fat, and satisfactory general health. According to the small-but-healthy hypothesis, these children are healthy, but their small size is often the result of decreased caloric and/or protein intake.

Of the two theories of human growth, the deprivation theory is the predominant one. It is assumed that every individual is born with a single, genetically determined growth po-

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Seven Sets of EPM



## Malnutrition: Definition, Incidence, and Effect on Growth

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tential. It is further assumed that a healthy and well-nourished child will grow to his or her genetic potential. In contrast, by definition, growth that is significantly below genetic potential indicates functional impairment. Of course, some individuals are normally small, but this may be difficult to determine. Nevertheless, in large populations, a skew of the distribution curve of height toward lower stature is perceived as functional impairment within that population.

In contrast to this view, the *homeostatic theory* of growth holds that the genetic endowment of the organism interacts with the environment in a system of cybernetic control to maintain homeostasis. According to this theory, the genetic growth potential of the deprivation theory is replaced by a broad array of potential growth curves in several anthropometric dimensions—a potential growth space, in other words. Within the bounds of this potential growth space, the growing organism may be mapped through various paths of size and shape in response to nutrition, disease, climate, activity, emotional stress, and other environmental influences.

The homeostatic theory postulates that a major control instrument is the regulation of growth rate with respect to the height of the child. If, for example, nutrient constraints are encountered at a given rate of growth, the rate is slowed to bring nutrient demand into equilibrium with supply. Similarly, the growth rate may be accelerated in response to overconsumption. According to this theory, neither nutrient constraint nor overconsumption is necessarily abnormal; they are merely adaptations. Through regulation of the speed of internal physiologic "clocks," short-term equilibrium can be established and the ultimate size and shape of the adult molded to the environment. Of course, there are bounds to these adaptive possibilities. If deprivation is severe, acute EPM may be superimposed on the expected short stature that may result from a modest decrement in caloric intake or utilization.

Thus, while the deprivation theory

postulates a continuous relationship between small size and the functional impairments of EPM, with the incidence and severity of deficiencies increasing as the size of the organism decreases, the homeostatic theory essentially postulates a discontinuous, threshold relationship. According to the latter theory, smallness is not necessarily correlated with functional impairment, although there is a high incidence and severity of functional impairment as the lower boundaries of size are transgressed. Thus, children with mild to moderate chronic EPM are likely to remain small but healthy. With regard to acute EPM and moderate to severe chronic EPM, the two theories are concordant.

If the homeostatic theory is valid, it explains why many small persons are not functionally impaired. In a study of Bangladeshi children, Chen et al found a high incidence of mortality in those with severe EPM, but no difference in mortality between children with mild to moderate EPM and normal children. Once some difficult statistical problems due to aggregation of individual variation in thresholds are better sorted out, similar threshold effects are likely to become apparent.

In summary, the essential difference between the deprivation and homeostatic theories is the difference between maximum and optimum. The deprivation theory states that the optimal size must be in accord with the maximum genetic potential. Hence, it follows that smallness is bad per se. The homeostatic theory, on the other hand, defines optimal size in terms of a functionally stable growth space that may be considerably below maximum height-for-age ratios. The lower boundary of the growth space, where significant functional impairments begin to occur, can be determined only by empirical research. Thus, while the deprivation theory deduces functional impairment from the premise that maximum growth is necessary to health, the homeostatic theory requires evidence of functional impairment to define EPM. To date, the incidence of malnutrition has been defined

primarily in relation to the deprivation theory.

The differences between the deprivation and homeostatic theories open important fields of research in pediatrics, genetics, endocrinology, and even economics. If it is found that the growth, and perhaps the shape, of the human body are controlled by factors other than nutrition and disease—genetic and environmental interactions, for example—then pinpointing the control mechanism and what it responds to becomes of great scientific interest.

In terms of nutritional policy, it seems clear that nutritional resources now being devoted to accelerating the linear growth of children should be reallocated to those children in clear and present danger of functional EPM: namely, those with serious to severe acute EPM (underweight for height). This approach would reduce the target population of nutritional programs to less than 10% of the children who are conventionally defined as having EPM. With proper management, the resources now largely being spent on accelerating linear growth in small but relatively healthy children could eradicate functional EPM. In fact, attempts to get children on higher growth curves before their poverty has been alleviated may put them out of equilibrium with their environment and do irreparable harm.

References will be supplied upon request to Dr. Blizzard.

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## Studies of Marginal Zinc Deprivation in Rhesus Monkeys: (IV) Growth of Infants in the First Year and (V) Fetal and Infant Skeletal Effects

Growth retardation, delayed skeletal maturation, and defective bone mineralization are reported in rhesus monkeys subjected to marginal zinc deprivation during the prenatal period and throughout the first year of life. The subjects were offspring of mothers given either a zinc-deficient diet (4 mg/kg of zinc) or a control diet (100 mg/kg of zinc) throughout gestation and lactation. At weaning, the offspring were fed either a zinc-deficient or control diet corresponding to the maternal diet. A complete morphometric examination, a quinine acceptance test for taste sensitivity, blood samples for trace metals, and bone x-rays were performed at various intervals during the first year of life.

At birth, zinc-deficient males had significantly lower body weights, crown-rump lengths, and femur lengths than control males. These data suggest intrauterine growth retardation. Reduced rates of weight gain and crown-rump length growth were reported in the zinc-deficient group compared with controls at 9 to 12 months of age. The diminished

rate of weight gain was positively correlated with reduced food intake, lower food-use efficiency, and decreased taste acuity at 1 year of age. Overall, zinc-deficient infants did not grow as well as the controls during the entire first year of life.

At birth, zinc-deficient infants demonstrated delayed skeletal maturation without defective mineralization. However, by 1 month of age, abnormal mineralization was reported in the zinc-deficient group. Specifically, there were changes suggesting rachitic syndromes with "frayed" metaphyses and "splayed" cortices.

As a result of these observations, the authors suggest that marginal zinc deficiency during gestation results in neonatal growth retardation that persists throughout the first year of life. Bone maturation delay and defective mineralization of the skeletal system also result from zinc deprivation.

Golub MS, Gershwin ME, Hurley LS, et al: *Am J Clin Nutr* 1984;40:1192. and Leek JC, Vogler JB, Gershwin

ME, et al: *Am J Clin Nutr* 1984;40:1203.

**Editor's comment**—These observations are important since they demonstrate that marginal zinc deficiency can lead to growth abnormalities in utero and to defective skeletal growth. These abnormalities resulted without inducing hypozincemia, but the mean values of the plasma zinc levels were lower in the zinc-deficient monkeys than in the control animals. Unfortunately, the maternal plasma zinc levels are not reported.

These data also demonstrate the need for zinc in skeletal mineralization and the regulation of bone formation. Radiographic findings of rickets were associated with zinc-deficient diets. Unfortunately, vitamin D levels were not obtained. The above observations may be important clinically, since decreased intake of dietary zinc is often seen during pregnancy. In childhood, marginal zinc deficiency is often seen in conditions associated with poor growth.

## Fear of Obesity: A Cause of Short Stature and Delayed Puberty

Fourteen of 201 children evaluated for short stature and/or delayed puberty over a 25-month period were found to fit a pattern of growth failure due to self-imposed restriction of caloric intake arising from a fear of becoming obese. Nine males and five females, ages 9 to 17 years, underwent a complete evaluation. They were all below the fifth percentile for weight and height. All showed deterioration of linear growth, which was preceded by one to two years of inadequate weight gain. The weight deficit for height was 5% to 23% of ideal body weight.

Seven of the older patients had delayed puberty. Physical examination and routine diagnostic laboratory examinations revealed no evidence of organic disease.

Review of the patients' 24-hour dietary intake by recall indicated that they ingested only 32% to 90%

of the recommended caloric intake for age and sex. Nine skipped meals regularly. They tended to reduce the amount of animal proteins in the diet, but consumed increased amounts of cereals, fruits, and vegetables. The seven-day record in nine patients supported the data obtained by recall.

An open-ended interview of all patients revealed no evidence of psychiatric disease or anorexia nervosa. As a group, these patients were good students with compulsively shy personalities. Seven underwent the Diagnostic Interview for Children and Adolescents, which also revealed no psychiatric disease. Three patients did show evidence of an oppositional disorder (usually, argumentative or confrontational behavior).

After receiving nutritional counseling and nonstructured psychiat-

ric counseling, the patients resumed an adequate caloric intake for age. Weight gain and a resumption of linear growth accompanied increased food intake, except in one female who underwent menarche and remained stunted.

Pugliese M, Lifshitz F, Grad G, et al: *N Eng J Med* 1983;309:513-518.

**Editor's comment**—This paper describes a newly recognized cause of poor growth in adolescence. It remains to be seen whether fear of obesity, which may be prevalent in our population because of concern over being fat, is a distinct disease entity with its own natural history. This entity could also be a mild variant of anorexia nervosa. Whether a caloric deficiency or the inadequate intake of a specific nutrient caused the poor growth remains unclear.

## Short Stature and Celiac Disease: A Relationship to Consider Even in Patients With No Gastrointestinal Tract Symptoms

Celiac disease (CD) as a frequent cause (8.3%) of short stature in an asymptomatic group of 60 short children is reported by Cacciari et al of Bologna, Italy. Duodenal biopsies were performed on 60 children (39 boys and 21 girls, 3½ to 18 years of age) who were less than the third percentile, who had no apparent cause for their short stature, and who had no gastrointestinal symptoms. All were tested for human growth hormone (hGH) release using arginine and L-dopa, and for xylose absorption, antireticulin antibodies, hemoglobin, and serum iron. The migration inhibitory factor (MIF) was tested in those with duodenal pathology and in a limited number of the others. Anthropometric measurements and skeletal maturation were assessed in all.

Five children (two girls and three boys) had total villous atrophy. These five did not differ in delay of bone age, height SD score, weight SD score, height age/bone age, or height age/weight age from the 52 patients for whom no cause of short stature was found. Surprisingly, the height age/weight age was  $1.03 \pm 0.17$  SD for the five children, which indicates no malnutrition for the group. Data for individuals are not given.

The data regarding antireticulin antibodies, xylose absorption tests, MIF tests, hemoglobin, and basal iron did not differentiate completely those patients with villous atrophy. For example, only three of five patients had abnormal xylose tests and antireticulin antibodies. Only two had positive MIF tests, decreased basal iron levels, or a history of frequent diarrhea during infancy. The article does not clarify whether the same patients had the same laboratory abnormalities. All five did have delayed bone age.

The authors state that the results do not allow statistical interpretation and absolute conclusions, but they do allow certain conclusions: (1) the incidence of celiac disease may be significant in a population of short children who are asymptomatic; (2) at present, the only way to produce a definite diagnosis in all children with celiac disease is to perform

intestinal biopsy; (3) if biopsies are done only in patients with a history of diarrhea in the first two years of life, and/or the presence of antireticulin antibodies, and/or an abnormal xylose test, the number of biopsies that need be done for diagnostic purposes is significantly reduced. Four of the five would have been identified by the presence of at least one of these three factors, and only 22 biopsies would have been done in the 60 patients; and (4) growth hormone (GH) secretion is normal in these patients with celiac disease.

Cacciari E, Salardi S, Lazzari R, et al: *J Pediatr* 1983;103:708.

**Editor's comment**—The data are intriguing. This is not the first report, as the authors readily state, of diagnosing celiac disease in children with short stature and without symptoms of gastrointestinal disease.

Groll et al reported that eight of 34 children with short stature and without gastrointestinal disease had intestinal biopsies characteristic of CD, and seven grew significantly on a gluten-free diet (*Lancet* 1980; 1:1097).

A little disturbing is the absence of repeat biopsies in either study to demonstrate alterations of histology toward normal. This would have been particularly helpful in the current study, as three of these patients had some adolescent changes during the observation period. Thus, one may not be able to exclude attributing the changes in height and weight to adolescent development.

In the United States and Canada, celiac disease is reported to occur less frequently than in Europe. It is therefore possible that we are missing asymptomatic cases. Letters to the editor concerning this topic are invited.

## Bone Marrow Transplantation in the Maroteaux-Lamy Syndrome (Mucopolysaccharidosis VI)

The authors report the use of bone marrow transplantation as treatment for the severe form of Maroteaux-Lamy syndrome in a 13-year-old girl who continues to show improved biochemical and clinical status 24 months after transplantation.

Bone marrow transplantation is now the treatment of choice for many leukemias, aplastic anemias, and immunodeficiency disorders. In experienced hands, when using marrow from HLA-MLC-matched sibs, complication rates and survival times have become quite acceptable. The possibility of using bone marrow transplantation for inborn errors of metabolism has been discussed for many years. Recently, bone marrow transplantation has been used, with encouraging results, for one form of osteopetrosis (an inherited disorder with osteoclast dysfunction) to restore the marrow's osteoclast-monocyte population.

Selective enzyme deficiencies such as Maroteaux-Lamy syndrome would appear to be candidates for

this type of treatment. Maroteaux-Lamy syndrome, for which there is an animal model, is a lysosomal disorder that spares the CNS. Using the feline mucopolysaccharidosis VI model, bone marrow transplantation experiments demonstrated that transplanted reticuloendothelial and hematopoietic cells could return to almost normal the biochemical and clinical abnormalities present in affected animals.

With this background, a 13-year-old girl with the severe form of Maroteaux-Lamy syndrome was identified for bone marrow transplantation. Her disease had become life-threatening with the development of frequent apnea episodes and severe congestive heart failure. Her sister, who was HLA-DR-identical, was the bone marrow donor. The patient was pre-treated with busulfan and a graft-versus-host preventive regimen.

Her response to therapy was monitored by clinical response, liver biopsy changes, white cell enzyme assays, urinary mucopolysaccha-

ride output, and electron microscopic (EM) studies of liver cells, bone marrow cells, peripheral blood leukocytes, and platelets. After engraftment, blood-group studies demonstrated the presence of only donor cells.

Peripheral leukocytes, which had been severely deficient in arylsulfatase B prior to bone marrow transplantation, showed normal activity by two months after transplantation and remained normal for the 24-month observation period. Liver biopsies revealed apparent repopulation with Kupffer's cells after bone marrow donation, with the ratio of arylsulfatase B to arylsulfatase A activity increasing from 3% to 16% of normal activity. Accumulation of mucopolysaccharides in hepatic Ito cells decreased so that no storage material was seen 148 days after transplantation. No storage material was seen on EM studies in hepatocytes. Kupffer's cells, or endothelial cells in posttransplantation biopsy specimens. Urinary excretion of mucopolysaccharides decreased and was within normal limits by 100 days after transplantation.

Pulmonary hypertension, cardiomegaly and thickening of ventricular walls, and congestive heart failure had been present prior to transplantation, but resolved completely 15 months after transplantation. Pulmonary function also returned to normal by this time, and apneic episodes ceased. No change in radiologic abnormalities of the bones could be demonstrated, but there was subjective improvement in the range of motion in most joints. Visual acuity improved, but glaucoma and corneal clouding remained unchanged. There was a marked decrease in hepatic mass, and the spleen returned to normal size post-transplant. Intellectual status remained normal, but the general sense of well-being was markedly improved. Now 24 months post-transplant, the patient has shown remarkable improvement in severely affected areas without any evidence of deterioration in new areas.

**Editor's comment**—This report describes an exciting new mode of therapy for some inherited disorders with inborn errors of metabolism. However, it is important to emphasize that this mode of therapy will be appropriate only in selected diseases that involve either bone marrow elements or reticuloendothelial cells which, when transplanted from an unaffected individual, can redistribute themselves in the liver, lung, and intestines of the recipient. Thus, disorders involving CNS deterioration are probably not appropriate candidates for treatment with bone marrow transplantation. The long-term outcome for an individual treated with this mode of therapy is

still unknown. There is no question that the natural history of diseases treated in this way will be altered. A new set of complications will arise.

As the authors also point out, bone marrow transplantation entails considerable risks of morbidity and mortality, as well as a large commitment of medical, financial, psychosocial, and other resources. A comprehensive evaluation and the presence of an HLA-identical sib are essential. Nevertheless, the morbidity or predictable mortality of the individual patient may be dramatically improved as in this reported case. This mode of therapy gives hope for previously hopeless disorders.

## Growth Retardation in Crohn's Disease: The Merits of Aggressive Nutritional Therapy

Growth retardation, defined as a cessation or slowing of linear growth to a rate below that expected for age and pubertal stage, occurs in 30% to 85% of children with Crohn's disease of prepubertal onset.

The author reviews several possible reasons for growth failure. Malabsorption does not seem to be a significant cause since most growth-retarded children have normal D-xylose absorption and minimal fat malabsorption. Decreased nutrient intake has been reported, with anorexia being an important component of Crohn's disease as well. Many patients experience early satiety or abdominal pain after meals, thus making it necessary for them to eat small, frequent meals. However, not all observers have reported low nutrient intake in all growth-retarded children with Crohn's disease. Of the hormonal factors studied in these children, somatomedin-C has been low. Zinc deficiency also does not account for growth retardation in all patients. The role of enteric protein loss is not understood, but many growth-retarded children are in positive nitrogen balance.

While the exact energy and protein requirements of growth-retarded patients with Crohn's disease are not known, the home use of nutritional support permits intake of adequate energy and protein to restore growth.

The author offers four methods for nutritional intervention: (1) increased oral intake, (2) supplementary formulas, (3) supplementary parenteral nutrition, and (4) total parenteral nutrition. The method chosen should depend on the individual patient and his needs. Lactose intolerance could restrict the range of oral formulations, but the author suggests that this can be overcome by adding commercial lactase preparations to the formulas. In the author's own experience, increased growth velocity (amount not specified) occurred when the caloric intake was raised from a mean of 1,245 kcal/d to the recommended 2,400 kcal/d.

Kirschner BS: *Manual of Clinical Nutrition* (suppl) 1983; 2(4):26.

**Editor's comment**—This paper is a good review of state-of-the-art approaches to nutrition and growth retardation in Crohn's disease. While the ultimate cause of the poor growth remains unresolved, nutrition appears to be an important component. Nutritionally induced remission of the disease after bulk nutritional supplementation, as well as improvement in growth, has been documented. More must be learned about specific nutritional deficiencies, (ie, magnesium and zinc) and ways to deal with the patient's inability to ingest adequate calories for growth.

## MEETING CALENDAR

**June 16-18** 45th Annual Meeting and Scientific Sessions of the American Diabetes Association. Baltimore Convention Center, Baltimore, Maryland. Contact: Carolyn Sciortino, ADA, 2 Park Avenue, New York, NY 10016

**June 19-21** 67th Annual Meeting of The Endocrine Society. Baltimore Convention Center, Baltimore, Maryland. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814

**June 22-25** Second Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology. The Hyatt Regency, Baltimore, Maryland. Contact: Dr. S. Raiti, Secretary, LWPES, 210 West Fayette Street, Baltimore, MD 20201

**August 1-3** Clinical Genetics for Practitioners. Postgraduate Course. East Beach Conference Center, Kiawah Island, South Carolina. Contact: Division of Continuing Education, Medical College of Georgia, Augusta, GA 30912

Genetics of Growth Hormone Deficiency by David L. Rimoin, M.D., Ph.D.

Robert M. Blizzard, M.D.  
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Support Groups for Growth-Deficient Children by Judith G. Hall, M.D.

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# GROWTH

## Genetics & Hormones

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Vol. 1 No. 3

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### Genetic Forms of GH-Deficient and GH-Deficient-Like Dwarfism

Causes of short stature related to human growth hormone (GH) can result from a variety of genetic and acquired interruptions in the hypothalamic-pituitary-peripheral tissue axis. The various genetic types of pituitary dwarfism can be classified on the basis of: 1) the level of the defect; 2) the mode of inheritance; 3) whether or not there is an obvious developmental or degenerative disease of the hypothalamus or pituitary; 4) whether the pituitary deficiency is monogenic (isolated GH deficiency) or multigenic; 5) whether there is a mutation or deletion in the GH gene; and 6) in those cases due to a defect in GH action, whether somatomedin levels are normal or decreased.

This article will review the six currently recognized types of inherited GH deficiency (see Table), the possibility of a genetically inherited GH-deficient-like syndrome attributable to biologically inactive GH, some syndromes of inherited GH-deficient-like dwarfism (such as Laron dwarfism and pygmy dwarfism, which are characterized by the inability to generate insulin-like growth factors [IGF] in response to GH), and IGF-resistant dwarfism.

Two of the six types of GH deficiency are associated with multi-tropic pituitary hormone deficiencies (MPHD), and the other four are associated with isolated GH deficiency (IGHD). No familial crossovers between MPHD and IGHD have yet been reported.

Inheritance is autosomal recessive (AR) in one type of MPHD and X-linked recessive in the other. There is both interfamilial and intra-familial variation of the associated hormonal deficiencies. In certain

families with the AR type, one individual may lack all of the tropic hormones, whereas another may lack only GH and gonadotropins. Similarly, there may be variability in the hormonal responses to the hypothalamic peptides (GRH) and thyrotropin-releasing hormone (TRH) in sibships. Rogol et al recently re-

ported studies in two sibships with three patients each. In each sibship, one patient responded to GRH with significant GH release and one responded to TRH with thyroid-stimulating hormone (TSH) release. This variability of response makes it difficult to determine whether the MPHD

*continued on p. 2*

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### The Genes Controlling Growth Hormone Production, Secretion, and Action

Six peptide hormones normally participate in the regulation of growth hormone (GH) production, secretion, and action. These are GH-releasing hormone (GRH) and somatostatin or GH-releasing inhibiting hormone (GRIH) from the hypothalamus, two GH molecules (22K and 20K) from the pituitary,

and two insulin-like growth factors (IGF-I and IGF-II) in the blood and some peripheral cells. During pregnancy, a seventh peptide hormone, human chorionic somatomammotropin (hCS), is produced and contributes to maternal GH activity. Advances in molecular genetics have made it possible to study the genes for each of these seven hormones. The purpose of this presentation is to discuss the chromosomal locations of the genes for each of these peptides, known abnormalities of the genes, and the consequences of those abnormalities.

Recombinant cDNA probes have been developed for GRH and GRIH. The former gene is located on chromosome 20 and the latter on chromosome 3. Preliminary studies have not disclosed examples of GRH gene deletion as a cause of isolated GH deficiency or multi-tropic pituitary hormone deficiency. Currently, GRIH is a gene without a recognized heritable disease phenotype.

All the genes for GH and hCS are  
*continued on p. 4*

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## Genetic Forms of GH-Deficient and GH-Deficient-Like Dwarfism

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is hypothalamic or pituitary in origin. Computerized tomographic skull scans were normal in two of the six patients. In the remaining four, the sella was either small or empty. Further studies using prolonged GRH stimulation might be helpful in elucidating the site of the lesion. The GH and GRH genes were present in the genomes of all six patients.

Zipf et al reported a third sibship in which MPHD was associated with X-linked recessive hypopituitarism. The deficiency of tropic hormones was variable, although GH and gonadotropin deficiency were constant. The authors suggested that the heterozygous state in the female does not result in any detectable abnormality. In this sibship, the mother had no easily identifiable X-chromosome genetic marker that would permit identification of the carrier state, such as XGA heterozygosity or color blindness. No restriction fragment length polymorphism (RFLP) studies have been reported in genetic MPHD, Type II. Since no clinical or endocrine differences exist between either of the two genetic forms of MPHD and the more common acquired form, genetic counseling is difficult.

Like MPHD, IGHD occurs much more frequently as a sporadic and acquired entity than as an inherited disease. Of the four currently recognized types of genetically inherited IGHD, only one (IGHD I-A) has been identified at this time with a demonstrable defect of the genomic material. Phillips et al studied nuclear DNA from eight individuals with IGHD I-A who were homozygous for DNA deletions varying in size from 6.7 to 7.6 kb, each of which included the hGH-N gene (see article by Parks on page 1).

IGHD I-A was described by Illig et al as AR and considered to be distinctive from other types of IGHD because of the total absence of circulating immunoreactive GH and the appearance of high concentrations of GH antibodies following GH therapy in these patients, rendering them GH-resistant. A number of families of different ethnic backgrounds have been described with this syndrome. Not all have developed antibodies and/or resistance to GH. An Argentinian child main-

tained a satisfactory response despite antibodies to GH. Several Israeli children with IGHD I-A did not form antibodies to hGH and their responses to treatment were as good as those seen in patients with other forms of GH deficiency. The originally described phenotype is not specific for IGHD I-A. A technique called Southern blotting for GH genes is required to establish the diagnosis.

IGHD I-B, also referred to as Type I, is inherited as an AR trait. Affected patients are hypersensitive to insulin and have glucose intolerance associated with insulinopenia. Puberty occurs spontaneously but is frequently delayed until the late teens or early twenties, and often appears abruptly during the first months of GH therapy. Rogol et al documented significant GH release following GRH administration, and Rimoin et al demonstrated normal GH-staining granules in the pituitary gland of an affected individual, suggesting that the basic defect is in the hypothalamus. Linkage studies utilizing RFLPs of the GH gene have not demonstrated a linkage in IGHD I-B. No abnormality of genomic material, including the GRH gene, was demonstrated by Rogol et al.

Patients with IGHD I-B usually grow spontaneously at rates that are slow but not as slow as the rates of patients with IGHD I-A or II. They also respond well to GH therapy. They usually do not exhibit the dramatic growth failure and/or phenotype of IGHD I-A and II and are hard to differentiate from those with ac-

quired GH deficiency. Consequently, the genetic incidence is difficult to determine.

IGHD II was first studied by Rimoin and Merrime in a family with three affected generations. Rogol et al restudied this family 20 years later, at which time a GH-deficient child had been born into the fourth generation. No abnormalities of the GH or GRH gene were observed. GRH administration produced no release of GH except in the 3-year-old in the fourth generation whose GH rose to 3.6 ng/ml. When initially studied, this family had increased rather than decreased insulin response to glucose and arginine, as is usual for most patients with GH deficiency. However, there have been reports of families with dominant inheritance who have the insulinopenia and metabolic features of IGHD I. Therefore, Type II, as currently defined, is a poorly delineated autosomal dominant type of GH deficiency, and further studies in multiple families are needed to clarify the heterogeneity of the entity or entities.

IGHD III is an X-linked dominantly inherited type of GH deficiency. Fleischer et al initially described a kindred of two brothers and their two maternal uncles. These individuals had isolated GH deficiency and hypogammaglobulinemia. Two of the four had recurrent pulmonary infections that abated with gammaglobulin therapy. Three had panhypogammaglobulinemia and absent circulating B cells, and the fourth had normal IgA and IgM lev-

Table Genetic Growth Hormone Deficiency

|                 | Inheritance | GH gene defect | GH-RH gene defect | Comment                 |
|-----------------|-------------|----------------|-------------------|-------------------------|
| MPHD*           |             |                |                   |                         |
| I               | AR          | ND             | ND                |                         |
| II              | X-linked    | ?              | ?                 |                         |
| IGHD*           |             |                |                   |                         |
| I-A             | AR          | hGH absent     | Probably normal   | Develop GH antibodies   |
| I-B (or Type 1) | AR          | ND             | ND                |                         |
| II              | AD          | ND             | ND                |                         |
| III             | X-linked    | ?              | ?                 | • Hypogammaglobulinemia |

\*Multitropic pituitary hormone deficiency  
 ?Isolated GH deficiency  
 AR = Autosomal recessive

AD = Autosomal dominant  
 ND = Not demonstrated

els but decreased levels of circulating B cells, T-cell function and number were normal. This kindred appeared to have a distinct X-linked recessive form of IGHD that was associated with hypogammaglobulinemia.

There have been reports of several patients with the clinical features of IGHD who achieve normal plasma immunoreactive GH levels following stimulation, who have low basal levels of IGF-I, and who respond to GH administration with normal IGF-I levels and a significant increase in growth rate. It was postulated that these individuals secreted an abnormal GH molecule that was biologically inactive, but immunologically cross-reactive. However, Phillips could find no trace of a defect in the hGH-N gene after careful DNA analysis in one such patient. He postulated that the hGH receptor in the liver rather than the hGH gene may be involved. In some of these patients, a deletion on chromosome 13, rather than on chromosome 17 where the hGH gene is located, was found.

Valenta et al recently reported a patient with normal levels of immunoreactive GH but decreased radioreceptor-assayable activity. This patient differed from those de-

*continued on p. 4*

### Address for Correspondence

Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

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## Meet the Editorial Board Associate Editor:



Alan D. Rogol, M.D., Ph.D.

Dr. Rogol is Professor of Pediatrics, Chief of the Division of Endocrinology and Metabolism, and Associate Professor of Pharmacology at the University of Virginia School of Medicine, Charlottesville. Before coming to Virginia in 1975, he served as a Lieutenant Commander in the Public Health Service.

A native of Seymour, Connecticut, Dr. Rogol received a Bachelor's degree in chemistry from the Massa-

chusetts Institute of Technology in 1963. In 1970, he earned a doctorate in physiology from Duke University and his medical degree from Duke University Medical School, Durham, North Carolina. He served both his internship and two years of residency in pediatrics at the Johns Hopkins Hospital, Baltimore, Maryland. He was also (for two years) a fellow in the Clinical Endocrinology Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland.

Dr. Rogol is the author or coauthor of more than 80 journal articles, reviews, and other publications and communications. He is a member or fellow of several professional societies, including the American Academy of Pediatrics, the American Federation for Clinical Research, the Endocrine Society, the Society for Pediatric Research, and the Lawson Wilkins Pediatric Endocrine Society.

## Associate Editor:



Judith G. Hall, M.D.

Dr. Hall has been Professor of Medicine and Pediatrics at the University of British Columbia and Director of the University's Clinical Genetics Unit at Grace Hospital, Vancouver, since 1981. For the previous nine years, she held similar posts at the University of Washington School of Medicine and the Children's Orthopedic Hospital in Seattle.

Born in Boston, Dr. Hall spent her teen years in Seattle. She returned to Massachusetts to attend Wellesley College, graduating in 1961. In 1965, she received a Master's de-

gree in genetics from the University of Washington in Seattle and, in 1966, her medical degree from the University's School of Medicine. She returned East again, this time to Baltimore, for six years of postgraduate training. She served a mixed medicine and pediatrics internship at Baltimore City Hospital and a two-year fellowship in medical genetics at the Johns Hopkins Hospital. After a two-year residency in pediatrics at the Hopkins-affiliated Harriet Lane Home, Dr. Hall was awarded a one-year fellowship in pediatric endocrinology at Hopkins Hospital.

Author, coauthor, or editor of more than 350 articles, abstracts, case reports, books, book chapters and sections, book reviews, and other communications, Dr. Hall is a member of the Editorial Board of the *Journal of Clinical Dysmorphology* and a reviewer for numerous medical journals and research grant agencies. She is currently Vice President of the Society for Pediatric Research and a member of the Board of Directors of the American Society for Human Genetics.

continued from p. 3

scribed above in that he had a normal plasma IGF-I level. However, when he was treated with exogenous GH, his growth response was excellent. Physical analysis of the GH in the patient's serum revealed a structural abnormality of the circulating GH, with most of the immunoreactive hGH migrating on gel filtration as large molecules.

These entities are probably heterogeneous, but the extent to which gene defects may be present is as yet unknown. Further studies are needed to define the pathophysiologic mechanisms.

Two syndromes with inherited defects of IGF-I generation are recognized. The first is Laron dwarfism; the second, pygmy dwarfism. Clinically, Laron dwarfs resemble patients with IGHD except that their GH concentrations are normal or elevated. This autosomal recessive syndrome was first described in Oriental Jews, but has since been found in numerous other ethnic groups. These patients have severely pinched faces, high-pitched voices and, in affected males, small genitalia; growth retardation is severe. Early development is generally slow, fontanelle closure is delayed, and symptoms of hypoglycemia are frequent. Glucose intolerance is present with hypoglycemia and hypopinsulinemia. Plasma GH levels, however, are elevated, although they are normally suppressed with glucose. Plasma IGF-I concentra-

tions are low and do not increase after GH administration. These patients are resistant to the growth-promoting effects of hGH administration.

Plasma GH appears to be qualitatively normal on the basis of serial immunoassay dilutions, electrophoresis, and molecular size distribution. Furthermore, substantial quantities of receptor-active GH have been found by radioreceptor assay. Using an erythroid progenitor technique, Golde et al found that Laron dwarfs had a specific cellular resistance to hGH. Liver cell microsomes from these patients do not bind hGH normally, although insulin does bind normally. Thus, the pathogenic mechanism in Laron dwarfism appears to involve a defect in IGF-I and IGF-II generation that is probably secondary to a universal defect in GH receptors.

The African pygmy resembles the pituitary dwarf in size and skeletal proportions, but does not have the latter's truncal obesity, peculiar facies, and wrinkled skin. GH levels are normal in pygmies after insulin-induced hypoglycemia and arginine infusion, but like IGHD patients, pygmies are insulinopenic and hypersensitive to the effects of exogenous insulin. They are completely unresponsive to the lipolytic, insulinotropic, and nitrogen-retaining properties of GH, and initial studies suggested that bioassayable somatomedin levels were normal. Thus, it was felt that short stature in

pygmies was due to a peripheral unresponsiveness to somatomedin. Re-examination with the new IGF immunoassays, however, has indicated that pygmies have a primary deficiency of IGF-I, but normal levels of IGF-II. Furthermore, IGF levels did not increase following GH administration. A number of Caucasian patients with similar primary deficiencies of IGF-I have also been described, suggesting that individuals who clinically and metabolically resemble pituitary dwarfs but who have normal levels of immunoassayable GH should have their IGF levels and responsiveness to GH carefully evaluated.

Several patients with proportionate dwarfism, elevated IGF-I concentrations, and normal or elevated levels of circulating hGH have recently been described. These patients are said to have IGF-resistant dwarfism since IGF-I levels were elevated regardless of whether they were determined by bioassay, radioreceptor, or radioimmunoassay. Cultured skin fibroblasts from a patient have shown a 50% decrease in IGF-I binding, suggesting defective IGF-I receptors as the cause of the IGF-I resistance. Whether or not there is a difference between patients with normal plasma GH levels and those with elevated levels has yet to be ascertained.

David L. Rimm, M.D., Ph.D.

References will be sent upon request to Dr. Blizzard.

## The Genes Controlling Growth Hormone Production, Secretion, and Action

continued from p. 1

located on the long arm of chromosome 17. The five genes (hGH-N, hCS-L, hCS-A, hGH-V, and hCS-B) lie in the same 5' to 3' transcriptional orientation over a distance of 48 kb (one kb is 1,000 base pairs), as shown in the Figure. There is greater than 90% homology of the base sequences among these genes so that cDNA probes for either hGH or hCS recognize all members of the hGH and hCS gene cluster.

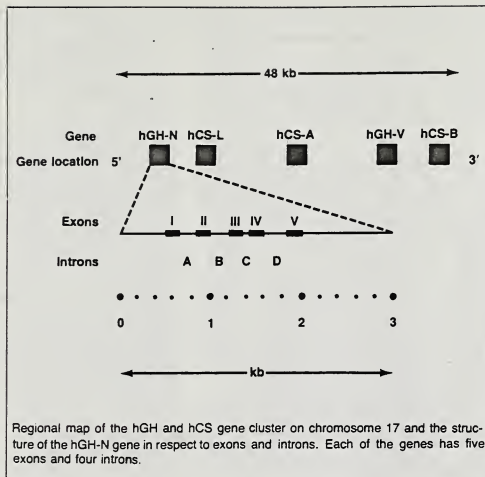
Each gene contains five exons (I to V in the Figure) that are interrupted at identical locations by four introns (A to D). The primary transcriptional products are pre-messenger RNA molecules with se-

quences representing both exons and introns. Intron sequences are excised and exons are spliced together to form mature messenger (m) RNA. Over 90% of the hGH pre-messenger is processed to form the mRNA for the 217 amino acid precursor of 22K hGH, which is the major GH molecule and has 191 amino acids. The remainder undergoes alternative splicing at the 3' end of intron B to yield mRNA for a smaller 202 amino acid precursor of the 176 amino acid 20K hGH, which has less growth-promoting activity than 22K hGH. The hGH-V peptide has been produced in a heterologous expression system. It has a potency in the radioreceptor assay

similar to that of hGH, but it cross-reacts poorly with antibodies directed to hGH in the radioimmunoassay.

Homozigosity for a deletion within the hGH-N gene accounts for the GH deficiency in Type I-A isolated (IGHD). (See GH deficiency article by Rimm on page 1.) The other four GH-related genes on chromosome 17 are intact. Prior to the discovery of this gene defect, Illig referred to the disorder as IGHD I-A, with the "A" referring to the blocking antibodies that affected patients tended to produce when exogenous GH was administered. The "A" now stands for absence of both gene and peptide. The pheno-





type of postnatal growth failure indicates that the hGH-V gene expression, if it occurs, is not sufficient to promote normal growth in childhood.

The carrier state can be identified by restriction endonuclease analysis, a technique described in the addendum, which is available upon request. Since all patients with gene deletion do not have the phenotype initially described by Illig et al, and since all patients with this phenotype do not have a deletion of the hGH-N gene, examination for absence of the hGH gene—utilizing the Southern blotting technique—is indicated in siblings of patients with IGHD. This test is also useful in detection of carriers and in prenatal diagnosis.

Analysis of restriction fragment length polymorphism (RFLP) can also be used to determine whether hGH-N gene mutations analogous to those seen in  $\beta$  thalassemias could account for the decreased GH production seen in Type I-B IGHD and Type II IGHD. If this were the mechanism of the disease, then the affected children would have inherited the same hGH-N genes from their parents. In I-B IGHD pedigrees, the hGH and hCS restriction fragment sizes are normal, and a

majority of affected sibling pairs are discordant for inheritance of hGH and hCS RFLP markers. Absence of linkage between hGH genes and disease shows that mutations causing I-B IGHD, and probably Type II, involve regulatory genes that are distant from the hGH-N gene.

The hCS-A and hCS-B genes share in the production of hCS, known also as human placental lactogen. They are expressed by syncytiotrophoblast cells of the placenta. The hCS-L gene is disabled by a single base change at the beginning of intron B. Substitution of an A for a G prevents normal splicing of premessenger RNA. Deletions have been described at the 3' end of the gene cluster. These produce an abnormal hormonal phenotype, but not a disease. One in 10,000 pregnancies is associated with the complete absence of immunoassayable hCS. A similar number of pregnancies have hCS levels of 1  $\mu$ g/ml at term (normal = 3 to 9  $\mu$ g/ml). Birth weights, lengths, and postnatal growth patterns have been normal. The mothers have had no difficulty in breast-feeding their infants. The explanation for absence of hCS in the maternal circulation is fetal homozygosity for deletions of the hCS-A, hCS-B, and hGH-V genes. Repro-

ductive fitness in the absence of hCS production is not too surprising when viewed in the context of the evolutionary history of hCS. Complex GH clusters containing CS genes are a recent development. This pattern of gene organization is limited to primates. In other evolutionary lines, CS genes either do not exist or are derived from prolactin genes.

The genes responsible for IGF-I and IGF-II are located on chromosomes 12 and 11. They have not been studied extensively in growth disorders. An IGF-I gene abnormality is a possible explanation for the African pygmy phenotype and some other types of short stature associated with low IGF-I and normal or elevated hGH levels. The phenotype of isolated IGF-II deficiency has not been described. Somatic overgrowth in the Beckwith-Wiedemann syndrome may be related to the IGF-II gene. Some children with this syndrome have a partial duplication of chromosome 11 that may include the IGF-II locus.

Two future developments may be anticipated. The first is gene cloning to demonstrate subtle abnormalities in hGH-N gene structure (accounting for abnormal hGH efficacy or potency) as a cause of short stature. The second is the cloning of cDNA or genomic DNA fragments related to the hGH receptor gene. This gene is the most likely site for mutations causing Laron type dwarfism. We can expect that molecular genetic analysis will provide information about the causes of other growth disorders, and that its applicability to diagnosis, choice of treatment, and genetic counseling will increase.

John S. Parks, M.D., Ph.D.  
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References supplied upon request to Dr. Blizzard. An addendum regarding RFLP and GH-related genes will also be sent upon request.

*Dr. Parks is a guest contributor for this issue. In addition to his faculty appointment at Emory University School of Medicine, he is Director of Emory's Clinical Research Center.*

## Pituitary Growth Hormone and Creutzfeldt-Jakob Disease

All clinicians who have provided human growth hormone (GH) therapy to patients in the past need to be aware of the concern that has arisen during the last several months. There is the possibility that some batches of GH extracted from human pituitaries are contaminated with an infective agent that can lead to neurodegenerative disease.

Earlier this year, the National Institutes of Health (NIH) and the FDA were notified that a patient in California who had been treated with GH between the years 1966 and 1976 had died of a rapidly progressing degenerative neurologic disease and was found at autopsy to have typical changes of Creutzfeldt-Jakob disease (CJD) in his brain. CJD is a rare condition; it is usually sporadic and usually presents between 55 and 65 years of age, with a rapidly progressive degenerative course (cerebellar ataxia leading to death over six to 18 months) and with pathognomonic spongiform changes of the brain on autopsy. It is a transmissible disease that is very closely akin to scrapie in sheep and to a specific type of encephalopathy in mink.

CJD has been studied intensively for many years because, theoretically, it would be preventable if an infective agent could be isolated and treatment developed. However, the infective agent is elusive, and it is unclear whether the disorder is caused by a slow virus or by a sub-viral pathogen (a protein called a prion, which is a newly defined class of proteinaceous infectious particles thought to be capable of being infective without the presence of nucleic acid). The infective agent is also resistant to the usual sterilizing procedures, such as those using formaldehyde, alcohol, or glutaraldehyde, and even to fixation, but it is susceptible to bleach (1 mol/L NaOH and one hour in an autoclave at 120 °C). Currently, there is no therapy for CJD, and no remissions have occurred. The incubation period may be several decades.

Dr. Carleton Gajdusek at the NIH, who has been involved for many years in research on CJD, estimates that one to two people per million in the general population have changes characteristic of CJD in

their brains at death. Because of the long latent period, as many as one in 6,000 to 10,000 people might carry the infective agent, although they may not manifest any symptoms. Thus, many of these people would die of other causes before CJD became manifest. GH has been produced by extracting between 5,000 and 16,000 pituitaries obtained at autopsy; there is a chance that one of these individual pituitaries carried the CJ infective agent, thus contaminating the entire lot. In North America, the average child on GH treatment will have received therapy for four years and will have received GH from several different lots. Thus, it is possible that many individuals treated with GH may have been exposed to the CJ infective agent.

Because of the potential implications of a transmitted neurodegenerative disorder in patients treated with extracted GH, several pediatric endocrinologists, officials from the NIH, the FDA, and the National Hormone and Pituitary Program (NHPP), as well as representatives of commercial suppliers of human growth hormone, assembled for an emergency meeting in April 1985. At about that time, it was recognized that two other patients who had been treated with GH had died of rapidly progressing neurologic degenerative diseases during the past year. One had not had an autopsy; the autopsy on the other patient was diagnostic of CJD. The incidence of CJD occurring in individuals under 30 years of age is less than one in 10 million; thus, to have three cases in one year in the United States, all of whom have had therapy with extracted GH, was a matter of great concern.

The technique for extracting GH changed dramatically in 1977. Prior to 1977, the method of extraction was relatively crude. It is important to point out that the three CJD patients all began their therapy before 1977 but were *not* all treated with GH from one lot. They overlap at least two extraction lots. The commercial companies and pituitary agencies in most countries feel that it is unlikely that a virus would be present in their material purified since 1977. However, CJ infective agent is not just any virus, but rather

an unusual compound that is not yet understood. Thus, it is possible that even after 1977, extracted GH might be contaminated with the CJ infective agent.

The next question, of course, is: Will all individuals exposed to the infective agent develop the neurologic degenerative disease? There seem to be individual differences in response, both with regard to clinical presentation and length of incubation. However, as yet there is simply no answer to the question of who may develop the disease.

Because of this information and concern, in April of this year the FDA and the NHPP in the United States withdrew extracted GH for therapy of children who are GH deficient and for any other type of therapy, with the exception of children who have hypoglycemia as a result of their GH deficiency.

There have been more than 30,000 individuals treated with extracted GH since treatment programs began. What will happen to those individuals who were taken off GH therapy? They will stop growing temporarily and may have minor metabolic imbalances. However, it is well known that genetically engineered GH will be available for therapy within the next few months. It is anticipated that most individuals who have been off GH for a few months will have catch-up growth when restarted on therapy and will be minimally harmed by the hiatus in treatment.

Intensive investigation of the infective agent in CJD has been going on for many years. The application of molecular genetic techniques has allowed the isolation of the gene that produces the prion protein known to be associated with infectivity (if not the infective agent itself). This protein has been isolated and cloned. Interestingly, genetic information coding for the protein prion sequence is present in the normal human genome. However, it appears that the infective agent protein prion is more resistant to breakdown than the "normally" occurring protein. Through these investigations, it is hoped that we can develop the ability to diagnose infected individuals prior to symptoms and recognize which individuals are at risk.

These events have spurred further investigation. First, as soon as the potential danger was recognized, GH from each extraction lot was injected into various animals (hamsters, monkeys, etc) known to be susceptible to the CJ virus in the hope of identifying batches that carry the infective agent. Second, research on the infective agent (in terms of developing potential therapeutic antibodies or therapies) is rapidly proceeding. However, the results of these investigations will not be available for months or years.

It has been known from previous work on CJD and related diseases such as kuru and scrapie that animals who have had repeated injections of infective agent do mount antibodies to the scrapie-associated fibrils and associated proteins (PRP 27/30). Obviously patients treated with potentially contaminated GH have had repeated injections. For this reason, researchers at the NIH would like to receive serum samples from patients who have been given GH in the past; they will let you know what they find in your specific patient(s). They will be testing for antibodies to scrapie-associated fibrils. To participate, send 4 to 5 ml of frozen serum collect by Federal Express to Dr. Joe Gibbs, Building 36, Room 4A17, NIH, Bethesda, MD 20205 (phone: 301-496-4821); include age of patient, during what years he or she was treated and for how long, how much GH was given, and the lot number, if known. Also, please notify Dr. Paul Brown, Building 36, Room 5B25, NIH, Bethesda, MD 20205 (phone: 301-496-5291) of any patients under your care who have received GH in the past, and who have neurological symptoms suggestive of CJ disease.

In summary, it is unclear what the future holds for patients who have been treated with extracted GH. However, it is important to be aware of the concern, to continue to be informed, to apprise patients and families of the situation, and to participate in any epidemiologic studies that are undertaken. In general, it is important to be straightforward and honest with patients, but to assure them that a great deal is being done to evaluate the situation and investigate the questions that remain unanswered.

## Micropenis: (I) Adult Follow-Up and Comparison of Size Against New Norms; (II) Gender, Erotosexual Coping Strategy, and Behavioral Health in Nine Pediatric Cases Followed to Adulthood; and (III) Family Mental Health and Neonatal Management: A Report on 14 Patients Reared as Girls

In the *first paper*, Money et al review eight patients (22 to 31 years of age) with micropenis whom they have followed since early childhood. By definition, a micropenis is less than 2.0 cm (stretched length) in an infant. Seven of the patients had penises at least 2 SD below the mean and six were more than 3 SD below the mean. Five were treated during childhood with testosterone. Although penile growth occurred, it did not keep pace with body growth during puberty and adolescence. Thus, as young adults, all five again had a micropenis as compared with the average penile length in 65 normal adult males of  $16.7 \pm 1.9$  cm, which was significantly higher than previously published figures. Mean length, which was determined to supplement this study, was similar in the normal men, regardless of race, height, body habitus, or sexual preference.

The authors conclude that testosterone treatment in childhood does not result in increased penile length in adulthood and that testosterone-induced enlargement of the infantile micropenis is an artifact of the induction of an adolescent growth spurt of the penis.

The *second paper* documents the coping strategies encountered in nine patients (the eight described in the first paper, plus one who had undergone a phalloplasty following testosterone treatment) followed into adulthood. The authors emphasize that there is no single syndrome of micropenis. Rather, a micropenis is a birth defect found in a variety of syndromes and having several etiologies. In a majority of cases, it is an isolated defect, with or without defective testicular function, and may result from hypopituitarism. The chromosomal karyotype is usually 46 XY, but occasionally may be 46 XXY, 46 XX, or mosaic.

During childhood, six of the subjects took precautions to avoid exposure during urination, and all avoided genital nudity. Despite pre-

cautions, five reported being teased viciously. Eight avoided juvenile sexual play. As adults, seven of the nine subjects were dissatisfied with the size and appearance of the penis. (The most extremely dissatisfied patient was the one who had undergone phalloplasty.) Of the remaining two patients, one had the second largest penis in the study. The other had multiple visible disfigurements characteristic of the Robinow syndrome.

As a strategy for erotosexual coping, several patients who needed exogenous androgen therapy for virilization deferred treatment and remained juvenile in appearance. Five were interested in sports and typical male activities as children. Teasing was minimal in this group and cross-dressing did not occur. Three associated more with girls than boys and subsequently had homosexual life-styles. Erotic inertia and deferred erotosexual participation with a partner were the most prevalent coping strategies in seven, and one initiated erotosexual contacts, but anticipated rejection. The remaining patient was the only one who took the initiative in erotic activity, relying on multiple partners and transient encounters.

Since four of the nine patients were associated with homosexuality and/or divergent sexual imagery, the authors hypothesize that having a micropenis may dislocate the normal juvenile experience of age-mate, rehearsal play, and imagery, and thus increase the chances that heterosexual orientation will be dislocated as well.

The *third article* describes 14 patients with micropenis who were assigned to the female sex. Ten were assigned by 12 days of age, and the other four by 29 months of age. Early decision is extremely important to avoid re-announcement of a baby's sex, always a crisis for the parents, regardless of their capacity to deal with it.

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The ignorance and/or reluctance of pediatricians, urologists, and obstetricians to diagnose micropenis and to advise accordingly was evident in the majority of cases. Although sexual deformities or malfunctions are still customarily considered stigmatizing in our society, it is still possible for many parents to cope, particularly with the help of professionals who can educate the parents and assist them in reaching a decision for gender assignment. The authors also emphasize that siblings are not usually included in the education process but should be.

Successful differentiation of feminine gender identity is contingent on the consistency of rearing the child as a girl, social determinants of gender role identity, and genital ap-

pearance. Female-appearing genitals can be created surgically during infancy. Late in adolescence, a coitally functional vagina can be created. Typically, there is no sacrifice of fertility, as sterility is likely to occur with micropenis. During adolescence, female hormones are administered so that the physique and appearance will be feminine.

The authors conclude that the functional morphology of the genitalia is a better criterion for sex reassignment than is the chromosomal or gonadal status. When a micropenis is vestigially small, it can be surgically reconstructed with vaginoplasty into a clitoris, whereas nothing can be done to make it coitally functional as a penis. Thus, a male baby with a micropenis can have a more satisfactory life as a girl and woman.

Money J, et al: (I) *J Sex Marital Ther* 1984;10:105; (II) *Compr Psychiatry* 1985;26:29; and (III) *J Prev Psychiatry* 1981;1:17.

**Editor's comment**—These data have been awaited for a long time. They are in accord with the editor's belief that patients with micropenis can be reared more satisfactorily as females than males. The one possible exception may be the patients who have micropenis in association with growth hormone (GH) deficiency. We feel this group may be different and diverse. We have at the University of Virginia four such patients, all of whom are receiving GH. In two, the penis grew significantly while the remaining two continue to have micropenis.

## Growth Hormone Secretory Dynamics in Turner's Syndrome

Ross, Long, Loriaux, and Cutler have examined growth hormone (GH) output, somatomedin-C determinations, and bone ages in 30 patients with Turner's syndrome (TS), ages 2 to 20 years. The findings have been compared with those of 17 normal subjects, ages 4 to 17 years. The mean GH concentrations during day and night (specimens collected every 20 minutes), the peak amplitudes, and the peak frequencies were similar in girls with TS who were less than 8 years of age and in age-matched controls. The mean 24-hour GH levels in this group were actually higher in patients with TS than in controls ( $4.6 \pm 0.7$  ng/ml v  $2.9 \pm 0.2$  ng/ml), although these values were not statistically significant.

TS patients who were more than 9 years old had lower mean GH concentrations during both day and night, compared with age-matched controls ( $p < 0.005$ ). Patients also had a significant decrease in the peak amplitude of GH release as compared with normals. Interestingly, when the TS patients between the ages of 9 and 20 were compared with each other, there was no significant difference between the day and night mean GH levels, peak amplitudes, or peak frequencies.

Normal females in this age range have greater nocturnal elevation and amplitude, but not peak frequency.

All 21 patients with TS who were stimulated with arginine and insulin had peak GH concentrations  $> 10$  ng/ml. Serum IGF-1 concentrations were stated to be significantly decreased in those with TS between 6 and 12 years of age when compared with normals. However, none of the IGF-1 determinations was in the GH-deficient range.

The authors also present data indicating that bone ages are delayed in TS children of all ages. The delay in 14 girls, 6 to 10 years of age, was  $1.4$  yrs  $\pm 0.3$  SEM. The difference in bone ages between the normal population and the patients with TS increased during the adolescent years. The mean values for patients with TS, 11 to 17 years of age, were decreased by approximately three years, as compared with controls.

A significant increase in GH secretion during normal puberty has been observed in some, but not all, normal subjects. The authors propose that in these sexually infantile girls the role of estrogen would be consistent with the observation that integrated concentrations of GH did not increase at pubertal age. They

also state that since short stature in children with TS is observed at all ages, the cause of short stature is most likely multifactorial. The authors conclude that a relative deficiency of GH in pubertal patients with TS may contribute to their adult short stature.

*J Pediatr* 1985;106:202.

**Editor's comment**—The data presented are not surprising, but documentation that there is a difference in GH secretion between normals and patients with TS during the adolescent years is a significant contribution. Although many earlier studies in normal children do not indicate an increase of mean GH concentrations in normals at the onset of adolescence, recent studies utilizing testosterone in boys with constitutional growth delay strongly suggested that more GH is released in the presence of testosterone, and other studies suggest estrogen increases GH concentrations.

In this study the authors found that the mean 24-hour GH determinations in their normal controls, 8 years of age and younger, was  $2.9 \pm 0.2$  ng/ml, v  $5.7 \pm 0.8$  ng/ml in the



## Late-Onset Adrenal Steroid 3 $\beta$ -Hydroxysteroid Dehydrogenase Deficiency: (I) A Cause of Hirsutism in Pubertal and Postpubertal Women

The physical signs and symptoms, as well as abnormalities in glucocorticoid and mineralocorticoid hormonal levels, have been well documented in the "classical" forms of congenital virilizing adrenal hyperplasia. Within the past decade it has become increasingly clear that genetic defects of an adrenal steroidogenic enzyme such as 21-hydroxylase or 11 $\beta$ -hydroxylase can be manifested during peripubertal life and that the steroidogenic enzyme defect may be one of the causes of androgen excess in peripubertal and postpubertal women. In 21-hydroxylase deficiency, the mild enzyme defect manifested at puberty results from an allelic mutation at the 21-hydroxylase locus.

A mild defect in 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) in adult women with hirsutism has also been found and may not be recognized until later in life, when symptoms of excessive androgen production occur. Thus, it is possible that allelism at the 3 $\beta$ -HSD locus occurs and is responsible for a classical severe form that presents at birth, and for a milder nonclassical form that presents later in life and causes peripubertal-onset hirsutism.

The present study was conducted on 30 normally menstruating women (controls) and 116 postmenarcheal women with either long-standing or slowly progressive excessive hair growth. None had ambiguous genitalia at birth by history. All had an adrenocorticotrophic hormone (ACTH) stimulation test, and blood samples were analyzed for glucocorticoids, mineralocorticoids, sex steroids, and their precursors. Partial 3 $\beta$ -HSD deficiency was suspected in hirsute women in whom the  $\Delta^5$  precursors and the ratios of  $\Delta^5$  steroids to their reduced products all increased after ACTH stimulation to more than 2 SD above the mean for normal women.

Sixteen of the 116 hirsute women were classified as having nonclassical (partial) adrenal 21-hydroxylase deficiency based upon very low  $\Delta^5$ -17-hydroxypregnenolone ( $\Delta^5$ -17P) to 17-hydroxyprogesterone (17-OHP) levels following ACTH administration. An additional 17 hirsute women, including three sisters, met all criteria for partial adrenal 3 $\beta$ -HSD deficiency—the  $\Delta^5$ -17P and dehydroepiandrosterone (DHEA) levels and the ratio of  $\Delta^5$ -17P:17-OHP were all significantly elevated following ACTH when compared with normal women. These women were classified as having partial adrenal 3 $\beta$ -HSD deficiency.

Eighty-three of the 116 hirsute women had no apparent adrenal steroidogenic defect. Many had classical or other types of polycystic ovarian disease.

Women with partial 3 $\beta$ -HSD deficiency had an exaggerated diurnal variation in  $\Delta^5$ -17P, with the major peak at 8 AM higher than in any of the normal women. These high levels of

the  $\Delta^5$  steroids were readily suppressed with glucocorticoid therapy in those with 3 $\beta$ -HSD deficiency.

In retrospect, of the 17 hirsute women with partial 3 $\beta$ -HSD deficiency, seven had final heights at least two to five inches below their parents' height. Five had pubarche between ages 5 and 8.5 years and six between 10.5 and 12 years. None had telarche before pubarche. Reliable data could not be obtained from the other six women. The onset of hirsutism or acne in all 17 occurred between 12 and 20 years of age. Baseline urinary 17-ketosteroid excretion was elevated in the majority, but was suppressed by dexamethasone therapy.

Pang S, Lerner AJ, Stoner E, et al: *JCEM* 1985;60:428-439

**Editor's comment**—These data, along with those from several other laboratories, indicate that partial 3 $\beta$ -HSD deficiency is a common (approximately 12%) cause in this referral population of hirsute women. The most valuable hormonal tests in differentiating patients with 3 $\beta$ -HSD deficiency from normal women and from patients with variant 21-hydroxylase deficiency or hirsutism without an adrenal steroidogenic defect, are the ratio of  $\Delta^5$ - $\Delta^4$  steroids and the high level of precursor  $\Delta^5$  steroids after ACTH stimulation. ACTH stimulation and dexamethasone suppression, plus the characteristic adrenal circadian rhythm of the steroids, indicate an adrenal source of the elevated  $\Delta^5$  steroids due to partial 3 $\beta$ -HSD deficiency. Hirsutism in these women may result from the peripubertal conversion of  $\Delta^5$  steroids to  $\Delta^4$  steroids in situ at the target organ—for example, the hair follicle. The 83 women without defect in steroidogenesis probably represent a spectrum of ovarian disorders (many had cystic ovarian changes) that together represent the largest cause of peripubertal hirsutism.

Since adrenal steroid biosynthetic disorders are readily treated, they should be considered during an evaluation by physicians who see female adolescents with severe acne and hirsutism.

9- to 17-year-old controls. The mean GH levels in TS decreased between childhood and adolescence from  $4.6 \pm 0.7$  ng/ml to approximately 2.5 ng/ml. The difference in the secretion of GH by the girls with TS in the two age ranges is not statistically significant. The observed difference in the mean GH secretory rates, therefore, is primarily related to an increase in GH secretion in normal female adolescents and is not surprising.

The discrepancy of the somatomedin-C determinations during adolescence is also probably related to the absence of sex hormones in the TS patients. The authors found a mean level of approximately 0.85 U/ml for the 11 TS patients who were 11 years of age and older. If estrogen were administered to girls in this age group, the somatomedin-C levels would very likely increase and approach those seen in normal female adolescents.

That bone age is delayed in TS patients is also not surprising, since sex hormones contribute to skeletal maturation after the age of about 9 years. Patients with TS do not have sex steroids present, and, therefore, the clinical observation of delayed skeletal maturation discussed by the authors is expected.

## Ketoconazole in the Management of Precocious Puberty Not Responsive to GnRH-Analogue Therapy

Precocious puberty is characterized by the intermittent pulsatile secretion of luteinizing hormone (LH) that reflects the episodic release of gonadotropin-releasing factor (GnRH or LHRH) from the hypothalamus. The pharmacologic principle employed therapeutically is that continuous infusion of GnRH (or the daily administration of a long-acting analogue) leads to subsensitivity (down regulation) of GnRH receptors on the gonadotrophs, thus annulling the release of the gonadotropins. Although most children with precocious sexual development will have the normal pubertal process turned on early (idiopathic precocious puberty), some boys have what appears to be autonomous Leydig cell function with low basal and GnRH-stimulated gonadotropin output (so-called testotoxicosis, or a form of gonadotropin-independent precocious

sexual development). These youngsters would not be expected to respond to long-acting GnRH-analogue therapy.

The authors treated three such boys with the antifungal preparation ketoconazole. All had failed to respond to GnRH-analogue therapy. Ketoconazole treatment (200 mg every 12 hours) was started at least one month after discontinuation of the GnRH-analogue therapy. Within 24 hours, the testosterone concentrations fell significantly (less than 20 ng/dl in two of the three subjects). Measurement of 17-hydroxyprogesterone concentrations revealed an inverse relationship to testosterone concentration. There were no significant changes in the low urinary levels of gonadotropins. Basal cortisol concentrations were unchanged, but the peak response to adrenocorticotrophic hormone (ACTH) was blunted. The testicular response to

human chorionic gonadotropin (hCG) was also unchanged following ketoconazole treatment. Striking improvements in behavior were noted within the first 48 hours of therapy, with disappearance of erections and masturbatory activity.

With increasing dosages of ketoconazole, the behavioral gains were sustained and the testosterone concentrations remained low. The height velocity was significantly diminished from 15 cm/yr to 6 cm/yr.

Holland FJ, et al: *N Eng J Med* 1985; 312:1023-1028.

**Editor's comment**—Most commonly, isosexual precocious development is due to central precocious puberty—that is, the normal pubertal mechanisms are activated too early. The efficacy of GnRH stimulatory analogue (agonist) therapy has been well documented. However, it is ineffective in patients with gonadotropin-independent sexual precocity. Ketoconazole was chosen because data suggest that this agent may interfere with testosterone biosynthesis through relatively selective effects on the C17-20 lyase step in steroid hydroxylation.

These preliminary data, which show reductions in height velocity and in the rate of bone maturation, are promising for boys with gonadotropin-independent sexual precocity. Although ketoconazole therapy ought to be effective in idiopathic precocious puberty, it would appear that GnRH-analogue therapy is preferable—there is low toxicity and, by now, a good deal of experience. Although none of the boys exhibited hepatic toxicity to ketoconazole, treatment of adults with this hepatically metabolized agent has been associated with abnormalities in liver enzyme levels. Thus, for the rare disorder of testotoxicosis, and possibly for other forms of gonadotropin-independent sexual precocity, ketoconazole is logical and effective therapy. Because of the drug's potential hepatotoxicity and possible adrenal toxicity, patients being treated with it require intensive follow-up.

## Infants With Birth Weights Less Than 1,001 g: Survival, Growth, and Development

At the University of North Carolina, 56 infants who weighed 1 kg or less and who were born in 1980 were cared for in the Newborn Intensive Care Unit. A surprising 52% survived the first year. Most of those who did not survive died during the first seven days.

Twenty-five infants were measured between birth and 16 months of age. Catch-up growth was apparent in many, but even when the growth plots were adjusted for age, 11 of 25 were below the fifth percentile for weight (four of the 11 were believed to be small-for-gestational-age infants). The heights appeared to be comparable to weights. Small head circumference at 12 to 16 months was closely related to low weight. Six of the 11 infants had chronic respiratory failure and five did not.

Development remains guarded, but optimism is reflected in the data. Twelve of 15 infants tested for hearing and language were found to be normal. Nineteen, including three of four survivors with birth weights less than 801 g, were free of neuro-

developmental difficulties, as defined in the study. They had physical and mental development indices of 86 or greater for their adjusted ages, and only two had permanent visual impairment. Four infants were mildly handicapped, and four were moderately to severely handicapped. A correlation between head circumference and a developmental handicap was apparent when the infants were tested at 12 to 16 months. The authors readily admit that the follow-up was short and that some children now classified as normal will probably be handicapped in the future, since learning disabilities cannot be predicted at this early age. Moreover, hearing deficits, now unrecognized, may subsequently become apparent.

Kraybill EN, Kennedy CA, Teplin SW, et al: *AJDC* 1984;138:837.

**Editor's comment**—There is indeed reason for optimism. This group of patients and similar groups must be studied for an extended time. The editorial board will review this topic in further detail in future issues of this publication.

## Growth Patterns in the Hemoglobinopathies:

### (I) Growth Patterns by Age and Sex in Children With Sickle Cell Disease and (II) Growth and Sexual Maturation in Thalassemia Major

The first report evaluates by age and sex the growth patterns of 133 children enrolled in the Sickle Cell Anemia program at Children's Hospital in Pittsburgh. These children were representative of the total population aged 1 to 18 years with sickle cell disease (SCD) in the metropolitan area. Eighty-three children (62.4%) had homozygous sickle hemoglobin (SS) and 50 (38.6%) had a variant hemoglobinopathy, such as sickle-cell hemoglobin C disease (SC), sickle cell thalassemia (S-Thal), and sickle cell plus hereditary persistence of fetal hemoglobin (S-HFP).

The median height and weight of males fell below the 50th percentile at all ages between 2 and 18 years. Height and weight deficits increased with age, with values eventually falling below the fifth percentile in the 14- to 17-year age group. The median height and weight of female patients at ages 2 to 18 years followed a similar pattern, with median height and weight falling below the 50th percentile at all ages. A trend toward increasing deficits with increasing age was also seen. The overall growth deficit in the female patients was less pronounced at all ages. This basic growth pattern was seen in all patients with SCD (regardless of subtype), except for significantly higher weight in female patients with a variant hemoglobinopathy.

This study provides evidence of growth impairment in a large sample of children with SCD. The growth deficit, which increases with age, is more pronounced in males. Height and weight deficits appeared to begin as early as 2 years of age; the increasing deficits in height and weight noted in males between the ages of 14 and 17 years and in females between 10 and 12 years of age were associated with delayed onset of puberty. The authors constructed growth velocity curves that demonstrated the significance of the delayed pubertal growth spurt; maximum height and weight velocity occurred later and the magnitude

of the spurt was depressed. Final adult heights of these patients were significantly decreased, with the mean height and weight of adult males falling below the tenth percentile.

In the second report, growth and sexual development were evaluated in 250 adolescents with  $\beta$  thalassemia major. These represented all patients above the age of 10 with transfusion-dependent thalassemia major who were receiving treatment at the thalassemia clinics of five teaching hospitals in northern Italy. Mean pretransfusion hemoglobin concentrations had been kept at greater than 9.5 g/dl during the previous five years and desferrioxamine had been administered for the previous seven to ten years. Thirty-seven percent of the thalassemic children were found to be 2 SD below the mean for normal height. After age 14 years, the percentage of children with short stature reached 62% for males and 35% for females. As in the normal population, thalassemic children with parents of short stature tended to be shorter than those with taller parents. Throughout childhood and adolescence, children with thalassemia were shorter than normal, but their weight was found to be adequate for their height.

Eighty-three percent of the males and 75% of the females had delayed skeletal maturation. Pubescent changes were absent in 30% of the females and 67% of the males between 12 and 18 years of age. Indeed, only 11% of females less than 18 years of age had experienced menarche. Cardiac arrhythmias were reported in 22% of the patients and cardiac failure in 5.6%. Several patients had diabetes, and thyroid function was frequently lower than normal.

Thus, growth retardation and delayed or absent puberty are common findings in children with transfusion-dependent thalassemia. The authors suggest that patients with lesser iron levels because of more intensive chelation therapy do not

fare any better than those on less adequate chelation therapy with regard to sexual maturation. Menarche does not seem to be any more prevalent today among girls between ages 12 and 14 who are on chelation therapy than it was in a group of female patients now older than 18, but not receiving adequate chelation therapy.

Phebus CK, Gioninger MD, Maciak BJ: *J Pediatr* 1984;105:28-33, and Borgna-Pignatti C, De Stefano P, Zonta L, et al: *J Pediatr* 1985;106:150-155.

**Editor's comment**—As these two reports demonstrate, short stature and delayed adolescence are common problems in the hemoglobinopathies. This, of course, is true of all chronic disorders, be they hematologic, infectious, gastrointestinal, or cardiopulmonary in origin. In transfusion-dependent thalassemia, however, much of the growth delay had been attributed to a defect in the hepatic biosynthesis of somatomedin and to iron deposition in the pituitary, resulting in deranged function of the hypothalamic-pituitary axis. Thus, hemosiderosis had been considered one of the major problems resulting in growth and sexual delay (and other endocrine problems) in thalassemia. Hemosiderosis, however, was not significant in the sickle cell study, although growth was significantly retarded in the SCD subjects. It is clear from these studies that individuals with SCD do not achieve normal adult height, in contrast to earlier reports from Jamaica suggesting that adults with SCD may attain normal or even greater than normal height. The specific reasons for the poor growth and delayed sexual development in children and adolescents with SCD, however, are not clear.

## MEETING CALENDAR

**October 9-12** American Society of Human Genetics Annual Meeting Salt Lake City, Utah. Contact: Ms. Gerry Gurvitch, Administrative Director, American Society of Human Genetics, 1550-B Monona Drive, Derwood, MD 20855 (301-424-4120)

**October 14-18** 37th Postgraduate Assembly of the Endocrine Society Sheraton Bal Harbour Hotel, Miami Beach, Florida. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

**October 19-24** American Academy of Pediatrics Annual Meeting San Antonio Convention Center, San Antonio, Texas. Contact: Division of Meeting Services, American Academy of Pediatrics, 141 Northwest Point Road, Elk Grove Village, IL 60007 (312-228-5005 or 800-433-9016)

**December 6-8** Advances in Pediatrics II. Postgraduate course Focus on allergy, adolescence, learning disabilities, nephrology, and newborns. Williamsburg Inn, Williamsburg, Virginia. Contact: American Academy of Pediatrics, Division of Continuing Education, P.O. Box 927, Elk Grove Village, IL 60007

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## In Future Issues

The Syndrome of Psychosocial Abuse Dwarfism: An Update by Charles Annicello, Sc.D., and John Money, Ph.D. • The Use of Estrogens to Inhibit and Stimulate Growth by Jürgen R. Bierich, M.D.

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# GROWTH

## Genetics & Hormones

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## Abuse or Psychosocial Dwarfism: An Update

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Abuse or psychosocial dwarfism is unique among syndromes of growth failure because it may be reversible. The child's failure to grow in stature, intellect, and social behavior because of abuse or neglect will persist irreversibly unless the child is rescued from the abusive environment. Sadly, the abuse usually occurs in the home.

Parents who abuse their children so contravene our society's idealization of the sanctity of the family that the evidence of abuse is either euphemized as discipline or, if that fiction cannot be maintained, prosecuted as a crime. Though child abuse is acknowledged in traditional children's literature, it is attributed to witches and wicked stepmothers; biological mothers are exonerated. A century ago, when society first recognized that children had a right *not* to be abused, "respectable" parents were not blamed for instances of child abuse, but parents who were illiterates, drunkards, prostitutes, or mental defectives were. Today it is known that parental child abuse cuts across social, economic, religious, and racial lines. Well-educated, nondrinking, pious parents of middle- and upper-class backgrounds may also be child abusers.

It is interesting to note that the victim in the first probable recorded

case of abuse dwarfism was from a noble family. Kasper Hauser, the victim, was found abandoned in Nuremberg during the 19th century. Although 17 years of age when found, he was small and his speech was poorly developed. After he was rescued, his speech improved, as did his statural and social growth. He subsequently described years of confinement.

### Causes and Classification

In the first half of the 20th century, the syndrome of abuse dwarfism was synonymous with maternal deprivation and hospitalism, even though height and weight measurements were not reported. Data on the syndrome of hospitalism were from investigations of institutionalized infants and children

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## The Use of Estrogens to Inhibit and Stimulate Growth

Jürgen R. Bierich, M.D.

*Associate Editor—Growth, Genetics and Hormones*

### Introduction

Estrogens exert a dual action on growth: small doses stimulate and high doses inhibit. While treating tall girls with high doses of the hormone is generally accepted today, low-dose therapy for retarded growth is still considered investigational and requires further study.

### Inhibition of Growth With High Doses of Estrogens

The use of high-dose estrogen therapy for growth inhibition goes back to Goldzieher (1956), who treated 14 excessively tall girls with 2 mg/d of stilbestrol. Since then, numerous variations of this approach have been applied by many investigators. However, the use of stilbestrol, an artificial estrogen that does not occur in nature, has been completely abandoned since it became apparent that it may induce vaginal carcinomas in female off-

spring if taken during pregnancy. The stilbenes will therefore not be discussed further in this article.

A number of studies assessing estrogen treatment for growth disorders have been conducted since 1962. Most of the data concern girls in whom therapy commenced at age 12 to 13 years, and thus present valid comparable observations. A mean estrogen-mediated height reduction of 4.5 cm was achieved within an average of 23 months.

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# Abuse or Psychosocial Dwarfism: An Update

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who exhibited characteristic behavior patterns and unresponsiveness despite adequate nutrition and medical care. Classical descriptions of this hospitalism syndrome were written by several groups.

Dwarfism undoubtedly has occurred under conditions of abuse and neglect in institutional settings. However, abuse dwarfism is typically recognized as occurring in the home. Paradoxically, the hospital provides a positive environment with good medical care, nutrition, and sleep, and the advantages of one-to-one "parenting" and education from a concerned staff.

When abuse dwarfism was recognized as a syndrome during the 1960s, investigators speculated that the chief etiologic factor might be maternal and/or emotional deprivation. This was consistent with the concepts and nomenclature developed by Spitz, Bowlby, and Ainsworth. However, two of the early papers on the syndrome by Powell et al concluded that emotional deprivation was the appropriate diagnostic designation. Before the identification of the specific diagnostic link between child abuse and reversible dwarfism, there were various terms for the syndrome—namely, environmental failure to thrive, deprivation dwarfism, psychosocial failure to thrive, and psychosocial deprivation dwarfism. Taxonomically, the syndrome today is usually known as abuse dwarfism, or psychosocial dwarfism.

The range of abuse inflicted on victims of abuse dwarfism includes disturbances of various intrusive and deficient sensory stimuli, particularly those related to isolation, food restriction, and direct trauma to the body. Adverse stimuli in the abusive environment presumably affect loci in the CNS that control statural growth. Discontinuation of abuse is accompanied by neurochemical changes that improve growth.

## Catch-Up Growth After Rescue

The components of growth failure persist and eventually become irreversible, unless the child is rescued from the abusive environment. The earlier the rescue from abuse, the

greater the amount of physical, mental, and behavioral catch-up growth that will be achieved. Many patients have shown dramatic catch-up growth even though their adult height fell below the mean for the general population. Of the 50 patients on record in the Johns Hopkins Hospital psychohormonal research unit, the two most severely affected dramatically exemplify the reversibility of statural growth impairment, but only to a degree. Following rescue, one boy (rescued at age 16) grew 13 inches in three years. The other patient, a girl rescued at age 8, grew 10.5 inches in only one year. However, their final adult heights were 5'4" and 4'10 1/2" respectively. (The average adult height is 5'10" for males and 5'5" for females.)

Dramatic improvements in intellectual growth have also occurred following rescue. The greatest magnitude of change, from an IQ of 36 to an IQ of 120, occurred in a girl

who was tested initially at 3 years, 8 months and subsequently at 13 years, 11 months. She was among 23 patients who qualified for an investigation of IQ change among abuse victims with various periods of persistent and continuing rescue. At rescue, the average IQ was 66 and, after different periods of persistent rescue for each individual, the average IQ for the group was 90. The change represents an average shift from mental retardation to normal intelligence. The longer the time in rescue, the greater was the increase in IQ (Table 1). In general, the findings revealed persistent impairment of IQ associated with abusive environments, in contrast to improvement of IQ in rescue environments even when rescue was found to be only partially satisfactory.

The question of whether permanent impairment of intellect could occur was addressed in an investi-

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**Table 1** IQ Elevation After Rescue (N = 23)

| IQ   | Before rescue | After rescue | Increase in IQ | Age before rescue* | Age after rescue* | Increase in age* |
|------|---------------|--------------|----------------|--------------------|-------------------|------------------|
| Mean | 66            | 90           | 24             | 7, 7               | 12, 8             | 5, 1             |
| SD   | 16            | 21           | 21             | 4, 7               | 5, 11             | 3, 1             |

$r = 0.78$ ;  $P < 0.005$

\*Age in years, months

**Table 2** IQ Elevation After Rescue: Younger and Older Patients (N = 14)

| Age at rescue | Baseline IQ* | Follow-up IQ* | IQ elevation* |
|---------------|--------------|---------------|---------------|
| <5 1/2        | 71 ± 21      | 104 ± 11      | 33 ± 24       |
| >5 1/2        | 63 ± 15      | 78 ± 16       | 16 ± 7        |

\* Mean ± SD

**Table 3** Means and Correlation of IQ and HQ Increments Accrued During Follow-up (N = 32)

| Follow-up status | IQ*     | Height quotient |
|------------------|---------|-----------------|
| Before rescue    | 69 ± 17 | 55 ± 17         |
| After rescue     | 88 ± 18 | 82 ± 11         |
| Difference       | 19 ± 22 | 27 ± 18         |

$r = 0.42$ ;  $P > 0.01$

\*Mean ± SD

gation of IQ change that compared younger v older patients. The younger the age at rescue, the greater the gain in IQ (Table 2). Each group had the same amount of time for catch-up change in IQ.

Steels published a history-making monograph in which he described the outcome of a controlled study of a permanent and gross degree of mental retardation as a sequel to infantile institutional abuse and neglect. Dennis, in *Children of the Crèche*, further demonstrated that intellectual growth was stunted and the IQ permanently reduced by as much as 50% as a sequel to uninterrupted, life-long institutional abuse and neglect. For those institutionalized foundlings who were adopted and integrated into normal family life, the earlier the adoption, the earlier the resumption of normal intellectual growth and the higher the ultimate level of adult IQ.

The correlation between child abuse and a reversible failure of statural and mental growth was first ascertained at Johns Hopkins in a longitudinal follow-up of a severely affected patient. However, until 1983, there were no systematic statistics on the specific relationship between the rates of intellectual and statural catch-up growth. At that time, height quotients (HQ) were

compared with intelligence quotients (IQ). Arrested and subsequent catch-up growth in stature were compared with arrested and subsequent catch-up growth in intelligence. Growth in both areas caught up at similar rates (Table 3).

The odd or antisocial behavior that abuse victims often exhibit—for example, eating from garbage cans, drinking from toilet bowls, and excessive eating and drinking, possibly followed by vomiting—is also reversible upon rescue. Other reversible behavioral symptoms include enuresis, encopresis, social apathy or inertia, crying spasms, insomnia, eccentric sleeping and waking patterns, pain agnosia and self-injury, all of which occur in the growth-retarding environment of abuse.

After children are rescued from abuse, their sleep characteristically changes from poor to good. This change correlates with a measured increase in statural growth. Interestingly, secretion of plasma human growth hormone (hGH) consistently relates to slow wave sleep and synchronized deep sleep stages (EEG stages 3 and 4) in normal children. Findings by Taylor and Brook in 1984 revealed that the postrescue reversal of stage 4 sleep impairment was associated with improvement in both hGH secretion and statural growth in a group of patients with abuse dwarfism.

Hyporeactive response to pain, or pain agnosia, is another phenomenon that reverses following rescue. Prerescue reports from parents and others note that the children did not complain, cry, or shed tears when punished, and generally did not react or complain when hurt, injured, or venipunctured.

Social maturation in children with abuse dwarfism is also retarded so that social age, including academic age and psychosexual age, is deficient. Although measurement problems exist, a project is under way to investigate psychosexual development in a small group of older patients.

### Endocrine Function and Reversibility

Modern genetic theory avoids dichotomizing genetic and environmental factors and postulates a genetic range of reactivity that re-

sponds to prescribed environmental cues. Factors that influence statural, intellectual, and socio-behavioral growth are implicit in the social environment. Child abuse constitutes one environmental factor that constricts the prescribed environmental boundary and inhibits its growth. Rescue from abuse may release the inhibition and widen this boundary.

Some of the hypothalamic/pituitary details of how growth is arrested and then resumed have been specified. Under conditions of abuse, the pituitary gland and probably the hypothalamus become dormant and hormonally hypofunctional. It fails to secrete growth hormone (somatotropin). If abuse continues until the expected time of puberty, the gonadotropic hormones (LH and FSH) are not secreted. Thus, the ovaries or testes fail to secrete their own sex hormones, and sexual maturation lags. To a lesser degree, pituitary secretion of adrenocorticotrophic hormone (ACTH) is also suppressed, but usually not lethally. It is reasonable to postulate that hypothalamic releasing hormones may be suppressed or that somatostatin is excreted in excess.

Knowledge about the influence of hypothalamic hormones or derivatives on learning and retention of learned facts in animals is accumulating rapidly. Neurohormones such as ACTH and MSH (melanocyte-stimulating hormone) and/or neurotransmitters such as  $\beta$ -endorphin may govern both hormonal secretion from the pituitary and growth of intelligence. Consequently, both pituitary secretion and growth of intelligence may be arrested in response to the external, socially imposed stimuli of child abuse and neglect.

The social mediation in this syndrome is evident in the interactions between the parents and the child, particularly with respect to pathological behavioral dynamics on the part of the parents. Both parents often collude as child abusers and frequently lie about how the symptoms of abuse occurred. The mother typically initiates abuse but cannot give a rational explanation for doing so. A study of the family dynamics in two severely affected patients re-

*continued on p. 4*

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### Address for Correspondence

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## Abuse or Psychosocial Dwarfism: An Update

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vealed that the mothers had a "sin" that was symbolically being atoned for or expiated by the sacrifice of their children. In both cases, the "sin" pertained to the mothers' own births out of wedlock; in one case the birth was a sequel to incest.

Impaired growth persists as long as the child is sacrificed as a proxy to atone for the mother's feelings of guilt. Interestingly, even after the abuse is removed, a dependency or even addiction to abuse persists in most of these children. Addiction to being abused helps to explain a victim's resistance to amelioration and cure.

One of the justifications used by abusing parents is that their child instigates abuse. This claim may signify a failure of parent-child bonding, even from birth onward. Subsequently addicted, the abused victim responds to abuse by stimulating more of it. The neurochemistry

of this addiction theoretically could be linked to the neurosecretion of a brain endorphin with a morphine-like sedative effect. This morphine-like effect might be helpful in explaining the presence of pain agnosia before rescue and why abused children incite their rescue caretakers into being abusive after rescue.

What is still needed is an explanation of how forbidden and repugnant behavior becomes endorsed and practiced. For so complete a reversal of turning the repugnant into a sanctioned practice, Solomon formulated and tested the theory of opponent-process learning. Opponent-process learning is seen in action when fear and terror are converted into a daredevil act or when the tragedy of being abused is turned into the exhilaration of seeking abuse. Opponent-process learning also explains how apparently decent parents self-right-

teously justify their abusive behavior and perpetuate themselves as abusers.

The syndrome of abuse dwarfism has profound heuristic and theoretical importance for medical practitioners. It exemplifies, in the human species, the way that stimuli from the external social environment may act in concert with internal physiologic functions to program—or arrest—growth and development. The syndrome should be considered in the differential diagnosis of short stature whenever a child presents with a combination of short stature for age, IQ deficit, learning disability, and odd or bizarre types of behavior.

References will be sent on request to Dr. Blizard.

Drs. Annecillo and Money are guest contributors for this issue. They are internationally respected as authorities in psychosocial dwarfism.

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## The Use of Estrogens to Inhibit and Stimulate Growth

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Which factors predict successful treatment? The dosage of the estrogens is significant, as are a number of interrelated auxologic factors, such as chronological age, bone age, onset of menarche in the subject at the start of treatment, and the subject's so-called "growth potential." The last factor is the difference between height at the start of therapy and the predicted final height.

Relatively low doses of estrogens were given to 12- to 13-year-old patients by Bayley et al (1962), Frasier and Smith (1968), Neugebauer (1974), Colle et al (1977), van der Werff ten Bosch and Bot (1981), and Schambach and Nitschke (1985). In these patients, an average growth reduction of only 2.4 cm was attained, an unsatisfactory result. Better results, ie, a mean reduction of 7.2 cm, were achieved by Kuhn et al (1977), von Puttkamer et al (1979), Willig et al (1980), and Bierich (1978, 1983). Kuhn et al and Willig et al gave a dose of 0.5 mg/d of ethinyl estradiol, while von Put-

kamer et al and Bierich gave a dose of 7.5 mg/d of conjugated estrogens. These dosages are currently recommended for the treatment of tall stature.

### Age and Maturity at the Beginning of Treatment

According to Bayley and Pinneau, 10-year-old girls still have 13.8% of their height potential, while 13-year-old girls have only 4.2% left. Consequently, during the three years between 10 and 13, the growth rate is reduced by 9.6%. Whitelaw and Foster (1962) were the first to draw attention to the importance of age and the necessity for early treatment. The same has been demonstrated by Greenblatt et al (1966), Zachmann et al (1975), Kuhn et al (1977), Bierich (1978), Andersen et al (1980), and John and Schellong (1980). Starting therapy early increases the growth-inhibiting potential of high-dose estrogen. In most cases, estrogen therapy was started in the second and third Tanner stage of puberty.

A number of investigators, however, recommend that treatment be

started prior to puberty. Among them are Whitelaw and Foster (1967), Reeser et al (1979), and Schambach and Nitschke (1985). Based on reports from the two latter groups, it is apparent that one can treat prepubertal girls with much lower dosages of estrogens than those used in postpubertal girls. Reeser et al (1979) recommended only 0.2 mg/d of ethinyl estradiol, while Schambach and Nitschke recommended only 0.08 mg of mestranol. These investigators specifically intend to avoid the possible risks associated with the use of supraphysiological doses of estrogens. However, one has to cope with a mild form of precocity if the treatment is started prior to spontaneous sexual development.

### Side Effects

Three major side effects of high-dose estrogen therapy will be considered here: development of neoplasms, infertility, and thromboembolism.

Malignancies, which often develop in older women receiving es-

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trogens, are not encountered in adolescents. Carcinomas of the endometrium have been observed only in patients with Turner's syndrome who were treated continuously with estrogens alone (Levine, 1978). As long as the endometrium is periodically shed (the result of exogenous progesterone), development of precancerous atypia such as cystic glandular hyperplasia can be avoided. Therefore, progesterone (eg, 10 mg of medroxyprogesterone acetate) is routinely given during the last week of each four-week cycle, while estrogen is given continuously throughout the cycle.

Suppression of the gonadotropins is substantially limited to the period of treatment, and spontaneous menstruation recurs two to three months after therapy is discontinued. Adverse effects on subsequent fertility have not been reported. Persistent amenorrhea is extremely rare.

The acute risk of thrombosis is a matter of concern. It is well known that adult females using oral contraceptives are at higher risk for developing blood clots. The accelerated coagulation of the blood is caused primarily by the estrogen-dependent reduction in antithrombin III. Blomback et al (1983) also found a significant lowering of antithrombin III in adolescent girls treated with high-dose estrogen. If conjugated estrogens are given, however, these alterations in clotting factors do not seem to occur (von Petrykowski and Schmidt, 1983).

It is important to note that only one instance of thrombosis was reported in a large-scale inquiry conducted by Conte and Grumbach in 1978. That study analyzed data from 904 patients who were treated with estrogens for tall stature.

#### Mode of Action

Based on the favorable results of estrogen treatment in acromegalic patients, Goldzieher (1956) hypothesized that estrogens suppress the somatotrophic function of the adeno-hypophysis, ie, human growth hormone (hGH). Actually, the opposite is the case. With estrogen treatment, basal and stimulated hGH secretion increase. The specific action of estrogens primarily involves somatomedin, not growth

#### Letter From the Editor

Dear Colleague:

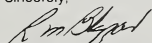
For the past six months, while human growth hormone (hGH) has been unavailable, there has been anxiety expressed by parents of GH-deficient (GHD) children that their children have been without treatment. Parental concern was partially alleviated on October 18, 1985, when the Food and Drug Administration approved a biosynthetic GH (Protropin®, Genentech, Inc.) for the treatment of children with GHD. This hormone will be available by prescription through pharmacies at hospitals where GHD children are typically treated.

The present formulation was tested in clinical trials in the United States and was demonstrated to be effective in stimulating growth in GHD children. Testing was done in 84 GHD children who received intramuscular injections three times per week at a dose of 0.1 mg/kg (0.2 IU/kg) for six to 36 months. Although antibodies developed in approximately 30% of the children, only one patient developed antibodies of the type and titer that produced slowing of growth (ie, antibodies associated with high GH-binding capacity). This patient subsequently responded to native hGH treatment with accelerated growth. No adverse effects of antibody formation on the immunological, cardiovascular, and renal systems were demonstrated.

Since this hormone is manufactured by a recombinant DNA process, there is no concern about contamination with the slow virus that causes Creutzfeldt-Jakob disease—the reason that native GH was withdrawn from distribution earlier this year. Recombinant DNA technology also ensures virtually limitless supplies of DNA biosynthetic GH, thus making it possible for all GHD children to be treated.

Physicians providing medical care to children should make every effort to identify—and provide proper evaluation and treatment for—GHD children. However, as a pediatric endocrinologist with many years of experience, I am concerned that this hormone may be abused and given indiscriminately to children who may or may not benefit from it. Hopefully, physicians will resist the pressures to prescribe GH for children who have not been adequately diagnosed as GH-deficient.

Sincerely,



Robert M. Blizzard, M.D.  
Chairman, Editorial Board

hormone itself. Wiedemann and Schwartz reported in 1972 that estrogens decreased the sulfation factor (the biologically active somatomedin) in serum in acromegalic patients. Von Puttkamer et al (1977) showed that serum somatomedin concentrations were markedly lowered in tall girls who received high doses of estrogens for months. After six months of treatment, the levels were only 57% of pretreatment values. These findings were confirmed in 1981 by Gourmelen et al. They explain that reduced growth velocity in girls with open epiphyses may be due to the decrement of serum so-

matomedin concentration, and not to a mechanical barrier that prevents longitudinal bone growth, although the latter possibility is not excluded.

#### Promotion of Growth With Low Dosages of Estrogens

The natural model of growth promotion by estrogens is the pubertal growth spurt (PGS) in girls. Although growth velocity is slow prior to puberty, it reaches a maximum of approximately 8 cm per year during the course of puberty. This rapid increase in height precedes men-

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## The Use of Estrogens to Inhibit and Stimulate Growth

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arche by at least a full year. PGS is absent in girls with gonadal dysgenesis. Possibly, this is because ovarian estrogens are not produced. Consequently, it has been discussed exhaustively whether the androgens from the adrenals cause the PGS in females. Today we know that PGS follows the onset of increased adrenal androgen secretion by a period of four to five years and correlates better with gonadarche. Another clinical indication of the determining role of the estrogens themselves is the normal adult height and the normal PGS in patients with testicular feminization (Zachmann et al, 1984). These "hairless" women or girls with a 46 XY karyotype produce in their testes large quantities of testosterone; however, since they have no androgen receptors, the androgens cannot exert their characteristic actions. Consequently, the PGS in females is primarily derived from estrogens.

Studies evaluating physiologic dosages of estrogen during puberty have been conducted by Rosenfield et al (1974, 1980). The investigators gave 1 to 2 mg depot estradiol intramuscularly per month to nine patients with Turner's syndrome who had an average chronological age of 15.9 years and a bone age of 12.5 years. This therapy led to normal sexual development and doubled the growth velocity and bone age velocity. During treatment, the serum somatomedin level rose markedly, resembling the normal pubertal increase. This finding is in sharp contrast to the reduction in somatomedin concentration to high doses of estrogens.

The question of optimal estrogen dose for the promotion of growth has been studied frequently in recent years. How low must the dose be if it is to stimulate growth? Interestingly,

doses as low as 0.05 mg ethinyl estradiol and 1.25 mg conjugated estrogens (hitherto applied as replacement therapy doses) are capable of inhibiting longitudinal growth. According to van der Werff ten Bosch and Bot (1981), growth velocity can be reduced by as little as 0.05 mg ethinyl estradiol.

Levine-Ross et al (1983) have correctly pointed out that peak height velocity during puberty does not occur at the time of menarche, but usually one year earlier. At this time estradiol concentration in plasma is approximately 20 pg/ml, or one sixth the adult level. These investigators measured the growth of the ulna in patients with Turner's syndrome who had received different estrogen regimens and determined that 100 ng/kg body weight was the optimal dose. The favorable results of the first short-term trials have since been confirmed by investigations of six months' duration. Other studies in which the same doses of estrogens were given produced similar results [Saghedi-Nejad et al (1984), Alexander et al (1984), and Rosen-dahl et al (1985)]. However, in all these investigations, the observation period was not long enough to draw any final conclusions or to make any definite statements on ultimate adult height.

### Mode of Action

How do low doses of estrogens stimulate growth? Aside from the enhancement of adrenal androgen synthesis, four principal interpretations are possible:

- Estrogens increase the secretion of hGH
- Estrogens stimulate growth *per se*
- Estrogens work synergistically with hGH
- Combinations of the above

During sexual development, spontaneous nocturnal hGH secre-

tion increases in both sexes to levels that are more than double the prepubertal values (Finkelstein et al, 1973; Bierich et al, 1985). Also, the conventional provocation tests for hGH induce considerably higher peaks after puberty (Frantz and Rabkin, 1965; Frasier et al, 1970) than before. These changes can be explained by a "priming" of the hypothalamus or hypophysis with the sex hormones. The well-known rise of serum somatomedin-C concentration during puberty is a sequel of the increased hGH secretion.

In the investigations of Levine-Ross et al, the greatest ulnar growth was seen with 100 ng/kg/d of ethinyl estradiol while the somatomedin levels reached their maximum with 800 ng/kg. This might indicate an estrogen-mediated, somatomedin-independent mode of growth stimulation.

In the male, the pubertal growth spurt is effected through two independent mechanisms: the androgen-stimulated hGH increase (see above) and a functional synergism between androgens and hGH (Zachmann et al, 1975). Androgens on their own may not stimulate growth. It can be presumed that a corresponding synergism also exists with regard to estrogens. Von Puttkamer et al (1977) reported on clear cut increments of spontaneous hGH secretion in tall girls receiving estrogen.

Additional mechanisms, which are not clear, may also play a role in the stimulation of growth by estrogens.

In summary, estrogens can stimulate or inhibit growth and can be used effectively in both instances. However, attention must be paid to the age of the patient, the type of estrogen used, and the dosage applied. Side effects of estrogen therapy for stimulation or inhibition of growth are minimal.

### In Future Issues

The Genetics of Insulin-Dependent Diabetes  
by Noel Maclaren, M.D.

The Effect of Insulin Control on Growth, IGF-I, and Growth Hormone  
by William Tamborlane, M.D.  
and Stephanie Amiel, M.B.

# Support Groups for Families of Children With Growth Problems

Judith G. Hall, M.D.

Associate Editor—Growth, Genetics and Hormones

Lay and support groups concerned with specific disease entities have blossomed in the past few years. Support groups for families of children with growth disorders have been active and productive. The purpose of this article is to make physicians who care for children with growth problems aware of these organizations and the resources they provide to families and other health professionals.

In general, there are five reasons why lay or support groups develop. First, they provide information and practical advice for the families and allow them to share personal experiences about specific conditions. Such groups can play an important role in helping a family adjust to the fact that they have a child with special needs. Second, the groups strive to educate the general public and physicians about these conditions. Many families have felt the frustration of not being able to find physicians who were knowledgeable about the rare conditions affecting their relatives. Supporting and advocating related medical research is the third reason. The fourth is to help provide care, therapy, and/or educational opportunities for affected individuals. The fifth reason is to provide an opportunity to socialize. Affected individuals and family members who face the same problems and share common experiences often become friends who wish to share their social experiences and good times together.

Several lay groups are described below. Most charge minimal membership dues and provide newsletters about recent developments in the area of interest. The newsletters usually include useful tips, a social calendar, and an update on other members. Most groups also raise money to promote research or to provide scholarship funds or medical care for affected individuals. Many publish outstanding booklets that are excellent sources of information for families and physicians, who especially need to be aware of such groups in order to

refer families and to utilize their resources. Physicians often play a critical role in guiding families to groups that can allow positive utilization of potentially hostile energy. Physicians should contact their local university-based genetics services for the names of other specific disease-related groups.

**Little People of America (LPA)** is a nationwide, voluntary organization dedicated to helping people of short stature. LPA is divided into districts (by geographic region) and smaller area chapters. Regional meetings, an annual national meeting, and a number of less formal gatherings are held periodically. The organization was founded in 1957 when the television and movie personality, Billy Barty, planned a meeting for short-statured individuals like himself in Reno, Nevada. Today, LPA has more than 4,000 members from all walks of life who are 4'10" or less.

Special needs for specific groups within LPA were recognized soon after its founding. A teen group and a group for young single adults were organized. The concerns of adult members prompted organization of committees and workshops on careers, exercise and fitness, nutrition, continuing education, social attitudes, and marriage and family counseling. An adoption committee finds adoptable short-statured children and promotes their availability to families who wish to adopt such children. In addition, many average-sized parents of short children (referred to as the "little littles" within LPA) had particular concerns, and organized an auxiliary to deal with these concerns.

An LPA Foundation has been formed to obtain and distribute funds for vocational training, scholarships, and the support of medical and scientific research. A Medical Advisory Board (MAB) serves as a resource for medical care and advice. Because LPA members often participate in a variety of research projects, the MAB reviews these projects for ethical and scientific merit.

LPA members have produced some excellent reading material. *The Idea Machine* provides information about handy gadgets and daily living tips for short-statured persons. A national newsletter, *LPA Today*, and district and chapter newsletters report on matters of interest to all members. The parents of "little littles" have written a booklet entitled, *My Child is a Dwarf*; it contains pertinent information about childhood development and special adjustments.

Physicians, paramedical professionals, and families are encouraged to write directly to LPA National Headquarters, P.O. Box 633, San Bruno, CA 94066, or to the Canadian counterpart at Little People of Canada, P.O. Box 453, Abbotsford, British Columbia V2X 2Z5, Canada.

**The Human Growth Foundation (HGF)** was organized in 1965 by parents whose children had severe growth problems of any type. Largely through the efforts of the HGF, growth hormone therapy was made available. From its inception, the HGF has worked hard to support basic and clinical research pertaining to growth disorders. The organization recently launched a program of career starter grants for professionals involved in growth disorder research.

The HGF has also produced excellent informational booklets on growth problems such as achondroplasia, Turner's syndrome, intrauterine growth retardation, short stature, and dwarfism. These booklets are an important resource to parents and children who face the difficulties associated with short stature. Inquiries may be addressed to The Human Growth Foundation, 4607 Davidson Drive, Chevy Chase, MD 20815 (301-656-7540).

In the United Kingdom, the **Association for Research into Restricted Growth (ARRG)** was founded in 1970 to promote investigations into the basic nature of growth problems, and to serve as a self-help

*continued on p. 8*

## Support Groups for Families of Children With Growth Problems

continued from p. 7

organization concerned with the well-being of people with restricted growth. The ARRG has also prepared a number of excellent pamphlets dealing with growth disorders. Information may be obtained from ARRG, c/o Miss P.R. Rutt, 24 Pinchfield, Maple Cross, Rickmansworth, Hertfordshire WD3 2TP, England.

There is also the **International Association of Little People (IALP)**, which was established to promote interaction between the organizations of various countries. The international organization has addresses of contact people in many countries. Those wishing information should write to Joy Campbell, International Correspondent, 5612A Hillsdale Boulevard, Sacramento, CA 95842.

Several groups have also been established for patients and their families whose short stature is associated with a specific disorder.

Although not all individuals with osteogenesis imperfecta are short in stature, they do have many medical and social problems. All types of osteogenesis imperfecta seem to be due to genetically determined collagen abnormalities. Complications include frequent bone fractures, dental anomalies, and deafness. Several lay and support groups addressing these problems have been formed. These groups and their addresses are **The American Brittle Bone Society**, 1256 Merrill Drive, Marshallton, West Chester, PA 19380; the **Osteogenesis Imperfecta Foundation, Inc.**, P.O. Box 838, Manchester, NH 03105; **Osteogenesis Imperfecta National Capital Area, Inc.**, Box 941, 1311 Delaware Avenue SW, Washington, DC 20024; and the **Canadian Osteogenesis Imperfecta Society**, Box 607, Station U, Toronto, Ontario M8Z 5Y9, Canada. All have newsletters that provide members with information about available aids, therapy, and new developments in research.

Individuals with Turner's syndrome have a special set of concerns, in addition to those associated with short stature. The **Turner's Syndrome Society** was founded to address these concerns. It has been extremely active in providing

information about problems specific to patients with Turner's syndrome by producing an excellent videotape and publishing an informative newsletter every few months. A booklet prepared by the Society, *The X's and O's of Turner's Syndrome*, is excellent for patients and families. Correspondence should be addressed to The Turner's Syndrome Society, c/o Susan Charney, York University, Administrative Studies Building, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada.

The mucopolysaccharidoses and mucopolipidoses are rare hereditary disorders with enzyme deficiencies in which abnormal compounds collect in the cells of various body tissues. Most of these disorders result in short stature and are associated with a variety of other problems. To promote research and public awareness, the **Society for Mucopolysaccharide Diseases** was founded. Groups have formed in the United States and Canada. They are **The MPS Society, Inc.**, 552 Central Avenue, Bethpage, NY 11714 and **The Society for MPS**, c/o Sheila Lee, 382 Parkway Blvd., Flin Flon,

Manitoba R8A 0K4.

This list of lay and support groups is not all-inclusive. Rather, it is intended to alert physicians to the availability of this type of resource for patients and families. Some families are hungry for information; it is wise that they be put in contact with these groups. Other families are initially resistant to joining but should be encouraged to seek information intermittently; over the long run, they will gain from the availability of reliable information and from the knowledge that they are not alone. When patients and families do become knowledgeable about rare disorders, it is important that physicians not feel threatened. Instead, they should help to put that knowledge into perspective since a lay person without a medical background can have unrealistic expectations and often needs to be reminded not to lose sight of the "whole child." On the other hand, lay groups need the support of the medical profession (and of individual physicians to serve as medical advisors) so they can work effectively in dealing with the problems associated with short stature.

### Letter From the Editor

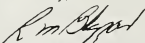
Dear Colleague:

In the four issues of *Growth, Genetics, and Hormones* published thus far, the Editorial Board has presented a number of pertinent articles as well as abstracts of special interest. We are particularly pleased that one of the abstracts that appeared in the first issue—Pseudopituitary Dwarfism Due to Resistance to Somatomedin: A New Syndrome—has elicited an "update" from Dr. Roberto Lanes of Caracas, Venezuela.

Dr. Lanes wrote to inform us that he has heard recently of two more patients with the syndrome. He and his colleagues reported one such patient in the *Journal of Clinical Endocrinology and Metabolism* in 1980 (50:485). Dr. Lanes noted in his letter that this syndrome is possibly more common than previously thought, posing a difficult problem for pediatric endocrinologists because affected children do not respond to any form of currently available therapy.

The Editorial Board thanks Dr. Lanes for his comments and encourages all of our readers to communicate with us regarding such events. We also invite you to share with us any comments you might have about specific articles or about the publication in general. We look forward to hearing from you.

Sincerely,



Robert M. Blizzard, M.D.  
Chairman, Editorial Board



## Effect of GH-Releasing Factor on GH Release in Children With Radiation-Induced GH Deficiency

Lustig and co-workers report five male children who had received cranial irradiation for extrahypothalamic intracranial neoplasms or for leukemia, and subsequently developed severe growth hormone (GH) deficiency. Each was challenged with supramaximal amounts of growth hormone-releasing factor (GHRF). Mean peak GH levels after GHRF rose to values higher than those evoked by levodopa or arginine ( $6.4 \pm 1.3$  ng/ml  $\nu$   $1.5 \pm 0.4$  ng/ml,  $P < 0.05$ ). The responses to GHRF were similar to those obtained in children with severe GH deficiency due to other etiologies. The results support the hypothesis that cranial irradiation in children can lead to hypothalamic GHRF deficiency secondary to GHRF-neuronal injury.

Lustig RH, Schriock E, Kaplan SL, et al: *Pediatrics* 1985;76:274.

**Editor's comment**—These data are consistent with previous reports of diminished GH secretion after cranial irradiation for neoplastic disease. However, they show pituitary responsiveness to GHRF and point toward the hypothalamus as the site of injury. The five patients with severe GH deficiency represent a rather small subset of those who have had cranial irradiation. It would be of interest to test a large number of long-term surviving children who had received cranial irradiation with submaximal amounts of GHRF to determine sensitivity as well as efficacy. Additional data—such as those from Blatt and co-workers (*J Pediatr* 1984;104:182) assessing the intrinsic secretory pattern of GH—would round out this study to define the neurosecretory system alteration for the GH "system" following a single protocol of cranial irradiation.

## Skeletal Age Changes in Puberty

A study by J.M.H. Buckler of Leeds, England, was conducted in 34 Leeds schoolboys, 10.1 to 11.4 years of age. Height measurements were taken every four months, and bone-age x-rays were obtained annually for four to five years. Growth velocity and skeletal velocity using the Tanner Whitehouse 2 (TW2) method to evaluate skeletal maturation were compared to ascertain if skeletal maturation progresses consistently year by year through adolescence. The data indicate that skeletal ages advance more rapidly than chronological ages during adolescence and that there is a direct relationship between skeletal velocity and growth velocity. Peak skeletal age velocity advances almost simultaneously with peak height velocity ( $13.7 \pm 0.8$  years  $\nu$   $14.3 \pm 1.0$  years).

The TW2 standards for bone ages, when established, were obtained using groups of children at various ages. These children were x-rayed once and, therefore, the standards do not take into account this rapid advancement of bone age

at the time peak height velocity occurs.

In males who are growing rapidly, bone-age determinations that are done serially will advance at rapid rates. If this fact is not recognized, errors in interpretation may be made. Late developers will initially show a relative retardation of bone age, but their skeletal age will catch up when puberty ultimately occurs. In monitoring treatment, physicians sometimes attribute the rapid changes in skeletal age that occur at this time to incorrect treatment, when in fact these changes can be readily explained by the patient's stage of puberty.

Buckler JMH: *Arch Dis Child* 1984; 59:115.

**Editor's comment**—We have all observed that, in certain patients, skeletal maturation occurs very rapidly and out of proportion to the chronological time that has passed. Dr. Buckler has supplied an explanation for at least some of these observations.

## Pituitary Dwarfism in a Patient With Circulating Abnormal GH Polymers

Valenta et al describe the growth pattern of a short 14-year old boy, the son of relatively short parents. His height and weight were average for a 10-year-old, and he had growth failure for at least the previous three years, growing between 1.5 and 3.5 cm/yr. Despite Tanner stage III development of the genitalia and pubic hair and circulating sex-hormone levels corresponding to this stage of sexual development, he had not yet shown a pubertal growth spurt. The physical examination, blood chemistry analyses, and circulating pituitary and endocrine target organ hormone concentrations were normal. The responses to all pharmacologic stimuli for growth hormone (GH) secretion were normal (peak GH: 9.6 to 36 ng/ml) and the somatomedin C (SmC) concentration was 1.7 U/ml. There was a marked

acceleration of growth rate during exogenous GH therapy.

The circulating GH and the fractionated components (gel chromatography) were subjected to various immunoassays and bioassays to determine their activities. Using the IM-9 continuous cell line of cultured human lymphocytes (receptor assay, RRA), these investigators found an RRA/radioimmunoassay of 0.5 and the bioassayable activity (Nb-2 cell lactogenic assay) reduced by 25%. On column chromatography, the usual three peaks of GH species were noted—"Big-Big" [85,000 Daltons, (?) tetramer], "Big" [45,000 Daltons, (?) dimer], and "little" [20,000 Daltons, monomer]—but were present in unusual proportions—60% to 90% GH polymers rather than the more usual

*continued on p. 10*

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14% to 40% in plasma. Further physical analysis revealed that the units of the polymers were joined by disulfide (covalent) linkage rather than the more usual noncovalent ("stuck together") forces.

Valenta LJ, Sigel MB, Lesniak M, et al: *N Eng J Med* 1985;312:214.

**Editor's comment**—The chemical analysis of the circulating GH species is very thorough and makes a very convincing case for a distinct abnormality in the physical and chemical properties of GH. However, it is less certain whether these abnormalities were the cause of this young man's shortness.

From the few growth points mea-

sured early on, it seems likely that this boy did not have growth failure before the age of 8 or 9 years. This pattern would be distinctly unusual for a congenital growth problem. In addition, the baseline SmC concentration was at the upper limit of normal—1.7 U/ml—rather than subnormal, which would be the case if these molecular species were unable to cause the liver to produce SmC. The IM-9 cell receptor GH activity was low with respect to the immunoassay potency, but no mean and standard deviations are given for those GH components in normal serum. Could these values merely represent the "tail" of Gauss? Finally, the Nb-2 cell bioassay results reflect the lactogenic activity of circulating human growth hormone

(hGH) (after immune precipitation of the other lactogen, prolactin). Although the results may be low (no mean and standard deviations are given for normals), this activity may not reflect the growth-promoting activity of hGH. What clearly needs to be done with the GH molecules in this patient's serum is to concentrate them immunologically before testing the mixed and/or separated components in a bioassay for growth in hypophysectomized mice or rats or in the tibial-line assay. In summary, although this patient most probably has an abnormality in distribution of GH polymers, it is doubtful that the hypothesis of an abnormal circulating GH molecule of diminished biological activity has been proven.

## Fibrochondrogenesis—A Lethal, Autosomal Recessive Chondrodysplasia With Distinctive Cartilage Morphology

The lethal neonatal osteochondrodysplasias are a heterogeneous group of disorders that can be distinguished from each other by radiologic and histopathologic criteria. Fibrochondrogenesis, a neonatally lethal, short-limbed skeletal dysplasia, was first described in 1978 in a single patient who was the offspring of a consanguineous mating. This disorder was named "fibrochondrogenesis" because of a distinctive fibrosis of the growth plate cartilage. In these two articles, four more patients with this syndrome are described and the clinical, radiographic, and morphologic features are defined.

The main clinical features were short-limbed, rhizomelic-type neonatal dwarfism, a relatively large head, a round flat face with prominent eyes, cleft palate (in two of four patients), and a small chest. All four were sporadic, nonconsanguineous cases.

Radiographically, the long bones were short and dumbbell shaped, with broad metaphyses. The ribs were short and cupped. The iliac bones were small and rounded. Dif-

fuse platyspondylia was present, with superior-inferior clefting defects and pear-shaped bodies.

Histological examination of chondro-osseous tissue revealed peculiar pathognomonic abnormalities of the cartilage. The resting cartilage was hypercellular, with round or spindle-shaped fibroblastic-appearing cells. The matrix appeared to be fibrous, with dense septae. At the growth plate, the cells were clustered in irregular nests within a fibrous matrix. The bone appeared normal in structure. Transmission electron microscopy of the cartilage revealed a fibrous matrix surrounded by round or fibroblast-like chondrocytes. The fibrous-appearing matrix was composed of thick-banded, densely packed collagen fibers. Proteoglycan granules were deficient in these areas.

These findings suggest either a defect of Type II collagen synthesis or structure, or an abnormality in the aggregation of collagen fibers secondary to a deficiency or abnormality in proteoglycans. Although this disorder was first distinguished on the basis of the peculiar morpho-

logic cartilaginous abnormalities, the radiographic features have now been recognized as quite distinctive.

Whitely CB, Langer LO, Ophoven J, et al: *Am J Med Genet* 1984;19: 265-275; and Eteson DJ, Adomian GE, Ornoy A, et al: *Am J Med Genet* 1984;19:277-290.

**Editor's comment**—The 1983 Conference for International Nomenclature of Constitutional Disease of Bone (Paris) enumerated ten lethal osteochondrodysplasias identifiable in the newborn period. Several new osteochondrodysplasias are identified each year. An accurate diagnosis must be made to provide meaningful prognostic information and appropriate genetic counseling. The differential diagnosis of these disorders depends on clinical, radiographic, and/or morphologic criteria, since their basic biochemical defects have not yet been elucidated.

Most of the severe neonatal disorders can be diagnosed prenatally by careful serial ultrasound examinations during the second trimester.

## Late-Onset Adrenal Hyperplasia in Hirsutism

The investigators studied the incidence of late-onset adrenal hyperplasia as a cause of hirsutism, its association with the major histocompatibility complex, and its clinical expression. Their patient population included 400 women seen for hirsutism. Twenty-four (6%) were found to have late-onset adrenal hyperplasia.

Nonclassical, late-onset forms of adrenal hyperplasia, in which sexual ambiguity is not present at birth but virilization occurs during childhood or after puberty, have been described. However, these late forms are extremely variable in age at appearance, in degree of hyperandrogenism, and in association with abnormalities of the menstrual cycle. An elevated basal plasma 17-hydroxyprogesterone (17-OHP) level—especially its dramatic elevation after adrenocorticotrophic hormone (ACTH) stimulation—leads to the diagnosis of "partial" adrenal 21-hydroxylase deficiency.

All 400 women had ACTH stimulation tests in which 17-OHP and adrenal androgens were measured. In addition, the 24 identified as having late-onset adrenal hyperplasia due to 21-hydroxylase deficiency had human leukocyte antigen (HLA) typing since this form, like the classical form, is linked to the major histocompatibility complex. The families of these 24 patients underwent HLA typing as well.

Basal cortisol concentrations did not differ from normal values, but the increase after ACTH was significantly lower than normal. By contrast, 17-OHP levels were higher than normal and strikingly increased after ACTH. Plasma androstenedione was high in all but three patients and plasma testosterone levels, although often elevated, were normal in nine patients. Urinary excretion of 3 $\alpha$ -androstenediol was higher than normal in most cases.

HLA typing showed HLA B-14 in 75% of the index patients, but in only

11.7% of a control population. AW-33, B-14 was 40 times more common in the patients. The family members who had HLA typing were divided into three groups: HLA identical, one haplotype in common (heterozygotes), and no haplotypes in common (normal). As expected, the basal and post-ACTH 17-OHP concentrations in the heterozygotes were intermediate between those values in the normals and those in the homozygotes.

Kuttent F, Couillin P, Girard F, et al: *N Engl J Med* 1985;313:224.

**Editor's comment**—This study represents the accumulation of large amounts of interpretable data on hirsute women and their families. The results indicate the utility of the ACTH stimulation test as part of the diagnostic evaluation of hirsute women and, by implication, in children of both sexes with premature adrenarche.

More importantly, this study points to the remarkable variability in the expression of androgen excess in these women and their families. Hirsute women with similar androgen profiles can have totally dissimilar menstrual alterations. Siblings with HLA-identical haplotype may or may not have the same androgen profile or clinical presentation. The facts that both symptomatic and asymptomatic forms of late-onset adrenal hyperplasia occur in the same family, that they are biochemically identical and are linked with the same HLA antigens, and that they are strongly associated with HLA B-14, suggest that the genetic mutation in the symptomatic and asymptomatic forms is the same.

The consideration of late-onset adrenal hyperplasia should be mandatory not only in the hirsute adolescent and adult, but also in the child with premature adrenarche.

## Use of Plasma SmC/IGF-1 Measurements to Monitor the Response to Nutritional Repletion in Malnourished Patients

The purpose of this study was to evaluate the usefulness and sensitivity of somatomedin C/insulin-like growth factor I (SmC/IGF-I) concentrations as an indication of short-term nutritional rehabilitation in malnourished patients. Six malnourished adults were studied during a period of ten to 16 days of parenteral or enteral nutritional therapy. These patients had a variety of gastrointestinal disorders that led to malnutrition. A statistically significant increase in plasma SmC/IGF-I concentrations was reported from a baseline (mean  $\pm$  SD) of  $0.67 \pm 0.15$  U/ml to  $0.93 \pm 0.38$  U/ml after two days of refeeding. By the 16th day of nutritional therapy, the SmC/IGF-I had fallen to  $1.28 \pm 0.49$  U/ml from a peak of  $1.80 \pm 0.44$  at day 10. All patients were in positive nitrogen balance throughout the duration of the study. In contrast, the levels of prealbumin, transferrin, and retinal-binding protein did not reflect significant changes.

In summary, the findings of this study suggest that plasma SmC/IGF-I is a more sensitive indicator of improved nutritional status during short-term nutritional rehabilitation in malnourished patients than other plasma proteins that are frequently used to assess nutritional status.

Clemmons DR, Underwood LE, Dickerson RN, et al: *Am J Clin Nutr* 1985;41:191.

**Editor's comment**—This paper demonstrates the validity of SmC/IGF-I levels as an indicator of short-term nutritional status. This may, therefore, be useful in evaluating the response to treatment in various forms of nutritional dwarfism, as well as the compliance of the patients with dietary intervention.



## MEETING CALENDAR

**January 17-19** American Diabetes Association 33rd annual postgraduate course. The Waldorf-Astoria, New York, New York. Contact: American Diabetes Association, 2 Park Avenue, New York, NY 10016 (212-683-7444)

**February 4-7** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, California

**February 6-8** Canadian College of Medical Genetics. Spencer Hall, London, Ontario. Contact: Canadian College of Medical Genetics, Alberta Children's Hospital, 1820 Richmond Road SW, Calgary, Alberta T2S5C7

**April 12-17** American Academy of Pediatrics. Spring Session. Orlando, Florida. Contact: American Academy of Pediatrics, Division of Continuing Education, P.O. Box 927, Elk Grove Village, IL 60067 (312-228-5005 or 800-433-9016)

**April 28-30** 1st International Symposium on Serum Hormone-Binding Proteins. Contact: Dr. M.T. Forest, Inserm 34, Hôpital Debrousse, F-69322, Lyon, Cedex 05, France

**May 6-9** American Pediatric Society/Society for Pediatric Research. The Sheraton Washington Hotel. Washington, D.C.

**May 9** Annual Meeting of the Lawson Wilkins Pediatric Endocrine Society. The Sheraton Washington Hotel, Washington,

D.C. Contact: Dr. Salvatore Raiti, Secretary, LWPE, Suite 501-9, 210 West Fayette Street, Baltimore, MD 21201 (301-837-2552)

**June 8-11** March of Dimes Birth Defects Symposium. Westin Bellevue-Stratford, Philadelphia, Pennsylvania

**June 22-24** 46th Annual Scientific Sessions of the American Diabetes Association. Anaheim Convention Center, Anaheim, California. Contact: American Diabetes Association, 2 Park Avenue, New York, NY 10016 (212-683-7444)

**June 25-27** 65th Annual Meeting of The Endocrine Society. Anaheim Convention Center, Anaheim, California. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

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# GROWTH

## Genetics & Hormones

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## The Genetics of Insulin-Dependent Diabetes

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### Introduction

Insulin-dependent diabetes (IDD) is the predominant form of diabetes in children and young adults. Approximately 25% of patients with IDD do not develop symptoms until midlife or later. Over the past decade, it has been recognized that IDD is the result of a slow, indolent process that belies its often abrupt and dramatic clinical onset. A chronic inflammatory infiltrate of the pancreatic islets, consisting of lymphocytes and occasional macrophages and accompanied by loss of some 90% of  $\beta$  cells, is seen in the pancreas of IDD patients at the time of clinical presentation.

It also has become increasingly apparent that IDD is the result of an autoimmune process. At this writing, the first reports of amelioration of IDD in newly diagnosed patients by immunosuppressive therapy have appeared in the literature. However, such therapy may have only limited success when initiated at the time of clinical diagnosis, because irreparable loss of  $\beta$  cells already may have occurred. Thus, there is increased interest in early detection of IDD—by screening populations at

risk for circulating islet cell autoantibodies (ICA) as well as by identifying the relevant genetic factors that predispose to IDD.

The search for susceptibility genes is important, since the risk of developing IDD is 20 to 30 times greater for first-degree relatives of IDD probands than for individuals in the general population and more than one-third of identical twins affected by the disease are *concordant* for IDD. In contrast, at least half of such identical twins are *discordant* for IDD despite sharing identical genes at birth. This high rate of discordance argues that environmental agents are needed to initiate the pathogenic process in those who are genetically predisposed. However, convincing evidence that any one agent plays a major role in this regard is lacking.

### One Gene or Several?

Several European groups have reported that more males than females are affected by IDD, as indicated by a male:female ratio of 1.2:1. Although this observation has not been clearly supported by American studies, our own unselected group of patients in Florida has a 10% excess of male patients, with the greatest excess seen among patients in two distinct age groups: those with IDD onset before 6 years of age and those with late-adolescent onset. Thus, a consensus view would hold that male gender represents a definite, but minor, genetic influence on disease susceptibility.

Thyrogastric autoimmunity genes are another genetic system of interest in patients with IDD. For example, thyroid microsomal and gastric parietal cell antibodies occur in IDD patients at a rate four to six times

that of nondiabetic controls matched for age, sex, and race. Although the incidence of thyroid and gastric autoantibodies increases dramatically with age in the general population, such incidence in children with IDD is equivalent to that in the geriatric general population. A greater prevalence of these autoantibodies are also found in nondiabetic parents of children with IDD. If the dominantly inherited tendency for production of thyroid and gastric autoimmunities is separate from the major genetic influence for IDD susceptibility, then thyrogastric autoimmunity must predispose to IDD in its own right.

Indeed, data gathered by Riley et al have confirmed a report by Cudworth et al that thyroid and gastric autoantibodies in families with IDD do not segregate with human leukocyte antigen (HLA) haplotypes. In contrast, HLA haplotypes do segregate with the occurrence of IDD in multiplex pedigrees. Thus, thyrogastric autoantibody genes are distinct from those for IDD and may also convey genetic susceptibility to IDD. IgG heavy-chain allotypes may also influence the occurrence of IDD, as documented in Graves' disease.

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# The Genetics of Insulin-Dependent Diabetes

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## HLA-Associated Inherited Susceptibility

The above factors notwithstanding, the major genetic factor in the inherited susceptibility to IDD is that associated with the HLA system. A primary association of IDD with HLA-DR3 and HLA-DR4 has been reported by a large number of investigators. In our experience, only 5% of children and young adults with IDD lack one or both of these antigens, whereas approximately 40% of patients have both antigens—i.e., are DR3/DR4 heterozygotes. However, HLA typing of random individuals in the general population provides little information that would help to identify individuals at risk for development of IDD.

As shown in the table, the absolute risk for developing IDD, as determined in collaboration with Rotter et al, is no higher than 1 in 40 for the highest-risk HLA phenotype, the DR3/DR4 heterozygote. HLA-DR2 and HLA-DR5 are infrequently found in patients with IDD, and are thus associated with low absolute risk (about 1 in 2,500) for IDD.

When the absolute risk for IDD is applied to family members of a diabetic proband, the sharing of HLA haplotypes containing the IDD-associated DR3 or DR4 alleles conveys greatly increased risk for IDD. For an HLA-identical sibling of a diabetic proband, the absolute risk for IDD is approximately 1 in 7; it rises to 1 in 4 if the shared haplotypes contain both DR3 and DR4 antigens. This risk is ten times greater than that for persons in the general population who are also DR3/DR4 heterozygotes. This implies that HLA haplotypes containing DR3 or DR4 associated with observed IDD are different from those seen in the non-diabetic general population. This conclusion is reinforced by the fact that the frequencies of particular alleles at other polymorphic loci on chromosome six are shown to be in linkage disequilibrium with DR3 and DR4. Thus, A2, Cw3, and Bw62 are in linkage disequilibrium with DR4, since these particular alleles of the A, C, and B loci are found to be associated more frequently with DR4 than would be expected. This extended haplotype is present at an increased rate in IDD patients, and

**Absolute Risks for IDD for Caucasian Persons of Various HLA Phenotypes and Genotypes (Based on an IDD Prevalence Rate of 1 in 500)**

| HLA Phenotype |            | HLA Genotype |            |
|---------------|------------|--------------|------------|
| DR1           | 1 in 1,000 | DR3/DR3      | 1 in 125   |
| DR2           | 1 in 2,500 | DR3/DRX      | 1 in 500   |
| DR3           | 1 in 185   | DR4/DR4      | 1 in 147   |
| DR4           | 1 in 208   | DR4/DRX      | 1 in 476   |
| DR5           | 1 in 2,500 | DR3/DR4      | 1 in 42    |
| DR6           | 1 in 1,429 | DRX/DRX      | 1 in 5,565 |
| DR7           | 1 in 1,250 |              |            |
| DR8           | 1 in 556   |              |            |
| DR9           | 1 in 345   |              |            |

is much less common among DR4-positive individuals in the general population.

In addition, this haplotype and other extended haplotypes associated with IDD may involve particular complement allotypes. In our observations of more than 1,000 patients with IDD, we have found a distinct excess of DR1-positive patients among the 5% of IDD patients who lack both DR3 and DR4 antigens. This is consistent with the idea that the HLA-associated IDD susceptibility gene or genes are separate from DR, but are in linkage with DR3, DR4, and—in some instances—DR1. [Autoimmune Addison's disease occurring as part of the Type II autoimmune polyglandular syndrome (Schmidt's syndrome) is also associated with DR3 and DR4 antigens, although it appears to be unrelated to HLA when it occurs as part of Type I autoimmune polyglandular syndrome (moniliasis/hypoparathyroidism).]

## Predictive Value of HLA Typing

HLA typing of individuals at random gives little information to predict latent IDD, although certain HLA-DR phenotypes, such as DR2/DR2, DR2/DR6, DR2/DR5, DR5/DR5, and DR5/DR6, are very rarely associated with IDD. However, HLA typing in pedigrees affected by IDD can be valuable in identifying at-risk individuals according to the number of haplotypes shared with the diabetic proband. Since ICA has been shown to be a useful marker for IDD, it was of interest to learn whether the occurrence of ICA is restricted to those with inherited susceptibility for IDD. In our studies, such was the case. ICA among siblings of dia-

betic probands was limited to those sharing HLA haplotypes, being most common among the HLA-identical siblings and having an occurrence rate similar to that of IDD. Furthermore, ICA in the general population was restricted to those with HLA phenotypes containing DR3 and/or DR4, the IDD risk alleles. Therefore, in persons with ICA, HLA typing does not significantly add to the predictive value of this marker.

## Male Segregation Bias and Extended HLA Haplotypes

Warram recognized that fathers with IDD were some five times more likely than mothers with IDD to produce children of either sex with IDD. This remarkable finding has been confirmed by many others. In our studies, Vadheim has shown that HLA haplotypes bearing DR4 are preferentially passed on to offspring from fathers of children with IDD; this was not the case for the mothers. This implies that the "diabetes-prone" DR4-bearing HLA haplotypes from fathers might enhance the chance of conception or, alternatively, convey some survival advantage to the fetus in utero.

Alper has shown that extended haplotypes involving HLA-A, C, B, complement Bf, C2 and C4, and HLA-D loci, and even the more-centromeric erythrocyte glycoylase locus (GLO-1), participate in the HLA-associated susceptibility to IDD. Such extended haplotypes in linkage disequilibrium may be unusually resistant to recombinant events. However, the reasons for this and the implications for genetic susceptibility to IDD are not yet clear.

## Molecular Genetics of the HLA Complex

The class II antigens of the major histocompatibility complex are heterodimeric cell surface glycoproteins involving relatively conserved  $\alpha$  chains and more variable  $\beta$  chains. In man, the three major class II antigens have been identified as DR, DQ, and DP. The DQ loci are closely situated to DR; the DP loci are more-centromeric loci on the short arm of chromosome six (Figure 1). The DR cluster contains one, possibly two,  $\alpha$  and three  $\beta$  genes. One of these  $\beta$  genes may be a pseudogene and is therefore not expressed. There are two  $\alpha$ - and two  $\beta$ -coding DQ genes and two  $\alpha$  and two  $\beta$  DP genes.

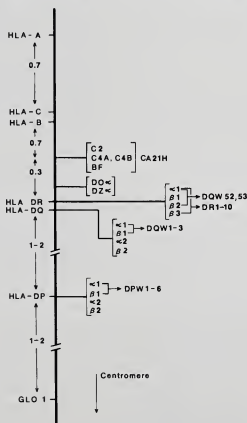
Whereas the DR antigens (DR1-10 and DRw52/w53) and the DQ antigens (DQw1-3) can be determined by serological typing (using maternal-derived alloantisera), DP typing can be performed only by using mixed lymphocyte reactions

or molecular biologic techniques. HLA is currently being studied at the genomic level through restriction fragment length polymorphism (RFLP) analysis of all of the HLA-D genes. This technique involves harvesting DNA from peripheral blood or from lymphoblastoid cell lines established by transformation of peripheral B lymphocytes with Epstein-Barr virus. The genomic DNA is digested with restriction endonucleases, which cleave the DNA at sites of specific base sequences; the resulting fragments are separated by size using agarose gel electrophoresis. Using the Southern blotting method, the DNA is transferred to nylon filters, which are then hybridized with  $^{32}\text{P}$ -labeled DNA probes (cDNA or genomic types) to identify the genes of interest. Using such an approach with DR $\alpha$  and DR $\beta$  probes, Erlich has identified RFLPs that differentiate DR3 haplotypes involving A1 1B8 DR3 haplotypes in diabetes-prone individuals

from other haplotypes.

Other investigators, including those in our laboratory, have concentrated on DQ $\beta$  and DQ $\alpha$  RFLPs associated with DR4. To date, DR and DQ RFLP analysis has identified HLA haplotypes that are more likely to convey inherited susceptibility to IDD than could be determined by serological typing for DR antigens alone (Figure 2). An absolute relationship between specific RFLPs of class II genes and IDD has not yet been found. However, as reported by Owerback et al, a 3.7 kb RFLP, seen when genomic DNA is digested with the restriction endonuclease Bam H1 and probed with a DQ $\beta$  cDNA, is infrequently found in DNA from IDD patients. To date, we have found this fragment to be absent on both DR4-bearing chromosomes in DR4/DR4 IDD patients. This implies that only DR4-bearing haplotypes that lack the 3.7 kb DQ $\beta$  RFLP convey susceptibility to IDD. The site and nature of the HLA-associated IDD susceptibility gene or genes has yet to be identified.

The HLA Region on the Short Arm of Chromosome Six



**Figure 1.** The map of the greater HLA genomic region is depicted. HLA-D molecules are heterodimers of  $\alpha$  and  $\beta$  chains. Transcomplementation products combining an  $\alpha$  chain coded by one chromosome with a  $\beta$  chain of the same antigen class from the other chromosome—as well as combinations of  $\alpha$  and  $\beta$  chains of a single chromosome—occur at each locus.

## Insulin Gene Polymorphism

The 5' flanking region of the human insulin gene is extremely polymorphic because of variable numbers of tandem repeats of 14-15 base pair oligonucleotide sequences. Although three classes of alleles have been defined, there is great variation within each class and thus there are many unique alleles. Interest in this region grew rapidly once its polymorphic nature was identified by Bell, because diabetes theoretically could result from defective regulation of insulin gene transcription. To date, non-IDD, atherosclerosis, hyperlipidemia, and IDD have all been reported to have associations with particular haplotypes, although these issues remain controversial. In our laboratory, Winter has identified a similar, but much abbreviated, polymorphism in the 5' flanking region of the insulin I gene in rats. Unlike man, rodents have two insulin genes. The insulin II gene is analogous to that in man. The spontaneously diabetic Biobreeding rat (which inherits diabetes as a recessive trait, according to Colle) has both IA and IB RFLP alleles in roughly equal numbers. However, IDD is not related to either allele.

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# The Genetics of Insulin-Dependent Diabetes

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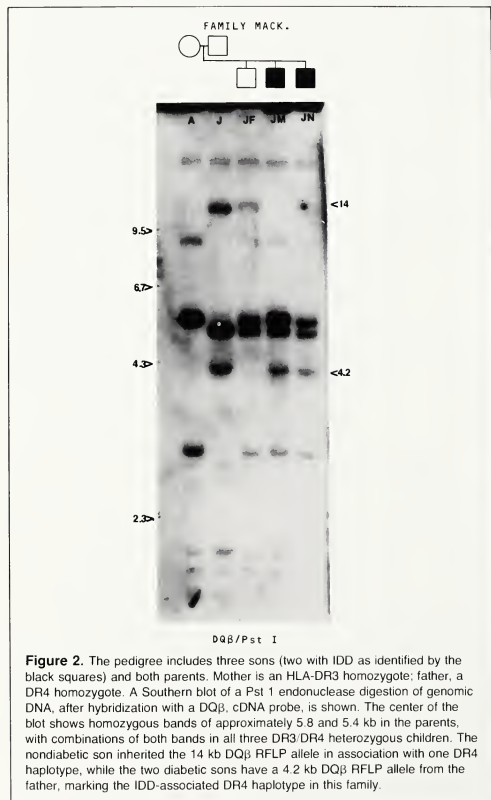
## Summary

Despite a growing body of literature on the genetics of IDD, the inherited predisposition to the disease cannot yet be identified with any great precision. There is at least one major susceptibility gene within the HLA-D region of chromosome six that is still to be mapped. There are other mi-

nor genes—those involving thyrogastric autoimmunities, male gender, insulin gene polymorphisms, and Ig heavy and light chain allotypes—that may affect the outcome. Immunoregulatory genes of the T-lymphocyte receptor will soon be subjected to study. In addition to these genetic influences, there is evidence that an environmental trigger also may be necessary for IDD to develop. It is anticipated that genetic and environmental interactions underlying IDD will soon be unraveled and translated into methods that will prevent disease.

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References will be provided upon request to Dr. Blizzard.



**Figure 2.** The pedigree includes three sons (two with IDD as identified by the black squares) and both parents. Mother is an HLA-DR3 homozygote; father, a DR4 homozygote. A Southern blot of a Pst 1 endonuclease digestion of genomic DNA, after hybridization with a DQB<sub>1</sub> cDNA probe, is shown. The center of the blot shows homozygous bands of approximately 5.8 and 5.4 kb in the parents, with combinations of both bands in all three DR3/DR4 heterozygous children. The nondiabetic son inherited the 14 kb DQB<sub>1</sub> RFLP allele in association with one DR4 haplotype, while the two diabetic sons have a 4.2 kb DQB<sub>1</sub> RFLP allele from the father, marking the IDD-associated DR4 haplotype in this family.

## In Future Issues

Hypophosphatemic Hyperphosphaturic Rickets: An Update by Harold Harrison, M.D.

Fetal Growth and Growth Factors by Joseph D'Ercole, M.D.

The Relationship Between Endurance-Type Training and Adolescent Development by Alan D. Rogol, M.D., Ph.D.

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# Diabetes Control and Growth Hormone: New Insights

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Type I diabetes is characterized by a variety of metabolic and hormonal abnormalities in addition to hyperglycemia. Elevations in plasma growth hormone (GH) levels frequently have been observed in Type I diabetics, but the mechanisms by which these elevations occur have been difficult to establish. Arguments that GH hypersecretion plays a role in perpetuating the metabolic derangements of diabetes have waxed and waned in popularity. The introduction of intensive insulin treatment regimens and other new *in vivo* techniques has provided considerable information about the complex interrelationships between metabolic control of diabetes and GH secretion and action. Studies in these areas have produced results that have implications concerning GH regulation in nondiabetic subjects as well.

## **Influence of Metabolic Control on GH Secretion**

Hansen was the first to show reversibility of GH hypersecretion in response to exercise by intensive treatment with multiple injections of insulin. Similarly, basal and post-exercise GH concentrations have been restored to normal following continuous subcutaneous insulin infusion (CSII) treatment, which also normalized the elevated mean 24-hour GH concentrations.

Several studies have tried to ascertain the level at which diabetes affects central regulation of GH secretion. Although there is little evidence to suggest that metabolic control of diabetes directly influences the pituitary, indirect effects have been demonstrated. For example, the pituitary response to

stimulation with GH-releasing factor is normal when diabetics are hyperglycemic, but not when they have normal plasma glucose concentrations. On the other hand, hypersecretion of GH has been observed in poorly controlled patients in response to several stimuli, such as arginine, L-dopa, and clonidine, that are thought to act through the hypothalamus. Furthermore, improved control with CSII reduces to normal the GH response to clonidine (an  $\alpha_2$ -adrenergic agonist), but does not affect the pituitary response to GH-releasing factor.

Deranged hypothalamic regulation of GH secretion in diabetes may be more selective than the above might indicate. For example, Simonson et al used a modification of the insulin clamp procedure to study the effects of improved metabolic control with CSII on the GH response to hypoglycemia, the classical hypothalamic stimulus. Surprisingly, the GH response to a lowering of plasma glucose from 90 to 50 mg/dL was normal when the patients were poorly controlled and suppressed to subnormal values after eight months on CSII.

## **Influence of Metabolic Control on Insulin-Like Growth Factors**

Because GH levels tend to be increased rather than reduced in poorly controlled diabetes, investigators began to look for possible diabetes-induced defects in other circulating growth factors, particularly insulin-like growth factor I (IGF-I). Early studies in diabetics receiving conventional treatment yielded contradictory results, with normal, elevated, and reduced levels of IGF-I being reported. These discrepancies may have resulted from differences in methodologies (eg, bioassay *v* radioimmunoassay), variability in the level of diabetic control, and failure to account for the increase in IGF-I that accompanies normal pubertal development. Thus, it is noteworthy that Blethen et al recently found a negative correlation between glycosylated hemoglobin and age-adjusted IGF-I values in conventionally treated adolescents with diabetes.

Recent studies of CSII have clearly demonstrated that inadequate insulin replacement results in a defect in IGF-I synthesis. Not only were IGF-I levels reduced in poorly controlled diabetic patients, as compared with age- and sex-matched normal controls, but IGF-I levels increased by 25% after only one week of CSII despite a fall in GH values. A further increase in IGF-I concentrations is observed with more prolonged improvement in metabolic control. Lanes et al showed that IGF-I response to exogenous GH administration was blunted in poorly controlled diabetics when compared to the response in relatively well-controlled subjects.

In contrast, metabolic control of diabetes appears to have little effect on IGF-II values. IGF-II levels have been reported by Amiel et al to be normal in both adults and adolescents with diabetes, and no change in mean values was noted after CSII. However, in the latter study, four of 19 patients with very low IGF-I levels also had depressed IGF-II concentrations (which returned to normal with improved metabolic control). Thus, except in the most severely affected patients, compensatory increases in GH appear to be sufficient to maintain adequate IGF-II production.

## **Influence of GH on Metabolic Control**

Hypersecretion of GH may also help perpetuate the metabolic derangements of diabetes to a much greater extent than is generally appreciated. In a recent study, Press et al administered exogenous GH as hourly intravenous pulses to a group of diabetics who were optimally controlled with CSII. Prior to GH administration, mean 24-hour plasma glucose and GH levels were within the normal range. However, when serum GH was raised to levels seen in poorly controlled patients, a progressive rise in plasma glucose was observed. Surprisingly, the hyperglycemia was primarily due to a marked stimulation of hepatic glucose production. GH is usually thought to increase glucose concentrations in diabetics, as in non-

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# Diabetes Control and Growth Hormone: New Insights

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diabetics, by inhibiting glucose uptake in peripheral insulin-sensitive tissues.

The adverse metabolic effects of GH demonstrated in this study were not confined to plasma glucose levels. Levels of circulating fatty acids, ketones, and branched-chain amino acids were also increased. Therefore, GH elevations can themselves produce the entire spectrum of abnormalities associated with poor diabetic control, despite previously optimized insulin treatment. It follows that some of the metabolic benefits of more intensive insulin regimens may be derived from GH-lowering effects.

The "dawn" phenomenon is a significant cause of glycemic lability in insulin-dependent diabetics. Plasma glucose and basal insulin requirements vary considerably during the night. Both reach a nadir between 2 and 4 AM and then rise together as daylight approaches. It was originally thought that these changes reflected diurnal fluctuations in plasma cortisol. However, a delayed anti-insulin effect exerted by the early nocturnal surges of GH may be a more likely explanation; however, not all investigators agree with this concept.

Diabetes in adolescents is particularly difficult to control. Although control problems are usually attributed to psychosocial and dietary factors, the hormonal changes of puberty might also play a role. If such is the case, the puberty-associated rise in GH would be expected to be a contributing factor. In a recent study, insulin sensitivity was determined in preadolescents and adolescents, with and without diabetes, using the euglycemic, hy-

perinsulinemic insulin clamp technique. It should be noted that insulin-mediated glucose metabolism was reduced in diabetic patients and healthy children with the onset of puberty. Furthermore, the degree of insulin resistance was directly correlated with mean 24-hour GH concentrations.

## Influence of Metabolic Control on Linear Growth

The observation that most conventionally treated diabetic children appear to be growing at a normal rate despite a host of metabolic and hormonal derangements is a testament to compensatory mechanisms that help sustain growth. However, Tattersall and Pyke found that patients who had developed diabetes before puberty were shorter as adults than their non-diabetic identical twins. The view that diabetic children may not be achieving their full growth potential is supported by studies that employ intensive treatment. Adolescents with diabetes—even those with normal stature and apparently normal growth rates on conventional therapy—show a sharp increase in growth velocity during treatment with either CSII or multiple injections.

Although it is attractive to speculate that the increased growth seen in well-controlled adolescents is the result of optimized therapy, such an interpretation is limited by difficulties in assessing growth velocity changes during puberty. To examine this further, we used a clonal stem cell assay for proliferation of erythroid progenitors (burst forming units-erythroid [BFU-E]) to determine the effect of CSII on cellular

growth *in vitro*. This assay system has been useful in assessing other causes of growth retardation. Blood was obtained from eight diabetic patients before and after one week of CSII. Numbers of BFU-E-derived colonies were not different from normal during conventional treatment, but increased sharply after one week of CSII. These changes in the *in vitro* cellular growth are strikingly similar to the long-term effects of intensive treatment on linear growth in adolescents. The ability to detect very rapid changes in cellular growth with the BFU-E assay illustrates the primary importance of improvements in fuel metabolism.

## Summary

Viewed together, the observations presented in this article have important clinical implications. In the poorly controlled diabetic patient, a vicious cycle—whereby hypersecretion of GH acts as a compensatory response to a reduction in IGF-I—may become established. The associated rise in GH causes a worsening of metabolic control, further impairing the somatotropic action of GH. The efficacy of intensive treatment may be due to an interruption of this cycle that lowers circulating GH concentrations. Normalization of hormonal milieu and improvement in growth rates are associated with good diabetic control. Consequently, physicians should make every reasonable effort to normalize glucose metabolism in juvenile diabetic patients. For those interested in reading further about the role of insulin in growth, an abstract entitled "Insulin as a Growth Factor" appears on this page.

References are available upon request to Dr. Blizzard.

## Insulin as a Growth Factor

In their review, Hill and Milner discuss various aspects of insulin's capability to promote growth (see related article by Tamborlane and Amiel on page 5 of this issue).

Insulin does influence *in vivo* growth. Although growth hormone (GH) is largely responsible for the rise and fall of insulin-like growth factor I (IGF-I), other factors also play a role, including insulin itself.

Disordered growth consequent to insulin dysfunction is frequently associated with a parallel change in circulating levels of IGF, suggesting a direct or indirect modulation of IGF production by insulin.

The similarity in structure between the IGFs and insulin allows low affinity binding between insulin and IGF receptors and vice versa. There are three individual receptors: one for

the action of insulin, one for IGF-I, and one for IGF-II. When stimulated, each of these is capable of inducing mitogenesis, and each is capable of accepting the other two growth factors if it is not already "occupied" by its primary growth factor. The authors emphasize that insulin, therefore, may exert direct mitogenic action—and consequently, growth—through either insulin or IGF recep-

tors. Their paper reviews experimental evidence (and draws some clinical parallels) in support of the concept that insulin has both direct and indirect roles in the control of normal body growth.

Insulin, in addition to its other actions, acts as a necessary mediator of IGF-I generation. Several mechanisms are possible. In rats, a decrease in the amplitude of GH peaks has been observed in the absence of insulin. However, GH administration does not stimulate IGF-I levels. Another probable mechanism is via a direct modulation of IGF release from the liver to other tissues. Insulin also may mediate IGF release indirectly by altering the GH/IGF axis, since there appears to be a severe reduction in the number of GH receptors in the liver of ketotic diabetic rats. Interestingly, in other studies cited by the authors, serum levels of IGF-I have increased and growth of the tibial epiphyses has occurred in hypophysectomized rats (GH deficient) whose pancreases were stimulated to release insulin by the administration of a sulfonylurea. This clear demonstration that insulin could modulate IGF release independent of GH indicates what may be an important mechanism for the growth retardation seen in experimental diabetes.

Another example of the important role of insulin in growth is the presence of inhibitory factors that oppose the action of both IGF and insulin in animals with chemically induced diabetes. As a consequence, tissue anabolism is impaired. The inhibitor is not specific to insulin and insulin-like peptides, but has a general depressive action on all aspects of cartilage metabolism. In the diabetic rat, this factor appears to originate in the liver.

Insulin dysfunction and postnatal and prenatal growth disorders are considered by the authors. Diabetes in childhood is the most common clinical example of disturbed growth due to abnormal insulin secretion. Growth may be subnormal for months before the diabetes is clinically manifested, and treatment is closely linked to the quality of diabetic control. Mauriac syndrome (in which the child is short, obese, and has a large liver with fatty infiltrations) results from excess dietary carbohydrate coupled with excess insulin and brittle control. The au-

thors cite a study by Winter et al of a 7-year-old with normal GH release in response to insulin hypoglycemia but with very low IGF-I values. However, IGF rose on each of two occasions when metabolic control was improved. Winter et al speculated that a block in the GH/IGF axis existed in poorly controlled diabetics since IGF-I did not rise with GH administration in this patient during periods of poor control.

Other studies from the literature cast further light on the relationship of diabetes and growth. One found that adult diabetics are consistently shorter than their identical but nondiabetic twins. Another found an inverse relationship between IGF-I and HbA<sub>1c</sub> in 40 diabetic children. Still another series found that GH values were higher than expected in diabetics with normal IGF-I values, suggesting a blunted response of IGF to GH concentrations.

The authors cite a study conducted by Rudolf, Tamborlane et al on the growth potential of children with relatively well-controlled diabetes (they measured growth velocity in nine insulin-dependent children before and after six months of intensive insulin treatment via pumps or multiple injections). During conventional therapy (injections of insulin once or twice daily), the mean growth velocity was 5.3 cm/yr, a rate within the low normal range, despite evidence of intermittent hyperglycemia. After a period of intensive management, in which the overall dose of insulin was not increased, mean plasma glucose fell from 270 to 105 mg/dl, and glycosylated hemoglobin fell from 12.4% to 8.4%; the mean growth velocity increased sharply to 9.4 cm/yr as the serum IGF-I level doubled. The rate of skeletal maturation did not increase. The conclusion was that improved metabolic control, even for children who were not obviously short, could substantially increase adult height potential.

A follow-up study examined the circulating IGF-I and II levels in diabetic children by specific radioimmunoassays. During conventional therapy, IGF-I was lower, but IGF-II was generally unaltered in 19 insulin-dependent diabetics as compared with nondiabetic controls. Following one week of intensive insulin therapy, IGF-I values increased by 25% despite a decrease

in the mean 24-hour levels of GH. Circulating IGF-II did not alter during intensive therapy. This study provided further evidence that the normal control of IGF-I by GH is disrupted in poorly controlled diabetics, and that this can be partially corrected by improved metabolic control. In contrast, endogenous hyperinsulinemia in childhood is not associated with a serious disturbance of growth. Blethen et al described seven children, less than 3 years of age, who had severe fasting hypoglycemia due to hyperinsulinemia. Neither IGF-I nor IGF-II differed from the values for age-matched control children.

It has been reported that cultured skin fibroblasts from patients with insulin- or non-insulin-dependent diabetes show increased sensitivity to insulin. Indeed, cells from diabetic patients were more sensitive to insulin than those from nondiabetics with respect to collagen synthesis. This may shed some light on the etiology of macroangiopathy in diabetes, since collagen comprises more than half the total protein present in human atherosclerotic plaques. Fibrous deposition in diabetics may originate from smooth muscle cells that proliferate in the subintima and deposit forms of collagen that are chemically distinct from those found in normal subjects. Insulin-dependent diabetics with atherosclerosis were also found to have higher circulating insulin levels than those without diabetes.

Leprechaunism, a rare dwarfing phenomenon seen in the neonate, is characterized by insulin resistance, poor stores of subcutaneous tissue, and intrauterine growth retardation. It is associated with various defects of the insulin receptor, including the absence of insulin receptors in some patients and postreceptor defects in others. Hill and Milner emphasize that insulin resistance at either a receptor or postreceptor site is seldom isolated from a resistance to the biologic actions of the other peptide growth factors, resulting in intracellular malnutrition, impaired growth, and, in many cases, early death.

In utero defects of insulin secretion and utilization are seen in syndromes other than leprechaunism. For example, the infant of the diabetic mother whose glucose me-

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### Insulin as a Growth Factor

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tabolism is poorly controlled is obese and often has visceromegaly. Although insulin is present in the human fetal pancreas as early as the tenth week of gestation, insulin release remains insensitive to glucose until the gestational age of approximately 28 weeks, at which time the preadipocyte matures into an insulin-sensitive cell capable of accumulating lipid. Most of the excess weight seen in the infant of a diabetic mother is fat accumulated during the last trimester of pregnancy. The less dramatic but unequivocal increase in somatic growth that occurs concurrently suggests that insulin has an additional direct or indirect role in protein synthesis and cellular proliferation. Enhanced fetal somatic development has been described in infants with nesidioblastosis or the Beckwith-Wiedemann syndrome, each of which is associated with hypersecretion of insulin. Conversely, in transient neonatal diabetes and in pancreatic agenesis, the newborn is characteristically small-for-dates, has poor muscle bulk, and has virtually no adipose tissue.

There are several pathways by which insulin can act as a fetal growth factor. First, it may alter cellular nutrition by increasing nutrient uptake and utilization. Second, insulin may exert a direct anabolic action via either the insulin or the IGF-I receptor. Third, insulin may modulate the release of IGF or other growth factors from fetal tissues. Although the authors found no direct mitogenic action of insulin on human fetal fibroblasts or myoblasts obtained from fetuses at less than 20 weeks' gestation, it is conceivable that insulin may exert a direct growth-promoting action during

later fetal development. Since the insulin receptor population may be abnormally elevated in some tissues of infants born to diabetic mothers, one can postulate that this, coupled with hyperinsulinemia, may result in a direct, pathophysiologic stimulation of human fetal somatic and skeletal growth.

Based on the experimental and clinical data regarding the endocrinology of the overgrowth seen in infants of diabetic mothers, two deductions seem reasonable: (1) Body length is increased slightly, if at all, even in the presence of extremely high insulin levels and a raised IGF level, suggesting that normal fetal growth is taking place close to its maximum potential; (2) modest hyperinsulinemia can result in organomegaly and obesity despite normal circulating IGF values. These effects appear to be due to either direct anabolic and lipogenic actions of insulin or to another, as yet unidentified, mediator.

The parallel changes in serum insulin and IGF levels, especially those seen in fetal growth retardation, suggest that some of the anabolic actions of insulin *in utero* may be mediated by a change in IGF release. In the fetuses of many species, including humans, body growth and circulating IGF levels do not depend on the presence of pituitary GH; in fact, growth persists after experimental decapitation in the rabbit or hypophysectomy in the sheep. The immaturity of the GH/IGF axis may be related to the observations that somatotrophic receptors do not appear in the liver of the sheep or rat until after birth. Any prenatal regulation of IGF release by insulin is therefore unlikely to be mediated by changes in GH secretion or by changes in the nature of the GH receptors.

The authors conclude that insulin functions as a growth factor at the cellular level and within the whole body. Yet, for many tissues, insulin does not appear to be the major circulating anabolic agent. The secondary position of insulin in the endocrine control of mammalian growth may derive from a diversification of biological function among the insulin-related family of molecules. In most mammalian species, the IGFs, and predominantly IGF-I, have evolved as the more potent mitogenic peptides while insulin fulfills a more acute metabolic function. Similarly, the IGF-I receptor, rather than the related insulin receptor, has become the most utilized initiator of a positive pleiotropic response. However, this is a gross generalization and, for particular tissues, such as the liver, insulin still may act as a potent mitogen via the insulin receptor. In addition, insulin may continue to exert control of the development of skeletal tissues, in association with intracellular nutrition, by regulating IGF release. Pathophysiologically, insulin may assume the role of a major growth-promoting agent if overproduction is associated with extensive binding to the IGF-I receptor, as may occur in the infant of the diabetic mother.

Hill DJ, Milner RDG: *Pediatr Res* 1985;19:879.

**Editor's comment**—The authors present an outstanding and complete review of insulin as a growth factor. This abstract discusses only a minor portion of the material covered, and the editor encourages all readers to review the article in its entirety.

### The Short Child With Subnormal Plasma Somatomedin-C (Sm-C)

The somatomedin-C (Sm-C), or insulin-like growth factor I (IGF-I), level is being used as a screening test for growth hormone deficiency (GHD). To evaluate its diagnostic value, the authors designed a protocol to evaluate: (1) the statistical tolerance limits for Sm-C in children of normal height

between 7 and 10 years of age; (2) the prevalence of subnormal Sm-C in children of the same age who are below the third percentile in height; (3) the prevalence of GHD in children with low Sm-C levels; and (4) the comparison of linear growth responses to hGH treatment between GHD children and hypsomatomedinemic, non-GHD short children.

Single Sm-C determinations were reported to be of limited value in diagnosing GHD. Only with an average of four determinations (taken at six-week intervals) in seven GHD children could all seven be said to have an average Sm-C level below the 95% lower limit of the tolerance intervals, as based on the mean of one, two, three, or four determinations.

In 97 short non-GHD patients whose Sm-C levels were measured

four times and then averaged, 45% (or 44 children) were below the 2.5 percentile established for normal children. Of these 44 children, who were considered to be hyposomatomedinemic, 12 were classified as GHD, seven as partially GHD, 20 as non-GHD, and five as intermediate in their responses or non-classifiable, as determined by the usual pharmacologic testing. Therefore, 19 of the 44 (or 43% of the hyposomatomedinemic children) and 19 (or 20%) of the 97 children with heights below the third percentile had some degree of diagnosable GHD. The anthropometric measurements and skeletal ages in relation to the chronological ages were identical in the 19 GHD and 20 non-GHD patients, as were the levels of Sm-C. Consequently, the authors deduce that approximately 20% of short children referred to them will be GHD.

Therapy with hGH was given to the GHD and non-GHD hyposomatomedinemic children for six-month alternating periods. During each period, one of four logarithmic dosages were administered: 0.16, 0.26, 0.43, or 0.70 U/kg/wk were given in equally divided doses Monday, Wednesday, and Friday at

10 PM. The results are shown in the table.

The authors report that the two intermediate doses produced significantly different growth rates in the two treatment groups. However, the largest dose produced comparable growth rates in both groups.

The authors speculate that there are several possible explanations for the low Sm-C determinations in the short non-GHD children, including: (1) a relationship to the delayed skeletal maturation, since Sm-C levels increase with age; (2) failure of nocturnal secretion of hGH; (3) impaired production of Sm-C; (4) a bioinactive GH; and (5) an altered Sm-C binding system.

The authors also speculate that non-GHD children will respond to GH therapy in many instances. At conventional GH doses (up to 0.43 U/kg/wk), the magnitude of the response seen in such children was less than 60% as great as that of their GHD counterparts; however, at the dose of 0.70 U/kg/wk, the responses of the four GHD children and the three non-GHD children were comparable.

Rudman D, Kutner MH, Chawla RK: *Pediatr Res* 1985;19:975.

## Behavioral Problems and Social Competence in Girls With True Precocious Puberty (TPP)

The authors evaluated 33 girls between 6 and 11 years of age with true precocious puberty (TPP) of various etiologies. At the time of presentation, 55% were above the 95th percentile for height-for-age; bone age was advanced by two to five years in all subjects. Before treatment, the parent(s) completed a 120-item child behavior checklist, from which a child behavior profile was generated. It consisted of three social competence scales, nine behavior problem scales, and two second-order factors (internalizing or externalizing scales). The personality profiles were compared with those of matched controls, and appropriate statistical data were extracted.

Many, but not all, of the girls were reported to have behavior problems. For example, 27% had a total behavior problem score at or above the 98th percentile for normals and many scored significantly higher than controls in all of the internalizing factors—eg, depression, social withdrawal (45% >97th percentile), somatic complaints (30%), and schizoid/obsessive traits. The incidence of hyperactivity and aggressiveness was significantly higher in TPP patients than in controls. The authors considered whether all these increases could be related to the expected changes of behavior that occur in adolescence and determined that such was not the case.

Other behavioral traits that were frequently observed in these girls included clinging to adults, feelings of worthlessness, sulking, fatigue, strange or unpredictable behavior, inability to sit still, daydreaming, crying, teasing, temper tantrums, and whining. They also tended to sleep less than most children.

Overall, the girls with TPP could be described as troubled, depressed, aggressive, socially withdrawn, and moody. The authors emphasize, however, that to view these children as psychiatrically disturbed and/or in need of psychiatric treatment is to misinterpret the findings. The behavioral "breakdown" reported may reflect the

*continued on p. 10*

| Dose hGH<br>(U/kg/wk) | Increase in growth velocity |                       |
|-----------------------|-----------------------------|-----------------------|
|                       | GHD                         | Non-GHD               |
| 0.16                  | 4.4 ± 0.7<br>(n = 8)        | 0.2<br>(n = 1)        |
| 0.26                  | 7.4 ± 1.2<br>(n = 9)        | 3.2 ± 0.7<br>(n = 16) |
| 0.43                  | 8.7 ± 0.9<br>(n = 12)       | 5.4 ± 0.7<br>(n = 12) |
| 0.70                  | 8.3 ± 1.1<br>(n = 4)        | 7.3 ± 2.0<br>(n = 3)  |

**Editor's comment**—Although the authors state that four Sm-C determinations were necessary to unequivocally diagnose GHD in the seven GHD patients studied for this purpose, a review of the data of the 28 determinations made in these patients reveals that only two determinations were greater than 0.30 U/ml. Therefore, 26 of the 28 determinations yielded values that were certainly compatible with GHD, although not diagnostic thereof. In

addition, the finding of a low Sm-C (<0.30 U/ml) by averaging four determinations in a short child would be associated with the diagnosis of GHD in only 40% of cases (19 of 44 children in this series). Therefore, the practicing physician can still effectively utilize a single Sm-C determination in evaluating the possibility of GHD. It should be noted, however, that the Sm-C determination is only one facet of the diagnostic evaluation of a short child.

**True Precocious Puberty***continued from p. 9*

mechanisms for maintaining homeostasis in an abnormal environment. These children need to cope with an age-appearance disparity that modifies the response of their social milieu. Adults expect children to perform tasks that are commensurate with height age. Consequently, these children may have an abnormal body image, lack self-confidence, or prefer to be by themselves. Their social withdrawal may well be related to the disparity of age and appearance and expected social behavior.

Sonis WA, Comite F, Blue J, et al: *J Peds* 1985;106:156.

**Editor's comment**—In their summary, the authors emphasize that a majority of the girls in their series did not have behavioral problems, although a significant and large minority did have a dysphoric and stressful adjustment.

Relevant to this report is one by Ehrhardt et al (*J Am Acad Child Psychiatry* 1984;23:1), entitled "Idiopathic Precocious Puberty in Girls: Psychiatric Follow-Up in Adolescence." This was a systematic, controlled study of psychopathology in 16 adolescent girls between 12 and 13 years of age, signif-

icantly older than the patients studied by Sonis et al. The average height (160.7 cm) was below the average height (166.4 cm) of the controls, as would be expected in females with a history of sexual precocity. Patients and controls were similar regarding various aspects of self-image, except for marginal differences in morals and sexual attitudes. The patients had a somewhat less positive attitude toward sexuality, rating having a boyfriend as far less important than did the controls. There were also marginal differences in intellectual and school status, with decreased popularity and less anxiety being associated with the patients. Conduct problems, antisocial behavior, inadequacy or immaturity, and socialized delinquency were marginally increased in the patients.

It is important to note that both sets of authors stressed that an increased incidence of definitive psychiatric disorders was not found. Both sets also emphasized the probability that the psychosocial concomitants of TPP, especially the reactions of families and peers, contribute to the behavioral outcome.

Both of these articles prompt the editor to recommend that psychologists, psychiatrists, or others with special expertise in TPP closely monitor and counsel patients with sexual precocity.

saline, no GH level increases were seen during saline infusion; in the other two, only minor increments were observed. Long-term GHRF infusion in the six patients significantly increased GH secretion. Four to 13 pulses were detected during sleep while the GHRF was being infused. The highest peaks varied from 3.5 to 10.3 ng/ml and the integrated GH secretions ranged from 13.1 to 40.2 ng/ml/h with a mean of 22.5 ng/ml. The subsequent bolus injections of GHRF induced GH increases in all ten patients. The peak levels observed in the patients after saline varied between 1.0 and 5.6 ng/ml, while levels after GHRF infusion varied between 2.5 and 13.5 ng/ml. The somatomedin-C values determined before and after GHRF were similar.

Hizuka N, Takano K, Shizume K, et al: *Acta Endocrinol* 1985;110:17-23.

**Editor's comment**—The authors have shown that GHRF infusion at a dose of 0.5  $\mu$ g/kg/h produced pulsatile GH secretion in patients with GH deficiency, and that the integrated area under the GH curve was much greater than that during saline infusion. With regard to the frequency of peaks, the secretion patterns resembled those previously observed in healthy subjects. However, with respect to the quantitative output, the secretion was much less, corresponding to approximately 30% of that seen in normal adults.

Three aspects of these studies require comment. First, the hypophysis displays a pulsatile form of GH output, although the stimulating agent, GHRF, is administered continuously. This leads to the conclusion that the mode of pituitary GH secretion is pulsatile per se. Second, the continuous administration of small amounts of GHRF for ten hours does not blunt the response to subsequent injections of standard doses of GHRF, whereas the infusion of larger amounts blunts the subsequent response (Vance et al: *JCEM* in press), which is probably due to refractoriness of the pituitary somatotropins. Third, somatostatin may be the controlling factor in GH secretion, since GHRF was infused at a constant rate in these studies of Hizuka et al, yet GH was released in a pulsatile fashion.

## Plasma GH and Sm-C Response to Continuous GHRF Infusion in Patients With GH Deficiency

The secretion of human growth hormone (hGH) is controlled by two hypothalamic hormones: growth hormone-releasing factor (GHRF) and somatostatin. The release of these hormones is in turn controlled by neurotransmitters in the central nervous system. The role played by the two hormones and the neurotransmitters in the pulsatile secretion of hGH is not yet clear. It is only known that most of the growth hormone (GH) pulses occur during the first hours of deep sleep. With the investigations presented here, Hizuka et al aim at a better understanding of the regulatory mechanisms involved and an improved

standard technique for the GHRF test.

In agreement with other investigators, the authors have shown previously that the majority of patients with idiopathic GH deficiency exhibit plasma GH increases following single or repetitive administration of GHRF-44. In the present protocol, the procedure is modified in favor of a combination of a ten-hour infusion of GHRF-44 at night with a subsequent bolus injection of the same hormone. Six patients with proven idiopathic GH deficiency underwent this protocol, receiving 0.5  $\mu$ g/kg/h GHRF-44 during the infusion and, subsequently, 2  $\mu$ g/kg as an intravenous bolus. Four other patients served as controls. They received a saline infusion over ten hours, followed by the same bolus injection.

In two of the patients receiving

## Somatomedin-C and Thymidine Activity in Appropriate and Small-for-Gestational-Age Human Newborns

During the past ten years, several reports on serum growth factors in newborns and premature infants have been published. In this report, two well-defined groups of term newborns are compared, using two different procedures. Ten infants had appropriate birth weights for gestational age (AGA);  $\bar{x} = 3,492 \pm \text{SEM } 1,388$ . Eleven infants were small for gestational age (SGA) with a mean birth weight of  $2,610 \pm 46$  g. Blood from the infants was obtained directly from neonatal vessels, rather than from the umbilical cord. Thymidine activity (TA) was determined by measuring the effect of the serum on thymidine incorporation into human lymphocytes activated by phytohemagglutinin. Somatomedin-C (Sm-C) was measured by radioimmunoassay (RIA) after separation from carrier protein. In addition, transferrin was determined using Mancini's technique of radial immunodiffusion.

The mean  $\pm$  SEM results obtained in the two groups are shown in the table.

| Measurements      | AGA             | SGA             | P               |
|-------------------|-----------------|-----------------|-----------------|
| TA (U/ml)         | $1.51 \pm 0.08$ | $1.04 \pm 0.11$ | <0.001          |
| Sm-C (U/ml)       | $0.52 \pm 0.03$ | $0.32 \pm 0.03$ | <0.001          |
| Transferrin (g/l) | $1.69 \pm 0.15$ | $1.61 \pm 0.13$ | Not significant |

The TA values in the SGA newborns correspond to normal adult values (1.0 U/ml), whereas those of the AGA infants are 50% higher (1.5 U/ml). The Sm-C levels, by contrast, are markedly lower (0.52 and 0.32 U/ml) in both groups of infants than in normal adults (1.0 U/ml). The transferrin levels were similar in both groups and significantly below the mean adult level.

Thiériot-Prévost G, Doffos F, Forrestier F: *Acta Endocrinol* 1985;110:32-35.

**Editor's comment**—The results presented here agree with previously reported results, obtained by radioimmunologic as well as biologic methods. The advantage of this study, however, is its simultaneous application of both assays, thus permitting their immediate comparison. TA values were positively correlated with the Sm-C levels in the AGA newborns ( $r=0.72$ ,  $P<0.05$ ) but not in the SGA group.

The significant difference of the TA values v the Sm-C-RIA values suggests that Sm-C plays a major role in the growth factors determined as thymidine activity, but is certainly not the only substance generating growth-promoting activity, as reflected by thymidine uptake. The importance of other factors, including the embryonic somatomedin described by Sara et al (1981), remains to be elucidated.

Obviously, there exists a relationship between impaired fetal growth and diminished Sm production and thymidine activity. Nevertheless, no individual correlation between the Sm levels and the birth weight was observed. The data on transferrin confirm previous investigations and demonstrate again that transferrin apparently does not play a direct role in fetal growth.

The entire subject of fetal growth and fetal growth factors remains a challenging field for investigation. Our understanding of the phenomena involved remains exceedingly limited.

## Effects of Intravenous, Subcutaneous, and Intranasal Administration of GH-Releasing Hormone-40 on Serum GH Concentrations in Normal Men

The effects of intravenous (IV), subcutaneous (SC), and intranasal growth-hormone-releasing hormone 40 (GHRH-40) on growth-hormone (GH) secretion were measured in normal adult volunteers. To better define the dose-response relationship between GHRH-40 and secreted GH, the circulating levels of immunoreactive GHRH-40 were quantitated. Normal men received either vehicle solution or GHRH-40

IV (0.003 to 0.1  $\mu\text{g/kg}$ ), SC (1 to 10  $\mu\text{g/kg}$ ), or intranasally (3 to 100  $\mu\text{g/kg}$ ). The table gives the results obtained during the two-hour period after IV administration or the three-hour period after SC or intranasal administration of GHRH-40.

In addition, significant dose-response relationships were documented between the maximal increments above basal in serum GH and GHRH-40 administered by all routes.

The mean peak plasma level of GHRH achieved after IV administration of 10  $\mu\text{g/kg}$  GHRH-40 was approximately 60 and 500 times greater than the mean levels achieved after the same dose SC

and intranasally, respectively.

Evans WS, Vance ML, Kaiser DL, et al: *JCEM* 1984;61:846-850.

**Editor's comment**—If chronic GHRH therapy is to become a reasonable alternative to GH therapy, one must be able to give appropriate quantities by SC or intranasal routes. The present preparation is active SC when given as 1 to 3  $\mu\text{g/kg}$  SC every three hours by micropump (see Thomer et al, *N Engl J Med* 1985;312:4). The present data indicate that the intranasal route is not yet practical. If the intranasal route is to be used, what is clearly needed are more lipid-soluble analogs (a peptide composed of the first 29 amino acids of GHRH is biologically active) or the fabrication of a lipophilic delivery system that allows the peptide to cross biological membranes. The dose-response relationships confirm that levels of GHRH of 40 to 60 pg/ml are necessary to evoke GH secretion.

| Route of administration | Dose ( $\mu\text{g/kg}$ ) | Maximal GH increment over basal (ng/ml) |
|-------------------------|---------------------------|---|
| Intravenous             | 0.1                       | 15.5                                    |
| Subcutaneous            | 3.3                       | 26.2                                    |
|                         | 10                        | 63.6                                    |
| Intranasal              | 30                        | 18.5                                    |
|                         | 100                       | 21.7                                    |



## MEETING CALENDAR

**April 12-17** American Academy of Pediatrics. Spring Session. Orlando, Florida. Contact: American Academy of Pediatrics, Division of Continuing Education, P.O. Box 927, Elk Grove Village, IL 60067 (312-228-5005 or 800-433-9016)

**April 28-30** 1st International Symposium on Serum Hormone-Binding Proteins. Contact: Dr. M.T. Forest, INSERM U34, Hôpital Debrousse, F-69322, Lyon, Cedex 05, France

**May 1-4** Postgraduate Course: Current Review of Pediatric Endocrinology. Washington, D.C. Contact: Dr. Salvatore Raiti, Suite 501-9, 210 West Fayette Street, Baltimore, MD 21201 (301-837-2552)

**May 6-9** American Pediatric Society/Society for Pediatric Research. The Sheraton Washington Hotel. Washington, D.C. Contact: William Berman, Jr., Department of Pediatrics, University of New Mexico School of Medicine, Albuquerque, NM 87131 (505-277-4361)

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**May 9** Annual Meeting of the Lawson Wilkins Pediatric Endocrine Society. The Sheraton Washington Hotel, Washington D.C. Contact: Dr. Salvatore Raiti, Secretary, LWPES, Suite 501-9, 210 West Fayette Street, Baltimore, MD 21201 (301-837-2552)

**June 8-11** March of Dimes Birth Defects Foundation Clinical Genetics Conference (With Focus on Muscle), Symposium, Westin Bellevue-Stratford Hotel, Philadelphia, Pennsylvania. Contact: Dr. Roy D. Schmickel, Conference Chairman, University of Pennsylvania. c/o March of Dimes Birth Defects Foundation, 1229 Chestnut Street, Philadelphia, PA 19107

**June 22-24** 46th Annual Scientific Sessions of the American Diabetes Association. Anaheim Convention Center, Anaheim, California. Contact: American Diabetes Association, 2 Park Avenue, New York, NY 10016 (212-683-7444)

**June 25-27** 65th Annual Meeting of The Endocrine Society. Anaheim Convention Center, Anaheim, California. Contact:

The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

**July 6-10** 26th Meeting of the Teratology Society. Park Plaza Hotel and Towers, Boston, Massachusetts. Contact: Alexandra Ventura, Administrative Assistant, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-564-1493)

**July 7-12** XVIII International Congress of Pediatrics. Sheraton Waikiki, Honolulu, Hawaii. Contact: Dr. Gerald E. Hughes, Director, Office of the International Congress of Pediatrics, American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

**September 22-26** 7th International Congress on Human Genetics. International Congress Center, West Berlin, Germany. Contact: Congress Bureau, DER-CONGRESS, Congress Organization, Augsburger Strasse 27, D-1000 Berlin 30 (Telephone: 030-24-60-11)

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# GROWTH

## Genetics & Hormones

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## Primary Hypophosphatemic Rickets And Growth Retardation

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Primary (hereditary) hypophosphatemia is the most common cause of rickets in the United States now that vitamin D deficiency is uncommon. Primary hypophosphatemia is also a cause of growth retardation, which may occasionally present before the deformities of rickets appear.

Winters and co-workers studied the genetics of primary hypophosphatemia in a large kindred. They concluded that the disorder was transmitted by a single gene as an X-linked dominant trait. In this kindred there was no male-to-male transmission, but all female offspring of affected males were also affected. Consistent with Lyon's hypothesis, the manifestations of the disorder were often less severe in females than in males.

Although the disorder in humans is often referred to as X-linked hypophosphatemic rickets, it has been transmitted by an autosomal dominant mechanism in other kindreds. In one such kindred studied by us, the affected father had one affected son, two affected daughters, and one unaffected daughter. No phenotypic differences could be detected between these individuals and those in kindreds in which an X-linked dominant gene was involved. Interestingly, a significant number of patients presenting with

hypophosphatemic rickets have no family history of the disorder, which suggests a spontaneous mutation. Follow-up of several such individuals who have since had affected offspring has confirmed the new mutation theory.

### Phosphate Concentrations and Renal Functions

The concentration of inorganic phosphate in plasma is determined primarily by renal function. The usual diet for humans is rich in

*continued on page 2*

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## Fetal Growth and Development: A Brief Survey of Cellular Mechanisms

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The growth and development of the human embryo and fetus result from an orderly series of events that occur between fertilization and birth and lead to the formation of many complex structures and a myriad of interrelating specialized functions.

Although there are many reports in the literature associating specific events and observations with modulation or alteration of fetal growth, much remains to be learned about the cellular and subcellular mechanisms governing fetal growth and development. However, recent advances in understanding the control of fetal growth have resulted from research designed to answer these fundamental questions:

- What substances stimulate cellular replication and/or differentiation?
- Do these agents act on all cells, or are specific factors required for each cell type?
- What governs the synthesis of growth regulators?
- How do these substances act on their target cells?

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# Primary Hypophosphatemic Rickets and Growth Retardation

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phosphate, a component of both animal and vegetable cells that is found in high concentration in cow's milk. Intestinal absorption of phosphate is influenced by vitamin D. However, even in vitamin D-deficient states, sufficient phosphate is absorbed to provide for tissue and bone phosphate needs if the diet is high in phosphate and if renal excretion of phosphate is appropriately reduced. Hypophosphatemia due to failure of intestinal absorption of phosphate can be induced by a very high intake of an aluminum or calcium salt, which precipitates phosphate in the intestinal lumen. The only other situation in which hypophosphatemia develops despite normal renal function is that of the prematurely born infant who is fed human milk and whose phosphate intake is low.

The metabolic abnormality in primary hypophosphatemia is impairment of tubular reabsorption of phosphate in the proximal convoluted tubule, the major site of phosphate reabsorption. This sodium-dependent phosphate transport system has a maximal rate that varies somewhat with the rate of glomerular filtration. The phosphate  $T_m$  expressed as mg P absorbed per dL of glomerular filtrate is highly correlated with serum P expressed as mg/dL. Parathyroid hormone (PTH) inhibits tubular reabsorption of phosphate and is an important factor in controlling serum phosphate concentration in normal individuals.

## Calcium and Vitamin D Concentrations

A defect of intestinal transport of phosphate is also present in primary hypophosphatemia and may be the basis of impaired absorption of calcium. Serum calcium concentrations are normal and PTH function, as measured by serum immunoreactive PTH concentrations (iPTH), is also normal. The only sign of impaired calcium absorption is low excretion of calcium in the urine. The eucalcemic state is due to the hypophosphatemia, which reduces the flow of calcium from extracellular

fluid into bone. Indeed, the lack of phosphate prevents bone mineralization and results in rickets.

The osteomalacia seen in patients with hypophosphatemic rickets is not prevented by physiologic amounts of vitamin D. Measurement of the concentrations of vitamin D metabolites in the serum of such patients has shown normal concentrations of 25-hydroxyvitamin D as well as 1,25-dihydroxyvitamin D. However, the concentrations of the latter are usually at the lower limit of normal. Since reduction of extracellular phosphate has been found to activate 25-hydroxyvitamin D, 1-hydroxylase in kidney tubule cells, the relatively low concentration of 1,25-dihydroxyvitamin D in patients with primary hypophosphatemia suggests that there is, in addition to the impairment of tubular transport of phosphate, a defect in the linkage between phosphate concentration and the formation of 1,25-dihydroxyvitamin D by tubule cells.

## Clinical Features and Diagnosis

**The growth retardation characteristic of the untreated subject** is believed to be secondary to hypophosphatemia, although the specific mechanism by which reduced concentrations of inorganic phosphate in extracellular fluid interfere with cellular growth is undetermined. Only part of the **reduced height** of these patients can be ascribed to the **bending and twisting deformity of the lower extremities** resulting from the impaired mineralization of the metaphyseal cartilage and the growing bone. Since 9 mg of phosphorus is retained in the cell for each gram of protein synthesized, it is likely that phosphate deficiency can inhibit cell growth through reduction of intracellular organic phosphate. Further evidence of the relationship between extracellular phosphate concentration and linear growth is seen in the acceleration of growth following treatment, as described later in this article.

**Reduced serum phosphate concentration** is, of course, the basis of the diagnosis of primary hypophosphatemia. Since physiologic serum phosphate concentrations are much higher in infants and children than in adults, appropriate standards must be used. Serum

phosphate concentrations below 4.5 P/dL in infants less than 3 or 4 months of age, as well as concentrations below 4 mg P/dL in older infants and children, should be regarded as abnormal.

If there is a **positive family history** for primary hypophosphatemia, **the diagnosis is confirmed by serial determinations of serum phosphate in the infant**. Prenatal diagnosis is not possible. Even if the mother is hypophosphatemic, placental transport of phosphate into the fetal plasma is not impaired. Thus, fetal growth and mineralization of the fetal skeleton are normal. For this reason, the diagnosis cannot be made in the early neonatal period either.

During the first weeks of life, the glomerular filtration rate is physiologically low and the serum phosphate concentration may remain in the normal range despite the phosphate reabsorption abnormality. Thus, unequivocal hypophosphatemia may not be detected until the infant is several months of age, particularly if the infant is fed a high-phosphate, cow's-milk-based formula.

Before a degree of hypophosphatemia that is considered to be diagnostic of the condition is reached, **serum alkaline phosphatase concentrations are often elevated**. This might be the initial evidence that the infant is affected and it presumably represents increased proliferation of osteoblasts in response to defective mineralization of bone. Possibly, the integrated 24-hour concentration of extracellular phosphate in these infants is below the concentrations necessary for mineralization of the rapidly growing skeleton even though a randomly determined serum phosphate level is not sufficiently low to be diagnostic.

If there is **no family history** of primary hypophosphatemia, the diagnosis is not usually made until **rachitic deformities** are noted. Since the most obvious deformities are in the lower extremities and develop after weightbearing, suspicion is not aroused until the second year of life **when bowing of the legs** (genu varum) or **knock-knee deformity** (genu valgum) develops, with the former being more common. Decreased serum phosphate concentrations, increased serum alkaline phosphatase levels, and

characteristic x-ray findings of irregular mineralization at the metaphyseal ends of the long bones rule out other causes of leg deformities such as Blount's disease, physiologic bowing, and chondro-metaphyseal dysplasia. A history of adequate vitamin D intake and/or exposure to sunshine argues against vitamin D deficiency as a cause of the deformities.

In rare instances, calcium deficiency may account for the rickets seen in infants who cannot tolerate milk and have not been given calcium supplements when placed on a milk-free diet. Measurements of the vitamin D metabolites, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in serum, and the IPTH concentration will differentiate primary hypophosphatemia from calcium deficiency or from vitamin D-dependent rickets (ie, rickets caused by defective conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D or by lack of end-organ receptors for 1,25-dihydroxyvitamin D). As mentioned previously, serum IPTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D concentrations are in the normal range, as is the serum calcium level, in patients with primary hypophosphatemia.

### Treatment

The treatment of primary hypophosphatemia consists of increasing phosphate intake to raise extracellular phosphate concentrations as well as taking adequate amounts of the active vitamin D metabolite, 1,25-dihydroxyvitamin D, or an analogous compound, dihydrotachysterol. The former is available commercially (as Rocaltrol) in capsules containing 0.25 or 0.5  $\mu\text{g}$  of active metabolite. This formulation is inconvenient for infants, who can be more readily treated with a solution of dihydrotachysterol in oil.

Both compounds act to increase intestinal absorption of phosphate and calcium. Unless adequate amounts of these compounds are given, ingestion of large amounts of phosphate will sequester calcium, thus preventing its absorption, and result in secondary hyperparathyroidism as measured by increased serum IPTH concentration. The appropriate dose of 1,25-dihydroxyvitamin D or dihydro-

tachysterol is that amount necessary to bring calcium absorption into the normal range. Calcium absorption can be indirectly assessed by measuring urine calcium excretion and serum calcium concentration. Depressed intestinal absorption of calcium is indicated by low urine output of calcium, whereas hyperabsorption of calcium results in excessive excretion of calcium and increased serum calcium concentration. The normal range of calcium in the urine in children is 1 to 4 mg/kg/24 h. If 24-hour collections are not possible, the calcium/creatinine ratio of a single voided specimen can be used as an approximation, the normal range being 0.05 to 0.25 mg calcium per mg creatinine.

The dose of phosphate is 1 to 1.5 g of phosphorus in four or five divided doses for infants under 2 years of age and 2 or 2.5 g of phosphorus per day for older children. The preparations available are

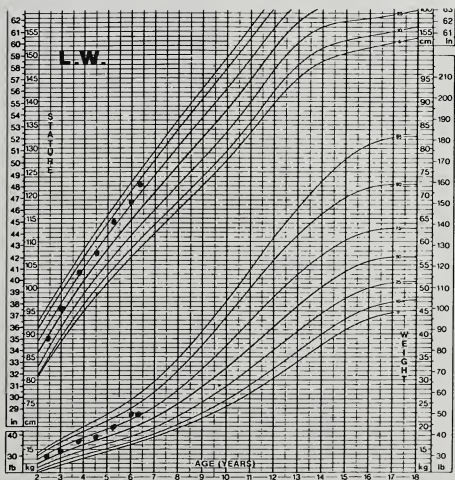
Neutra-Phos, Neutra-Phos-K, or K-Phos. My preference is for Neutra-Phos-K, which is buffered potassium phosphate.

Application of this treatment is often quite difficult. The large load of phosphate salt is unpalatable and its osmotic effect may cause hyperperistalsis and diarrhea. Some patients cannot tolerate the phosphate except in minimal dosage, but attempts must be made to increase the phosphate gradually until an adequate dose is achieved or tolerance is exceeded. Phosphate must be given in three or four divided doses per day to keep serum phosphorus levels in the normal range for a significant portion of each 24-hour period.

If treatment is adhered to, radiologically normal bone structure and excellent growth can be attained, as shown in Figure 1. L.W., the daughter of a hypophosphatemic mother who was 59 inches in height, was

*continued on page 4*

**Figure 1.** Growth curves of a child who was diagnosed as having primary phosphatemia at two months of age. Treatment was begun at the time of diagnosis.



Adapted from Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth. National Center for Health Statistics percentiles. *Am J Clin Nutr* 32:607-629, 1979 Data.



# Primary Hypophosphatemic Rickets and Growth Retardation

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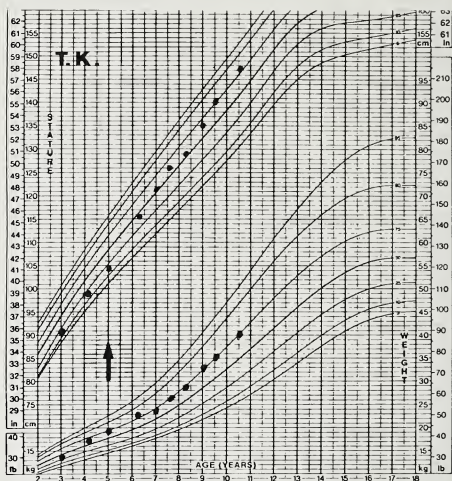
diagnosed as having primary hypophosphatemia at 2 months of age. Treatment was begun as indicated above. The child tolerated treatment, and there was excellent compliance.

In the case of T.K. (Figure 2), the family history was negative for hypophosphatemia. At 2 years of age, T.K. had marked genu varum and x-ray evidence of rickets, but a specific diagnosis was not made and appropriate treatment was not begun until she reached 5 years of age (indicated by the arrow). These two patients are admittedly the exceptions rather than the rule, but they clearly illustrate how proper treatment can improve growth and bone mineralization.

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**Figure 2.** Growth curves of a child who did not begin receiving treatment for primary phosphatemia until she was 5 years of age (the arrow denotes the beginning of therapy). Although this child exhibited clinical signs and radiologic evidence of rickets at the age of 2 years, a definitive diagnosis was not made until three years later.



Adapted from Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore RM. Physical growth. National Center for Health Statistics percentiles. *Am J Clin Nutr* 32:607-629, 1979 Data.

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# Fetal Growth and Development: A Brief Survey of Cellular Mechanisms

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The term "growth factor" has been used as a generic designation for any substance capable of inducing cellular proliferation and/or differentiation. Some growth factors act on a wide variety of cell types, while specific cells are the targets of others. All of the known growth factors may be important to the fetus. This survey will review some of the known growth factors and their actions on fetal growth, as well as point out their regulation by classical hormones and their probable paracrine and/or autocrine mechanisms of action. Insights into growth mechanisms derived from the study of oncogenes will also be explored, and speculation regarding the course of future research in fetal growth will be offered.

## Growth Factors With Broad Specificity

Epidermal growth factor (EGF), a 53-amino-acid peptide measuring 6,045 daltons (da), is a potent mitogen for cells of ectodermal and mesodermal origin. In the fetal lung, EGF stimulates branching morphogenesis and the proliferation of airway epithelium. It also appears to be involved in skin growth. EGF can be measured in amniotic fluid and is present in high concentrations in human milk, the latter suggesting that it might have a role in gut maturation. Tissue concentrations of EGF are regulated by androgens and thyroxine.

The somatomedins, or insulin-like growth factors, somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) and IGF-II, are peptides of 7,649 and 7,471 da, respectively. They are potent mitogens for a wide variety of cell types and have marked amino acid sequence homology with insulin. Both appear to be synthesized in many fetal tissues, and cell surface receptors that presumably mediate their mitogenic actions are ubiquitous in the fetus. The fetal rat has high blood concentrations of multiplication stimulating activity (MSA, the rat homologue of IGF-II) and abundant MSA cell surface receptors, suggesting an important role in prepartum rodents. While it is clear that Sm-C/IGF-I and,

to some extent, IGF-II as well, are regulated postnatally by growth hormone (GH) and nutritional status, placental lactogen may supplant GH in the fetus. It is also likely that nutrient supply plays an important role.

Platelet-derived growth factor (PDGF), a disulfide-linked, two-chain protein of approximately 30,000 da, is one of the several mitogens found in platelets. PDGF stimulates cellular proliferation in concert with other peptide mitogens, such as EGF and Sm-C/IGF-I. Based on experiments in cultured cells, it is now thought that PDGF is one member of a family of peptides that act by making resting cells capable of undergoing DNA synthesis when they are stimulated by other mitogens.

Fibroblast growth factors and endothelial growth factors are examples of mitogens with PDGF-like effects. The former has been purified from bovine brain and acts on cells of endodermal and mesodermal origin, while the latter are proteins purified from brain and platelets that presumably act specifically on vascular endothelial cells. PDGF is probably important in wound healing, given its high concentrations in platelets and their known role in clotting. In the fetus, peptides with PDGF-like actions may be necessary to stimulate the proliferation of many cell types.

## Growth Factors for Specific Cells

Erythropoietin, an acidic sialoprotein containing 166 amino acids, appears to be made in the kidney. It stimulates the mitosis and differentiation of red cell precursors in response to hypoxia. Its production is also stimulated by androgens and GH. Colony-stimulating factors are peptides and glycoproteins derived from a variety of tissues that stimulate white blood cell proliferation and differentiation.

Hematopoietic cells also synthesize several mitogens, of which the interleukins are perhaps the best described. Interleukin-1 is the designation for a group of peptides made by macrophages and other cells that are capable of a variety of actions on both T and B lymphocytes. Interleukin-2, a 15,500-da

peptide, is made by T lymphocytes and promotes their proliferation after antigenic stimulation. Interleukin-3, a 28,000-da glycosylated protein, stimulates the growth of immature lymphocytes.

Thymosins and thymopoietins are peptides made in the thymus; they promote the growth and differentiation of immunologically competent lymphocytes. Macrophage growth factors have also been described. They are made by mononuclear phagocytes and appear to exert their mitogenic effects on a variety of other cell types.

## Growth Factors Causing Differentiation

Nerve growth factor (NGF) is the best characterized of the growth factors that promote cellular differentiation. The biologically active form,  $\beta$  NGF, is a 13,259-da peptide that is 25% homologous with insulin. NGF promotes the survival, differentiation, and axonal outgrowth of sensory and sympathetic ganglia. It appears to be synthesized in the peripheral tissues that are innervated by these ganglia and is transported by retrograde movement to cell bodies where the signals for axonal growth are given.

Thyroxine may be essential for NGF synthesis in the fetal and neonatal central nervous system. When thyroxine blood concentrations are low in the neonatal rat, brain NGF concentrations are reduced. This may explain why hypothyroid fetuses and newborns have impaired brain development and neurologic handicaps.

Fibroblast pneumonocyte factor (FPF) is another example of a substance capable of differentiative action. It is made by lung fibroblasts in response to glucocorticoids and stimulates surfactant production by type II pneumonocytes.

## Other Stimulators of Fetal Growth

The study of cellular oncogenes and the proteins they encode provides clues to normal growth mechanisms in the fetus. Cellular oncogenes are stretches of genomic DNA (genes) containing nucleotide sequences that are homologous with nucleotide sequences of RNA in retroviruses

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isolated from naturally occurring tumors. These viruses, whose genetic information is encoded by RNA, can transform normal cells into neoplastic cells. Retroviruses possess enzymes, called reverse transcriptases, that are capable of transcribing RNA into DNA. Therefore, the genetic information encoded in the RNA of these enzymes can be incorporated into the DNA of the infected host cell. The portion of the retroviral RNA responsible for neoplastic transformation is termed the viral (v) oncogene.

It is now thought that v oncogenes originated from normal cellular genes, presumably by recombinational events occurring during the viral transfection of normal cellular DNA. By virtue of their presence in whole or in part in the genome of the retrovirus, these normal genes are called cellular proto-oncogenes, despite the fact that they are normal genomic components and, as such, have no role in oncogenesis. The proteins that these genes encode are called proto-oncogene proteins (pOGP). Because proteins encoded by v oncogenes are responsible for the abnormal growth of neoplastic cells, it follows that the pOGPs may be important in normal cellular proliferation. For example, the v oncogene *v-sis* is part of the RNA genome of the simian sarcoma virus and encodes a protein designated *p28-sis*. This protein is almost identical to the 109 N-terminal amino acids of the  $\beta$  chain of PDGF. The normal cellular proto-oncogene that encodes this protein is called *c-sis*.

There are many other examples of proteins encoded by v oncogenes that have normal cellular counterparts. Identification of v oncogenes and the proteins they encode is, therefore, likely to lead to a better understanding of normal growth mechanisms by revealing other proteins and their genes that are important in this process.

Other links between normal cellular growth and tumor growth come from the study of substances termed transforming growth factors (TGF). TGF $_{\alpha}$  is a 51-amino-acid peptide that was purified from the media of normal rat kidney cells after they were transformed by the

murine sarcoma virus. TGF $_{\alpha}$  shares 44% homology with human EGF, and when added to normal cultured cells together with another substance called TGF $_{\beta}$ , it induces transformation. TGF $_{\alpha}$  has been identified in many normal cells, suggesting that it may be a normal EGF-like growth factor.

## Difficulties in the Study of Fetal Growth

Until recently, many studies of the regulation of fetal growth focused on classical hormones. Endocrine mechanisms of regulation were anticipated. Specifically, it was expected that substances synthesized at sites distant from their sites of action would control growth. Few growth factors, however, appear to act in this manner. Rather, they act in a paracrine or autocrine fashion. Their sites of synthesis are distributed widely and their biologic effects occur on nearby cells or on their cells of origin.

While classical hormones are synthesized by and have biologic actions in the fetus, clear regulatory roles, such as those defined in post-natal growth, have been difficult to define in the fetus. Aside from the inaccessibility of the fetus, at least two reasons for this difficulty are apparent. Because the placenta can synthesize homologues of many classical hormones, it is difficult to distinguish *experimentally* the effects of hormones derived from the fetus and those derived from the placenta (and possibly from the mother).

In addition, many or most of the growth-promoting effects of classical hormones seem to be mediated by growth factors that act on the same tissue that produced them. For example, the maturational effect of thyroxine on the skin and central nervous system seem to be mediated, at least in part, by EGF and NGF, respectively. Growth stimulation by GH and placental lactogen is thought to be mediated by the somatomedins. Because growth factor synthesis and action in the fetus are likely to be dependent on multiple influences, the regulatory effects of classical hormones on growth factors are neither obvious nor easily delineated.

## Mediation of the Action of Growth Factors

For peptide growth factors to stimulate developmental change, the cellular apparatus effecting the biologic response must also be in place. All peptide growth factors are thought to initiate their actions by binding to cell surface receptors. In most instances, this is followed by phosphorylation of the receptor, as well as certain cytosolic proteins. Subsequently, a series of biochemical events occurs, leading either to mitosis or differentiation of the target cell. The precise nature of the events occurring in response to receptor binding has not been delineated completely. Evidence that such events are crucial is provided by findings that many proteins encoded by oncogenes and cellular proto-oncogenes are kinases, enzymes that phosphorylate proteins. Similarly, at least one v oncogene product (gp65 encoded by v *erbB*) is homologous to a portion of the EGF receptor.

## The Future of Research on Fetal Growth and Development

To understand fetal growth, we must understand the mechanisms that control transcription and translation of each protein involved in the developmental process. Important questions to be answered include:

- What mechanisms govern transcription of the genes that encode proteins that are important in development?
- How is transcription coordinated with the development?
- What turns the genes that are important in development on and off?
- What translational and post-translational events influence the gene product that is expressed at each phase of development?

In addition, we must understand the regulation of structure. Important information about the genes regulating the gross morphology of one complex species, *Drosophila melanogaster*, is now accruing. Within a 70-kilobase DNA sequence, termed the bithorax complex, there are specific genes that govern the development of the three thoracic and eight abdominal segments of the fruit fly. By investigating the genome of *Drosophila* mutants

that lack somatic segments or have segmental abnormalities, the DNA sequences (genes) that regulate some specific segments have been deduced. Messenger RNAs encoded by these genes are now being isolated and it is hoped that the expressed proteins will be identified. This will allow further study of mechanisms that lead to gross structural morphology. Perhaps the elucidation of mechanisms governing the sculpturing of the fruit fly will provide a basis for similar studies in man.

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## The Effect of Adrenal Androgens on Skeletal Maturation and Growth

There are few instances when the effect of gonadal steroids on growth and skeletal maturation can be distinguished from those of adrenal steroids. Wierman et al have elegantly examined the interrelationship of adrenal and gonadal function in 29 patients with sexual precocity by measuring DHAS, a steroid produced almost exclusively in the adrenals, in these patients before and during treatment with the LHRH analogue (LHRHa [D-Trp 6-Pro 9-NET]). The authors have correlated the findings with changes in skeletal maturation and predicted height, and they have established the following salient points:

- (1) Only 10 of 29 patients studied had coincident premature adrenarche as determined by adolescent

values of DHAS ( $\geq 60 \mu\text{g/dl}$ ).

(2) The use of LHRHa did not alter the DHAS levels in patients with adrenarche.

(3) The predicted heights of patients with sexual precocity but without adrenarche increased significantly more than those with adrenarche as a result of LHRHa therapy.

(4) The change in bone age/change in chronological age ratio was greater over a period of 1 to 4 years in patients with associated adrenarche than in those without.

(5) The presence of pubic hair did not correlate with DHAS levels before therapy.

(6) Sexual hair regressed in patients without adrenarche when treated with LHRHa but not in those with adrenarche.

On the basis of these findings the authors conclude:

- (1) Adrenarche is not under the control of gonadotropins and the

factor(s) that induce adrenarche remain obscure.

(2) The data presented suggest that adrenal androgens contribute significantly to epiphyseal advancement during adolescence.

(3) LHRHa therapy is potentially most effective in increasing the height of children with sexual precocity if they do not have adrenarche in association with the sexual precocity.

Wierman ME, Beardsworth DE, Crawford JD, et al: *J Clin Invest* 1986;**77**:121.

**Editor's comment**—This paper is well worth reading and digesting completely. It presents data that provide insight into the separate occurrences of gonadarche and adrenarche and it also reviews what we currently know and do not know about the relationship of adrenarche to skeletal maturation.



## Fetal Alcohol Syndrome: Two Reports

### I. Natural History: A Ten-Year Follow-up of 11 Patients

In 1973, Jones et al described 11 children with a common pattern of altered morphogenesis and central nervous system dysfunction. Their mothers were chronic alcoholics who continued to drink heavily during pregnancy. Since then, fetal alcohol syndrome has been identified in children from every racial group and in many countries. The teratogenicity of alcohol has been confirmed in laboratory studies involving many different species of animals, and a dose-response curve for prenatal alcohol exposure has been established.

In this report, the authors describe how the 11 children have developed physically and mentally over the past ten years. Two are now dead, one is lost to follow-up, and the remaining eight continue to be growth deficient (with respect to height, weight, and head circumference) and dysmorphic. Although most showed some catch-up linear growth during the first year of life, weight and head circumference decreased relative to the norms during this time in most of the children. Thereafter, length and head circumference remained relatively constant with respect to the norms, whereas there was some catch-up in weight with increasing age. There was relatively slow growth of the head after delivery. During childhood, the children were all strikingly underweight for height.

The major craniofacial features—especially the short palpebral fissures, hypoplastic philtrum, thin vermilion border of the upper lip, and flat midface—did not change during the ten years of follow-up. However, their noses changed, with more prominent growth of the nasal bridge. Cardiac anomalies, which consisted of an atrial septal defect in one patient, patent ductus arteriosus in another, and a grade 3/4 systolic murmur interpreted as a ventricular septal defect in six, have all resolved spontaneously or have become insignificant. Orthopedic complications were managed successfully in almost all patients by

casting or splinting.

The short palpebral fissures were thought to be secondary to the decreased growth of the eye. Frank microphthalmia was observed at necropsy in one of the patients. Chronic serous otitis media, probably secondary to eustachian tube dysfunction associated with maxillary hypoplasia, required medical and surgical procedures in four of the children.

None of the eight children followed had normal intellectual development. Four were mildly and four were seriously retarded. The degree to which postnatal environmental factors influenced the development of these children is difficult to assess. Mothers of three of the four seriously retarded children were so severely alcoholic that they died of alcohol-related causes within six years of giving birth.

The two major predictive factors concerning prognosis were the severity of the maternal alcoholism and the extent and severity of the initial pattern of malformation. The four children with the most striking craniofacial abnormalities had the most severe degree of microcephaly, the shortest stature, and the lowest intellectual function. The severity of maternal alcoholism appeared to be the most predictive factor in the backgrounds of the four most severely retarded children.

Streissguth AP, Clarren SK, Jones KL: *Lancet* 1985;2:85-91.

### II. Prospective Study of Children Exposed to Variable Amounts of Alcohol in Utero

Although it is well known that offspring of mothers who consume large quantities of alcohol during pregnancy are at high risk for physical and mental deficiencies, few prospective studies have dealt with the fetal effects of interrupted alcohol consumption during pregnancy as the result of an intervention program. The authors describe a Swedish antenatal program that was started to help pregnant women stop alcohol abuse with the hope of reducing the adverse effects of alcohol on the fetus.

A total of 40 children born to alco-

holic women (Groups 2 and 3) and 40 children born to nonalcoholic women (Group 1) attending the same local maternity health clinics for antenatal care were studied between the ages of 18 and 27 months. The mothers in Group 1 drank less than 30 g of pure alcohol prior to the first prenatal visit and abstained or minimized their consumption thereafter. Group 2 consisted of 25 children born to women who were classified as excessive drinkers and had an average consumption of 30 to 150 g of pure alcohol per day during the month before their first visit to the clinic. All mothers in this group markedly reduced their alcoholic consumption after their first visit and 19 abstained completely. Group 3 consisted of 15 children of alcoholic mothers who had an average consumption of more than 125 g of pure alcohol per day during the month before the first visit to the clinic. Nine mothers in this group stopped drinking alcohol during the first or second trimester, but the remaining six continued drinking throughout the pregnancy.

A statistically significant reduction in weight, height, and head circumference was seen in Group 3 children when compared with Group 1 children. Six of the 15 alcoholic women (Group 3) continued to abuse alcohol throughout pregnancy. Three of these women gave birth to children with abnormalities characteristic of fetal alcohol exposure; one child had the complete fetal alcohol syndrome. Only one child in Group 3 was normally developed in all physiological parameters and had normal behavior.

No fetal growth retardation was found among the children in Group 2, where the mothers reduced or ceased alcohol consumption after their first prenatal visit. Neither did these children show any other physical or physiological characteristic of fetal alcohol syndrome. About half of them, however, had retarded speech that the authors attributed to postnatal environmental influences. Indeed, signs of social instability, such as frequent separations between the parents and frequent registrations with the social welfare department, were seen in Group 2.

The authors suggest that fetal exposure to alcohol has a severe adverse effect on development, but

this can be significantly reversed by abstaining from alcohol in the first trimester of pregnancy. Cessation of alcohol abuse after the first trimester cannot reduce the documented increased risk of congenital malformations.

Larsson G, Bohlin AB, Tunell R: *Arch Dis Child* 1985;60:316-321.

**Editor's comment**—*Fetal alcohol syndrome has been well established as an important cause of congenital malformations, mental retardation, and prenatal and postnatal growth retardation. This syndrome is being diagnosed more frequently now that physicians are specifically questioning mothers-to-be about teratogenic exposure and, especially, alcohol exposure.*

*The study by Streissguth et al indicates the relationship between the severity of the maternal alcoholism and the severity of the physical and mental handicap. It also points out that the various features of this syndrome tend to be correlated to severity: degree of mental retardation, growth deficiency, and intellectual impairment. This study also points out the difficulty in diagnosing this syndrome after mid-childhood, since some of the typical facial characteristics change, specifically, the structure of the nose, while the markedly underweight appearance of the female children who had reached puberty disappeared. It is unknown whether this applies to the weight of males of pubertal age, since none of the boys had reached puberty. Moreover, it becomes increasingly difficult to obtain a doc-*

*umented history of maternal alcohol abuse as the children grow older.*

*The Swedish study documents the importance of discontinuing alcohol before pregnancy or at least during the first few weeks of pregnancy. It is interesting that those women who drank fairly heavily during the first month or so of pregnancy and then stopped, or markedly reduced their intake, had no physical abnormalities in their children. Those women who did not stop or reduce their drinking until the second or third trimester, however, continued to have children with abnormalities characteristic of fetal alcohol exposure. It is hoped that with the increased emphasis and warnings concerning alcohol exposure in utero, the incidence of this devastating syndrome will be reduced in coming years.*

## Reevaluation of Russell-Silver Syndrome

Russell-Silver syndrome is characterized by intrauterine growth retardation (IUGR) and postnatal growth retardation in association with asymmetry of the body, normal head size, triangular facies, and normal psychomotor development. In 1953, Silver first described two patients with IUGR, body asymmetry, and postnatal growth retardation. In 1984, Russell described another group of five patients who also had growth deficiency, triangular facies, and disproportionate shortening of the upper limbs. Only two of the five had limb asymmetry. Since these two patients appeared to have the same anomalies described by Silver, the clinical entity was named the "Russell-Silver syndrome."

Saal et al reevaluated 15 patients with Russell-Silver syndrome 2.9 to 13 years after their initial diagnosis. They observed great variability in each of the features of the syndrome, suggesting that Russell-Silver syndrome is not a discrete entity but a heterogeneous group of disorders. Most interesting are the data regarding eventual growth. Five of the 15 exhibited late catch-up growth and attained normal heights. Eight remained below the third percentile, but paralleled the growth curve. Six of the 15 had gross evidence of body asymmetry

at the time of diagnosis—four of the six continued to have a discrepancy in leg and/or arm length of more than 1 cm. One of the four had severe scoliosis.

Psychomotor development was abnormal in six of the 15. One of the six had seizures and six had café au lait spots, strongly suggesting neurofibromatosis. Psychomotor development in Russell-Silver syndrome had previously been thought to be normal, despite the frequent finding of gross motor delay in infancy. Eventual head circumference was normal in some and below the second percentile in others, unrelated to the degree of psychomotor delay. Russell-Silver patients are generally thought to have a normal head circumference. Eleven of the 14 patients originally described as having triangular facies were again so described on follow-up. Although hypogonadism and abnormal genital development had been described in several patients with Russell-Silver syndrome, all of these 15 patients had normal sexual development.

The authors conclude that the features of Russell-Silver syndrome are so diverse that it is highly probable the entity is a heterogeneous group of disorders. It is thus difficult to offer parents a clear prognosis. In addition, if the diagnosis is made too

loosely, the work-up for short stature may be prematurely terminated in some children; potentially correctable conditions could therefore be overlooked.

Saal HM, Pagon RA, Pepin MG: *J Peds* 1985;107:733.

**Editor's comment**—*Although the Russell-Silver syndrome has been considered a well-defined form of IUGR, it is clear that patients with a variety of forms of prenatal growth retardation have been lumped under this term. Conflicting reports of responsiveness to growth hormone therapy probably reflect this heterogeneity, especially in view of the fact that one third of the patients in this series obtained normal height without therapy. In the absence of a specific laboratory diagnostic test, the delineation of heterogeneity within a syndrome is difficult, but must always be kept in mind when offering parents a prognosis. In addition, the authors are correct in emphasizing that Russell-Silver syndrome can occur concomitantly with hypopituitarism or other causes of growth retardation. Cassidy et al recently reported (AJDC 1986;140:155) the seventh case of growth hormone deficiency in association with Russell-Silver syndrome.*

## Computed Tomography of the Foramen Magnum: Achondroplastic Values Compared to Normal Standards

Achondroplasia, the most common of the skeletal dysplasias, is an autosomal dominant disorder whose clinical manifestations include short-limbed dwarfism, large head, shallow thoracic cage, and characteristic radiographic findings. This disorder is characterized by a decreased rate of endochondral ossification and normal membranous ossification.

The foramen magnum has long been recognized to be small in achondroplastic individuals. The small size is secondary to deficient growth of the endochondral occipital, supraoccipital, and basioccipital bones, which form the boundaries of the foramen magnum. Neurologic abnormalities seen in achondroplastic patients have included respiratory embarrassment, quadripareis and paraparesis, obstructive hydrocephalus, and sudden death. Compression of the upper cervical spinal cord and caudal medulla due to the small foramen magnum has been seen at autopsy in several cases. Surgical decompression by suboccipital craniectomy and cervical laminectomy has been suggested for patients with evidence of neurological involvement.

In this report, the extent of foramen magnum stenosis in achondroplasia was quantified by measurement of the maximal transverse and sagittal lengths of the foramen magnum by computed tomography (CT) scan. These were compared against foramen magnum measurements for persons of normal stature.

CT scans of the foramen magnum were performed by scanning at 0° horizontally from the top of the hard palate through the foramen magnum to the occiput. Maximum transverse and sagittal foramen magnum length was measured on the axial bone window scan with a ruler. Measurements were obtained from people of normal stature and varying ages: the transverse dimension was measured in 164 cases and the sagittal dimension in 144. Mean normal values  $\pm 1$  SD were calculated at monthly intervals from birth

through 2 years of age and at two-year increments thereafter. It was found that the mean foramen magnum size did not change appreciably after 15 years of age.

These values were then compared with foramen magnum measurements from 63 patients with achondroplasia. Among this group, 41 patients had no neurological findings. Twenty-two had evidence of neurological dysfunction suggestive of foramen magnum compression based on history, physical exam, short latency somatosensory potentials, and/or polysomnography.

From the data, one can conclude that the normal foramen magnum grows rapidly in both dimensions from birth to 1 year of age and then continues at a greatly diminished rate until approximately 15 years of age. The foramen magnum in achondroplastic individuals was significantly smaller than that of normal people at all ages. Achondroplastics without neurological dysfunction had measurements within  $\pm 5$  SD of the normal mean for the transverse and  $\pm 4$  SD for the sagittal dimensions. Patients with neurological symptoms had significantly smaller measurements. Included in this latter group were seven patients with extremely small foramen magnum size and obstructive hydrocephalus. Significantly, foramen magnum size was not shown to correlate with head circumference in this or any other group studied.

The authors concluded that stenosis of the foramen magnum may be more widespread in achondroplasia than had been previously appreciated. Because significant morbidity and potential mortality are associated with this stenosis, CT scans may identify individuals at high risk for these complications. However, the efficacy and safety of surgical decompression has not yet been clearly documented. The authors recommend that CT scan of the foramen magnum be considered part of the comprehensive care of individuals with achondroplasia.

Hecht JT, Nelson FW, Butler IJ, et al: *Am J Med Genet* 1985;20:355.

**Editor's comment**—The recent recognition of late infantile mortality as a significant complication of

achondroplasia has made careful neurological surveillance of achondroplastic children imperative. This report documents the differences in foramen magnum dimensions between normal and achondroplastic children, and especially between achondroplastic children with and without neurological complications. Newer diagnostic imaging procedures, such as magnetic resonance imaging (MRI) scans, have also become useful in the evaluation of spinal cord compression due to upper cervical vertebrae or foramen magnum stenosis in achondroplasia or other skeletal dysplasias. Unlike CT scanning, MRI can easily distinguish among varying soft tissue densities and can identify kinking or impingement on the spinal cord at an early stage. Discovery of anatomic abnormalities should be followed up by neurological and neurophysiological evaluation, including somatosensory-evoked potentials. Definitive criteria for neurosurgical intervention, however, remain to be established.

## Impaired Calcitonin Secretion in Patients With Williams Syndrome

The Williams syndrome (WS) is characterized by prenatal and postnatal growth retardation, microcephaly, facial dysmorphism (the so-called elfin facies), congenital heart disease (most commonly, supravalvular aortic stenosis), and mental deficiency. In a number of patients, this condition has been associated with neonatal hypercalcemia. This latter finding led to the initial description in 1952 of WS as "idiopathic hypercalcemia of infancy." In older children, metastatic calcium deposits have been found in the kidney, and osteosclerosis has been seen on x-ray in the skull and the metaphyses of the long bones.

Because the etiology of the hypercalcemia seen in WS has been debated for a number of years, the authors examined several aspects of calcium and vitamin D metabolism in five children with WS and seven age-matched controls. At the

time of this study, all patients, whose ages ranged from 2 to 14 years, had normal serum calcium concentrations. After an overnight fast, all received a 3 mg/kg bolus of calcium (as calcium chloride) and blood samples were analyzed over a one-hour period for serum calcium, parathyroid hormone (PTH), and immunoreactive calcitonin. In addition, the response of WS patients to an infusion of 200 U of synthetic human PTH was determined by measuring 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels. Serum calcium and phosphate concentrations were also measured at three and 24 hours after PTH administration.

Patients with WS had a delayed clearance of infused calcium when compared to the controls. No significant difference between the groups was noted in the PTH response, but the WS patients had a blunted immunoreactive calcitonin response.

Serum creatinine, phosphate, and total protein values did not differ between the two groups. Finally, the rise in 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations seen at three and 24 hours after the PTH infusion was not significantly different in patients with WS from the response reported in normal adults.

The authors conclude that delayed clearance of the exogenously infused calcium in WS patients is due to deficient secretion of calcitonin rather than abnormalities of PTH or vitamin D metabolism. In support of their conclusions, they point out that children with hypothyroidism secondary to thyroid dysgenesis have a similar delay in clearance of infused calcium and a blunted calcitonin response. These children had little or no functional thyroid tissue, and presumably lacked calcitonin-secreting parafollicular cells (C cells).

Culler FL, Jones KL, Deftos LJ: *J Pediatr* 1985;107:720.

**Editor's comment**—This study provides a possible explanation for the hypercalcemia associated with Williams syndrome. The authors have shown that the abnormality in calcium metabolism seen in patients with WS and thyroid dysgenesis might both result from a common pathophysiologic mechanism, namely, a deficiency of calcitonin secretion. The pathogenesis of the calcitonin deficiency in WS and its relationship to its other clinical manifestations remain unknown. It is interesting that the calcitonin deficiency was present in these normocalcemic older children, whereas the hypercalcemia seldom manifests itself after infancy. To better understand the physiology, calcitonin secretion should be evaluated prospectively in infants with WS and concomitant hypercalcemia.

## Letter to the Editor

### Celiac Disease and Short Stature

In response to comments in the June 1985 issue of *Growth, Genetics, and Hormones*, I think you will find the following of interest. I recently reported [in the *American Journal of Gastroenterology*, October 1985, Volume 80, Number 10] a patient with what I believe to be the first asymptomatic celiac disease with short stature reported in this country.

The patient, a 6-year-old boy who was referred to me because of short stature, had no gastrointestinal (GI) symptoms except for a history of mild diarrhea during the first year of life. He was also biopsied by my colleague Dr. Hagos Tekeste on three separate occasions.

Initial evaluation showed a growth velocity of 4 cm/yr, a normal growth hormone (GH) response to clonidine stimulation, a low serum somatomedin-C (Sm-C) level, normal D-xylose absorption, normal quantitative stool fat collection (on a diet containing 50 mg of fat), and a

small bowel biopsy consistent with celiac disease.

Adherence to a gluten-free diet restored the biopsy to normal and improved the growth velocity to 6 cm/yr. The patient's Sm-C level, which had been at levels consistent with GH deficiency prior to treatment, rose promptly after gluten was excluded from his diet. Anti-gliadin antibodies were not obtained, but are presently being measured by another physician because the patient has moved to another area since the report was made.

### Dr. Blizzard's Comments

Dr. Holman's letter was prompted by two discussions of celiac disease that appeared in Volume 1, Number 2 of *Growth, Genetics, and Hormones*. The abstract of Cacciari (*J Pediatr* 1983;103:708) related that short stature was caused by celiac disease in 8.3% of children in an asymptomatic group of 60 short children.

In his article on nutrition, growth, and growth failure, Dr. Lifshitz pointed out that much attention has been given in Europe to the association between celiac disease without

I am convinced that this is a very common disorder and that many of the so-called variant short stature group described by Rudman and others—particularly those with low Sm-C levels—may, in fact, be patients in this category.

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Director, Pediatric/Genetics and  
Endocrinology Center  
Texas Tech University Health  
Sciences Center  
Amarillo, Texas

significant GI symptoms and short stature. Lifshitz believes that it is reasonable to suspect celiac disease in children who are short without an adequate explanation. He recommends that suspect patients be challenged with a high-gluten diet for four to six weeks and that an intestinal biopsy to confirm the diagnosis of celiac disease be done as well.

Readers of *Growth, Genetics, and Hormones* are encouraged to write and share their clinical experiences and interesting cases with their colleagues.



## MEETING CALENDAR

**July 6-10** 26th Meeting of the Teratology Society, Park Plaza Hotel and Towers, Boston, Massachusetts. Contact: Alexandra Ventura, Administrative Assistant, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-564-1493)

**July 7-12** XVIII International Congress of Pediatrics. Sheraton Waikiki, Honolulu, Hawaii. Contact: Dr. Gerald E. Hughes, Director, Office of the International Congress of Pediatrics, American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

**September 12-13** 8th Annual Emergency Pediatrics. Royal Sonesta Hotel, Cambridge, Massachusetts. Contact: Department of Continuing Education, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118 (617-638-4605)

**September 22-26** 7th International Congress on Human Genetics. International Congress Center, West Berlin, Germany. Contact: Congress Bureau, DER-CONGRESS, Congress Organization, Augsburgstrasse 27, D-1000 Berlin 30 (Telephone: 030-24-60-11)

**October 16-18** Pediatric Nutrition Update. San Francisco, California. Contact: Extended Programs in Medical Education, University of California, Room U-569, San Francisco, CA 94143-0742 (415-476-4251)

**November 1-6** Annual Meeting, American Academy of Pediatrics. Washington, D.C. Contact: American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-433-9016) (800-421-0589 in Illinois)

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Celiac Disease and Its Effect on  
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Pubertal Development  
in Endurance-Trained  
Female Athletes  
by Alan D. Rogol, M.D., Ph.D.

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# GROWTH

## Genetics & Hormones

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### Prader-Labhart-Willi Syndrome: An Overview

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Prader-Labhart-Willi syndrome (PLWS), also known as Prader-Willi syndrome, is the most common dysmorphic form of human obesity. The prevalence of PLWS, as estimated from referrals of infants for evaluation of hypotonia, is estimated to be between 1:25,000 and 1:30,000. The features include obesity, short stature, small hands and feet, hypotonia, hypogonadism, and mental retardation. Emotional and behavioral abnormalities, including severe overeating, are common.

#### Etiology and Genetics

The etiology of PLWS has been the subject of considerable interest for endocrinologists and geneticists. Most cases occur sporadically within families, although recurrence in sibships, as well as concordance in monozygotic twins, has been reported. Prior to the de-

scription of cytogenetic abnormalities in PLWS, the risk of recurrence within a sibship was estimated to be 1.4% to 1.6%. Studies of banded karyotypes have shown that many patients with PLWS have chromosomal abnormalities involving the proximal portion of the long arm of chromosome 15. The

most common abnormality is an interstitial deletion of band 15q11-13, although other abnormalities involving this portion of chromosome 15, including unbalanced and apparently balanced translocations, have been described.

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### Pubertal Development in Endurance-Trained Female Athletes

Alan D. Rogol, M.D., Ph.D.  
*Associate Editor—Growth,  
Genetics, and Hormones*

Although recreational sports have been available to young women for a number of years, the availability of competitive athletics and other strenuous training routines to females is relatively recent. With a large number of girls, adolescent females, and adult women participating in sports activities, a new set of issues relating to the effects of such training on the reproductive cycle has been raised. The

questions below represent a summary of these issues:

- What are the effects of preadolescent endurance-type training on the progression of pubertal development?
- Is menarche delayed by endurance-type training?
- Are physically active girls who mature later better suited for and, thus, more successful in endurance-type events?
- What are the effects of endurance-type training on the reproductive function of post-menarchal adolescents?

#### Pubertal Process

The normal pubertal process has been extensively reviewed by Tanner et al and will not be restated here, except to note that this pro-

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# Prader-Labhart-Willi Syndrome: An Overview

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The reported incidence of cytogenetic abnormalities ranges from 50% to virtually 100%, which may be due to differences in the patient population, the clinical criteria for diagnosis, or the cytogenetic techniques utilized. A study by Niikawa and Ishikiriya comparing karyotype findings in 12 "classic" PLWS patients with those in 15 patients with less typical disease found that all "classic" patients had deletions involving band 15q11.2, whereas six of the atypical patients had chromosome abnormalities. In another study, Butler et al (1986) demonstrated—surprisingly—that PLWS was diagnosed at an earlier age in cytogenetically normal patients than in patients with cytogenetic abnormalities (5 years, 4 months v 9 years, 4 months). They also report other clinical differences in patients with the deletion: lighter hair, eye, and skin color; greater sun sensitivity; and higher IQ. The need for high-resolution chromosome banding to detect some of these deletions and the occurrence of apparent mosaicism in some patients further complicate the interpretation of cytogenetic studies.

Most of the chromosomal abnormalities seen in PLWS, particularly the interstitial deletions of 15q11-12, have occurred in families in which both parents had normal karyotypes. Studies of chromosome markers to determine the parental origin of the abnormal chromosome have shown that it is usually of paternal origin. Butler et al (see Table) found 13 "informative" karyotypes for determination of parental origin of the abnormal chromosome 15; all the abnormal chromosomes were paternally derived and represented *de novo* deletions.

Chromosomal analyses have proved particularly helpful in assessing hypotonic infants in whom the diagnosis of PLWS is being considered. Such studies have enabled physicians to diagnose PLWS in infants who have not yet become obese. This could lead to

earlier intervention to try to control weight gain.

### Clinical Features

Despite the developments in the cytogenetic identification of PLWS, most patients are still diagnosed on the basis of clinical findings. The Table summarizes the clinical features as reported in four series of patients. As might be expected, those features by which the diagnosis is made are seen in most, if not all, patients. Apparent differences in the incidence of certain findings could reflect real differences in the patient populations, the clinical criteria used, or the age at diagnosis. Certain features (such as short stature or obesity) may not be apparent in younger patients, whereas other features (such as hypotonia) may become less obvious with age.

Fetal and neonatal hypotonia and feeding problems in the newborn are the earliest clinical features of PLWS and may be indications for chromosome analysis. The feeding problems usually involve an inadequate sucking reflex, sometimes necessitating alternative feeding regimens such as gavage or even gastrostomy. Muscle tone may improve with age, usually late in the first year of life.

The facial features of individuals with PLWS are characteristic and include a narrow bifrontal diameter, almond-shaped eyes with mildly upslanted palpebral fissures, a thin upper lip, and downturned corners of the mouth.

### Intelligence and Development

Delay in attaining early developmental milestones and mental retardation are common in PLWS. Part of the early developmental delay can be ascribed to hypotonia, but language and social skills are delayed as well.

The mean IQ for older children is generally around 65, although a wide range has been reported. Butler et al report that the mean IQ of the PLWS patients with a de-

letion of 15q is higher than the mean IQ of those without the deletion (69.6 v 59.2). In a survey reported by Holm (1981), 41% of the patients were normal or in the "borderline" range. Sulzbacher et al (1981) found that the intellectual performance of children with PLWS resembles the performance of children labeled "learning-disabled" more closely than "retarded." This observation may be important in school placement for these children.

### Obesity and Diabetes

Obesity is frequently the most obvious presenting sign of PLWS in older children and adults. An increased caloric intake and decreased caloric expenditure and requirements are believed to account for the obesity. Non-insulin-dependent diabetes mellitus (type II) is seen in patients with PLWS and is probably related to the obesity. Five of the first 46 reported patients developed diabetes mellitus, which was diagnosed at ages 11, 12, 16, 17, and 27 years. These patients were described as poorly responsive to insulin, but did not develop ketoacidosis. Ten additional patients from this group had abnormal glucose tolerance but not clinical diabetes. A 2½-year-old patient with PLWS and diabetes has also been described. The incidence of diabetes in patients with PLWS, based on a survey of physicians managing PLWS patients, has been estimated at 7%.

### Stature and Endocrine Abnormalities

Although short stature is commonly seen in individuals with PLWS, its basis is uncertain. The average adult height (59 inches for females, 61 inches for males) is well below normal. However, the bone age is usually equivalent to the chronologic age or is only slightly delayed. Studies of growth hormone dynamics show changes similar to those seen in obese controls who do not have PLWS, including "blunting" of the growth hormone response to several pharmacologic stimuli. Treatment

of one patient with exogenous growth hormone did not accelerate the growth rate. Similarly, no increase in linear growth has been observed after treatment with thyroid hormone. Even though a slightly greater thyroid-stimulating hormone (TSH) response to thyroid-releasing hormone (TRH) is seen in PLWS patients than in obese controls, the failure of thyroid treatment to increase growth velocity is not surprising.

Hypogonadism and cryptorchidism are frequently seen in patients with PLWS. Hypogonitalism is seen in both sexes but is more readily noted in males, in whom more extensive studies of gonadal function and histology are available. Histologic examination of the testes has been reported in 12 af-

fected males ranging in age from 4 to 28 years. The prepubertal males have generally shown normal histology for age. In older males, however, the findings have been abnormal, but varied. Sertoli cells have usually been present, but the number and distribution of Leydig cells and germinal cells have varied from patient to patient. Tubules are usually small or atrophic.

The extent of sexual maturation is variable in patients who are not treated with hormones. Menarche may be early, late, or normal, and normal menses as well as oligomenorrhea have been described. Several centers have reported "precocious puberty," usually in females with premature adrenarche. Adrenarche has been reported to occur as early as 5

years of age in PLWS patients, but usually has not been followed by premature menarche. Several patients have been described who had early adrenarche (ages 7 to 8 years), followed by menarche at age 10 years; gonadotropin levels, however, were low. These patients may have a variant form of PLWS, or their premature adrenarche and menarche may be explained by the action of adrenal steroids and the peripheral conversion of adrenal steroids to estrogens. Adrenal steroids could produce "adrenarche" (development of pubic hair and early axillary hair), and peripheral conversion (in adipose tissue) of adrenal steroids to estrogen could cause estrogenization of the uterus and result in

*continued on page 4*

**Table** Clinical Findings From Five Groups of Patients With Prader-Labhart-Willi Syndrome

|                                | Hall and Smith<br>(1972) | San Francisco<br>Study (1980) | Bray et al<br>(1983) | Butler et al<br>(1986) |          |
|--------------------------------|--------------------------|-------------------------------|----------------------|------------------------|----------|
| Number of patients             | 32                       | 20                            | 21                   | 21 (D)*                | 18 (ND)* |
| Gestation                      |                          |                               |                      |                        |          |
| Poor fetal vigor               | 74%                      | 85%                           | 84%                  | 90%                    | 81%      |
| Breech presentation            | 40%                      | 22%                           | 38%                  | 35%                    | 24%      |
| Nonterm delivery               | 43%                      | 43%                           | 33%                  | 55%                    | 77%      |
| Neonatal period and infancy    |                          |                               |                      |                        |          |
| Low birth weight<br>( $<5$ lb) | 21%                      | 41%                           | 20%                  | 5%                     | 11%      |
| Hypotonia                      | 100%                     | 100%                          | 100%                 | 100%                   | 100%     |
| Feeding problems               | 100%                     | 100%                          | 90%                  | 100%                   | 100%     |
| Delayed milestones             | 97%                      | 100%                          | 90%                  | 100%                   | 94%      |
| Central nervous system         |                          |                               |                      |                        |          |
| Mental retardation             | 97%                      | 100%                          | 100%                 | 100%                   | 100%     |
| Seizures                       | 16%                      | 20%                           | 20%                  | 25%                    | 22%      |
| Personality problems           | 71%                      | 60%                           | 71%                  | 71%                    | 72%      |
| Growth                         |                          |                               |                      |                        |          |
| Obesity                        | 100%                     | 100%                          | 100%                 | 86%                    | 88%      |
| Short stature                  | 94%                      | 90%                           | 90%                  | 43%                    | 50%      |
| Delayed bone age               | 50%                      | —                             | 90%                  | —                      | —        |
| Sexual development             |                          |                               |                      |                        |          |
| Cryptorchidism                 | 84%                      | 87%                           | 100%                 | 100%                   | 100%     |
| Hypogonitalism (males)         | 100%                     | 87%                           | 100%                 | 100%                   | 100%     |
| Menstruation                   | —                        | —                             | 33%                  | 43%                    | 25%      |
| Other                          |                          |                               |                      |                        |          |
| Strabismus                     | 40%                      | 67%                           | 95%                  | —                      | —        |
| Small hands and feet           | 79%                      | 100%                          | 100%                 | —                      | —        |

Adapted from Bray GA et al: *The Prader-Willi syndrome: A study of 40 patients and a review of the literature*. *Medicine* 1983; 62: 59-80.

\*D = deletion; ND = no deletion (of chromosome 15).



## Prader-Labhart-Willi Syndrome: An Overview

*continued from page 3*

breakthrough bleeding, which could be misinterpreted as menarche. One patient who was said to have had menarche at 11 years of age was found to have no rise in luteinizing hormone (LH) or follicle-stimulating hormone (FSH) after administration of luteinizing-hormone-releasing hormone (LHRH). Examination of her ovaries following her unexpected death revealed immature organs; there was no evidence of ovulation. It is possible that some of the reports of precocious puberty are correct, but such cases need to be carefully studied and documented.

Basal serum LH and FSH levels in PLWS patients have been low or inappropriately normal, given the low serum concentrations of gonadal steroid hormones. The LH response to injected LHRH in PLWS patients in their second or third decade has usually been subnormal. Response to clomiphene treatment in males has been variable, perhaps related to dosage and duration of treatment. Treatment of a 23-year-old male with clomiphene was followed by maturation of the testes and an increase in serum testosterone and LH. These findings suggest that the pituitary-gonadal axis in PLWS patients can be stimulated by clomiphene treatment to secrete gonadotropins and gonadal steroids. Treating males with chorionic gonadotropin has also stimulated secretion of gonadal steroids in some but not all patients.

Dynamic studies of adrenal and thyroid function have been normal in most patients with PLWS. Cortisol levels reflect a normal diurnal rhythm, but an impaired adrenal response to adrenocorticotrophic hormone (ACTH), as reflected by plasma cortisol measurements, has been reported. A slightly enhanced release of TSH in response to TRH injection has been described. Measurements of circulating thyroxine are usually normal.

Eight available autopsy reports of PLWS patients (ranging in age

from 3 to 45 years) have provided little insight into the pathogenesis of the syndrome. Although several of the clinical features and endocrine abnormalities associated with PLWS suggest a hypothalamic problem, examination of the hypothalamus and pituitary by routine methods has failed to show pathologic lesions. Detailed neuroanatomic studies in documented cases of PLWS are needed.

### Therapy

Therapy has generally been directed toward dietary restriction and behavior modification, with some encouraging short-term successes. Pipes (1981) reported success in 19 of 24 patients who were managed in an interdisciplinary nutritional management program and followed carefully. In 11 of 12 patients seen before the age of 7 years, prevention of obesity was reasonably successful (for up to nine years in follow-up). Less success was noted in older children who were quite obese before starting the diet/behavior modification protocol. The long-term outlook for this approach to obesity management has yet to be demonstrated.

Surgical procedures (gastric bypass and, less commonly, gastroplasty and jejunoileal bypass) have been used in patients with morbid obesity who could not be managed with diet and behavior therapy. Although there has been some success with gastric bypass, these procedures should probably be reserved for those patients with life-threatening obesity for whom alternative approaches are not successful.

Educational and behavioral needs should be met on an individual basis, with particular attention to the possibility that the patient might be misclassified as "mentally retarded" when "learning-disabled" might be the more appropriate assessment. It is apparent that patients with PLWS should be managed by a coordinated team that can jointly

approach their many medical, nutritional, and psychological problems.

### Conclusion

Despite the relatively extensive literature that has accumulated regarding PLWS, the physiologic and biochemical basis of the disorder remains unknown. Most of the published information about PLWS consists of small series of patients from a single institution or anecdotal reports of complications or findings in single patients. There is need for coordinated, collaborative prospective studies of virtually every aspect of this condition, particularly the natural history, anatomic findings, and effectiveness of therapy.

Previous reliance on strictly clinical criteria for diagnosis has made interpretation of the literature and comparison of separate studies difficult. The finding of a specific cytogenetic abnormality in many PLWS patients may help to standardize the patient population available for study so that results of collaborative efforts will be more meaningful. Even the isolated

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### Address for Correspondence

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case report, if well documented, can contribute to our understanding of this condition. Earlier diagnosis by use of cytogenetic analysis of hypotonic infants may allow for more rigorous attention to diet and behavior and could decrease the morbidity and mortality associated with PLWS. The possibility that at least part of the gonadal dysfunction is of later onset, and is perhaps responsive to pharmacologic intervention, suggests that anticipatory treatment might be helpful.

For further information (for patients and their families) please contact: The Prader-Willi Syndrome Association  
Gene Deterling, Director  
P.O. Box 392  
Long Lake, Minnesota 55356  
612-473-2793

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## Pubertal Development in Endurance-Trained Female Athletes

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cess is usually an orderly one and is marked by a high degree of variability in its onset and completion. Once entrained, however, the variability between stages, although present, is much less pronounced.

Puberty may be considered delayed in girls if they have not achieved breast budding by 13 years of age or if more than five years have elapsed between breast budding and menarche. If a girl has not begun developing breasts by the time she is 13 years old, one should suspect an abnormality in pubertal development and strongly consider further evaluation. Any female over 15 years of age who has not begun pubertal development, and in whom there is no explanation for its absence, must have medical evaluation.

## Sociologic and Phenotypic Effects on Athletic Performance

In addition to the impact of the physiologic processes of puberty on athletic performance, one must also be cognizant of sociologic and phenotypic factors that occur with or affect athletic performance. Malina et al substantially broadened the analysis of the interaction between training and puberty in their two-part hypothesis. The first part of their hypothesis suggests that the physical characteristics seen with delayed adolescent maturation in females may be associated with successful athletic performance in selected sports. The later-maturing adolescent girl is characteristically longer-legged, narrower-hipped, more linear in physique, lighter in weight (proportional to height), and relatively leaner than her early-maturing peers.

The second part of their hypothesis relates to socialization. The early-maturing girl is perhaps "socialized away" from competitive sports as she loses her litherness

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## Pubertal Development in Endurance-Trained Female Athletes

*continued from page 5*

and the physical structure that is conducive to athletic success. In contrast, the later-maturing adolescent may not experience the same social pressures or desires to "phase out" sports activities as those entering puberty at early or average ages and may be more motivated to compete athletically.

### The Effect of Nutrition and Endurance Training on the Reproductive System

Lower animals, subhuman primates, and humans depend strongly on adequate nutritional intake for the reproductive system to function normally. According to Malina's hypothesis, the timing of the onset of puberty is more closely related to body weight and nutritional status than to chronological age. Studies in humans first implicated a "threshold" body weight and then a "minimum" percentage of body fat as important metabolic signals that determine the onset of puberty in girls. More recent reports suggest that this concept is too simple to explain the physiology of pubertal development, although there is a strikingly significant correlation between the onset of puberty and the attainment of a specific body weight or percentage of body fat.

If the attainment of a specific body weight or a specific fat composition does not explain the physiology of adolescent development, what other signals might be responsible for triggering the maturation of the reproductive axis? Recent studies in the monkey have suggested that certain hormones or metabolic substrates such as insulin, glucose, amino acids,  $\beta$ -hydroxybutyrate, or glycerol act as humoral derivatives of body mass and serve as important cues to the reproductive axis to stimulate the onset of puberty. It has been suggested that changes in the concentrations of these metabolic fuels, which occur during the

transition from childhood to adulthood, provide a signal to the neuroendocrine centers that regulate reproductive function. This signal may be correlated with the decreasing sensitivity of the hypothalamic gonadotropin-releasing hormone (GnRH) neurons to the feedback inhibition of gonadal steroid hormones or with the intrinsic maturational events in the brain that occur prior to pubertal development.

In the human, the restoration of cyclic reproductive function in women with secondary amenorrhea is associated with either adequate caloric intake or decreased physical exertion. Similarly, delayed puberty and disruption of cyclic reproductive function in women are associated with inadequate caloric intake and increased physical activity. During starvation, the "nonessential" or potentially detrimental processes, such as gonadotropin secretion, are decreased, while those essential for survival, such as adrenocorticotrophic hormone (ACTH) or thyroxine secretion, are retained. Delay in pubertal development during famine and the restriction of fertility to those few months when nutrition is adequate have been noted in the nomadic Kung San hunter-gatherers who live in the Kalahari desert.

Energy expenditure by itself or in concert with low body weight and low percentages of body fat can delay puberty in adolescent female dancers and other endurance-trained athletes. The effects of exercise on pubertal development must be addressed with respect to the variations described within the majority of children who are not in training.

One can readily assess the impact of exercise upon the onset and progression of the pubertal process in girls because early pubertal development does not confer an advantage in many sports. To the contrary, late pubertal development may be characteristic for females in sports like gymnastics or ballet, in which the prepubertal body configuration and flexibility may confer a profes-

sional advantage. Consequently, several investigators have attempted to ascertain if prepubertal or peripubertal exercise and training affect the menarche. In the early 1970s, Malina et al showed that a group of track athletes had a later onset of menses than a group of more sedentary women used as controls. They also found that Olympic athletes, who were presumably more highly trained than other female athletes, had later menarche than high school or college athletes.

Warren and Frisch et al have done intensive studies regarding the onset and progression of pubertal development in groups of young ballet dancers. There are unequivocal data that menarche is delayed by one to three years among these dancers and that secondary amenorrhea is quite prevalent. Warren has carefully distinguished between delayed puberty and adrenarche. She found that the dancers had delayed breast development and menarche, which are mediated by ovarian steroid hormones, but normal pubic hair development, which is largely mediated by adrenal androgenic steroids. In addition, the young women had increased long bone growth, an apparent asset for performing. Although the data are not in question, the underlying mechanism is not clearly defined. Since some of these young women progressed rapidly through puberty or attained menarche when forced to stop exercising (usually due to an injury), and since they incurred no change in body weight or body fat when they stopped exercising, Warren favors the hypothesis that it is the energy drain of training and competition rather than diminished body weight or body fat that is the primary cause of delayed menarche.

Women who trained as swimmers or runners before puberty were evaluated by Frisch et al, who noted delayed menarche when these women were compared to women who began training after puberty. The investigators noted a 0.4-year delay in menarche for ev-

ery one year of prepubertal training. The implication is that puberty was delayed because of the early onset of training. However, it is also possible that the body habitus that is conducive to success at these athletic endeavors is also susceptible to delayed pubertal development.

In theory, endurance-trained female athletes might have a characteristic hormonal profile or characteristic hormonal responses to stimuli. Unfortunately, there are very few data in this area. Consequently, relating delayed menarche (or secondary amenorrhea) to exercise must be done by excluding other causes. A number of conditions, including chronic bowel inflammatory disease and anorexia nervosa, must be considered and ruled out in each endurance-trained adolescent athlete before one can ascribe the hypogonadal state to exercise itself. The evaluation of such patients is outlined in Table 1.

Cessation of menses is the most obvious effect of endurance training upon the reproductive system. However, more subtle effects, such as luteal phase defects and chronic anovulation, can occur with regular or mildly irregular cyclic vaginal bleeding. A wide range of the prevalence of "athletic amenorrhea" (secondary) has been reported: 1% to 43%, compared to a 2% to 5% incidence of amenorrhea in the general population. This broad prevalence range can be attributed to methodologic limitations. For example, the definition of amenorrhea varies from cessation of menses for four months to cessation of menses for 12 months. In any event, young competitive athletes have noted a much higher incidence of amenorrhea than older women who run recreationally.

Are menstrual cycle changes reversible? Reversibility of gonadal axis defects has been assumed, but not proven. In a single study, two marathon runners experienced amenorrhea during long-distance training after running the marathon. Coincident with decreased training mileage, the

**Table 1.** Clinical Evaluation of Delayed Adolescence in Girls

|  |
|--|
| <b>History</b>   |
| <ul style="list-style-type: none"> <li>• Age of onset of breast development<br/>pubic hair<br/>menarche</li> <li>• Family history of reproductive system problems</li> <li>• Linear growth history (growth chart)</li> <li>• Central nervous system symptoms or signs (eg, craniopharyngioma)</li> <li>• Pharmacologic agents (eg, phenothiazines)</li> <li>• Features of anorexia nervosa</li> <li>• Exercise habits</li> </ul> |
| <b>Physical Examination</b>  |
| <ul style="list-style-type: none"> <li>• Height, weight, body proportions</li> <li>• Status of pubertal development</li> <li>• Presence of uterus (rectal examination)</li> <li>• Features of chronic systemic disease</li> <li>• Anosmia (Kallmann's syndrome)</li> <li>• Evidence of intracranial disease</li> </ul>   |
| <b>Screening Biochemical Tests</b>   |
| <ul style="list-style-type: none"> <li>• Complete blood count, creatinine, liver enzymes, electrolytes, calcium, urinalysis, erythrocyte sedimentation rate</li> <li>• Endocrine studies—T<sub>4</sub>, prolactin, dihydroepiandrosterone sulfate (DHEAS) to assess adrenarche, bone age, serial luteinizing hormone (LH) concentrations (urinary or serum) over three to 12 months to assess pubertal progression.</li> </ul>   |
| <b>Other Tests</b>   |
| <ul style="list-style-type: none"> <li>• As indicated by suspicious preliminary findings</li> </ul>  |

menses returned in both. The following observations suggest that secondary amenorrhea related to exercise may be temporary:

- When training is interrupted, long-distance runners and ballet dancers resume menses without change in weight
- Menses return when training intensity is decreased below a "critical level"
- Resolution of amenorrhea occurs in rowers after the end of rowing season
- Normal reproductive function is seen in young girl swimmers 10 years after training.

Although one cannot say with certainty that these natural activities of young women have detrimental longer-term reproductive consequences, there are enough anecdotal data to assure these young women that they are not at great risk for reproductive system dysfunction when they elect to reduce their exercise load. Although

fertility is unlikely in these amenorrheic patients, it is prudent for them to employ some form of birth control since "athletic amenorrhea" is not an absolute safeguard against conception.

Even when these women are amenorrheic, the available data frequently do not indicate specific reproducible hormonal abnormalities—the serum estradiol levels are usually within the low normal range and the gonadotropin concentrations are in the mid-range or low-normal range for the follicular phase (although they are probably too low for the relatively low levels of circulating estradiol). The responses to exogenous GnRH are normal.

The possible mechanisms for the apparent hypogonadal state in endurance-trained women are outlined in Table 2 and serve as the framework for our own studies summarized below.

*continued on page 8*



# Pubertal Development in Endurance-Trained Female Athletes

continued from page 7

## Reproductive System Function in Amenorrheic Long-Distance Runners: Preliminary Results

My colleagues and I recently undertook a cross-sectional study of highly trained adult female athletes. We reasoned that if we could not define an alteration in hypothalamic-pituitary-ovarian function in this highly selected group, we would have great difficulty in defining alterations in girls and young women whose exercise habits were less strenuous. We also reasoned that similar alterations might delay adolescent development in prepubertal girls who were involved in endurance training. Thus, our preliminary data might, by analogy, give us clues to the alterations in the physiologic pubertal process in these young women athletes.

Certain long-distance runners with secondary amenorrhea or severe oligomenorrhea show an unambiguous decrease in pulsatile luteinizing hormone (LH) secretion, despite mean serum gonadotropin, prolactin, and sex steroid levels that are normal. The reduction in LH pulse frequency is associated with normal or increased pituitary responsiveness to GnRH and intact ovarian estradiol secretion in response to GnRH-induced endogenous LH release. These observations implicate an alteration in the brain's regulation of pulsatile LH secretion (see Table 2).

By analogy, I would consider that endurance-type training in the prepubertal or peripubertal female might delay the normal acquisition of pulsatile gonadotropin secretion that occurs at puberty and maintains the prepubertal condition—few pulsations of low amplitude—for an indefinite period. However, it seems quite clear that the maturational processes in the brain continue naturally during this period of heavy exercise since puberty may be telescoped into a brief period (six

**Table 2.** Possible Mechanisms for Hypogonadism in Endurance-Trained Athletes

### Brain Mechanisms

- Altered frequency or amplitude of basal pulsatile gonadotropic secretion
- Loss of estrogenic positive feedback
- Enhanced sensitivity to estrogenic negative feedback

### Pituitary Mechanisms

- Impaired synthesis/release of gonadotropins
- Biologically subactive gonadotropins

### Gonadal Mechanisms

- Diminished target-organ sensitivity

### Other

- Hyperprolactinemia (multiple mechanisms)
- Beta-endorphin hypersecretion
- Variable metabolic clearance rates of gonadal steroid hormones

to 12 months) when endurance-training adolescents diminish their training schedule. Although one cannot locate precisely the altered portion of the reproductive system, abundant data implicate diminished hypothalamic GnRH release as the cause of "athletic amenorrhea" and delayed pubertal progression in young female athletes who are engaged in endurance training. At present there are no data to support or implicate any of the other mechanisms listed in

Table 2 as etiologic factors for the amenorrhea in these runners, although one cannot totally rule out these conditions without further study.

In summary, the limited data available indicate that there may be decreased pulsatile release of GnRH and subsequent diminution of pituitary gonadotropin secretory episodes. The control mechanisms as they relate to energy expenditure and nutrition remain speculative.

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## IN FUTURE ISSUES

Diarrhea and Growth in Third World Countries  
by Leonardo Mata, M.D.

Intrauterine Growth Retardation: Adaptation or Pathology?  
by Joseph B. Warshaw, M.D.

The Concepts of Genetic Linkage  
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Genetic Linkage in Endocrine Disease  
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Celiac Disease and Its Effect on Growth  
by D.H. Shmerling, M.D.

## Prospective Screening for Down's Syndrome Using Maternal Serum AFP

Maternal serum alphafetoprotein (MSAFP) has been used for many years to screen for neural tube defects. Recently, an association between low MSAFP levels and fetal chromosomal anomalies has been observed. It has been postulated that maternal screening for neural tube defects could be used not only to look for high AFP levels, which indicate a risk of a neural tube defect, but also for low values to identify prospectively fetuses with Down's syndrome. In this paper, the physicians responsible for the Connecticut genetics program reviewed their data from MSAFP screening for neural tube defects during the last four years. The normal values of alphafetoprotein must be adjusted for gestational age, maternal weight, and maternal age.

Women 35 years of age and older at the time of delivery have traditionally been offered prenatal diagnosis for chromosomal abnormalities. However, women less than 35 years old have not been offered prenatal diagnosis routinely because of the relatively low risk of having a child with a chromosomal abnormality. These authors suggest that when an inappropriately low MSAFP level is found in women under 35, a second trimester amniocentesis should be offered. They calculate that if their criteria for identifying low MSAFP levels are used, and if women under 35 with low MSAFP levels are offered amniocentesis, one amniocentesis out of 350 would be positive for a chromosomal problem. The present screening policy for chromosomal anomalies in the fetuses of women over 35 years of age is estimated to identify only 10% to 20% of all Down's syndrome pregnancies. Using low MSAFP levels to screen mothers under 35 years of age would be expected to identify an additional 20% to 25% of cases. Thus, the authors suggest that using the combined approach of amniocentesis or chorionic villi

sampling in women over 35 plus MSAFP screening with subsequent amniocentesis for those with low values could be expected to identify up to 50% of all children with Down's syndrome prior to 20 weeks of gestation.

Baumgarten A, Schoenfeld M, Mahoney M, et al: *Lancet* 1985;1:1280-1281.

**Editor's comment**—With the institution of maternal screening for alphafetoprotein in most states, it is anticipated that many fetal abnormalities will be identified. This type of program, which is aimed at identifying neural tube defects, will certainly detect a large number of fe-

tuses with anencephaly and spina bifida in the absence of a positive family history. In addition, a number of other abnormalities associated with high alphafetoprotein levels (eg, Turner's syndrome and hydrops) will be found.

One outgrowth of the MSAFP screening program has been the recognition that a low MSAFP level may also have important diagnostic ramifications since fetuses with Down's syndrome have, on the average, low alphafetoprotein levels in amniotic fluid and maternal serum. The pathogenetic mechanism is unclear, but the potential usefulness of screening is obvious. The costs of such a program may be enormous, but it would appear that if these authors' calculations are correct, the cost:benefit ratio favors this approach.

## Growth Without Growth Hormone: Evidence for a Potent Circulating Human Growth Factor

The investigators present a case report of a boy with poor growth and growth hormone (GH) deficiency who, at 4½ years of age, began to grow spontaneously at an accelerated rate (more than 7 cm/yr for more than five years). His bone age rapidly advanced from 3.6 to 12 years and he became massively obese. Repeat GH testing showed inadequate responses to pharmacologic stimuli, whether measured in the immunoreceptor or radioreceptor (IM-9 cell) assay. Somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) levels were within the hypopituitary range when measured by radioimmunoassay.

Laboratory investigation was undertaken to try to determine the etiology of the patient's accelerated growth. Relative somatomedin bioactivity by the embryonic chick pelvic rudiment method was nearly the same as that of a reference pool from normal children. The patient's serum had very great activity in an assay of erythroid progenitor cells (measuring

burst-forming units), indicating the presence of a circulating growth factor different from those usually described.

Geffner ME, Lippe BM, Bersch N, et al: *Lancet* 1986;1:343-347.

**Editor's comment**—This single case report may provide evidence for the growth observed in some children with intracranial tumors before or after therapy. Some children grow well despite low levels of GH and Sm-C. Many are obese, as is this child. His serum obviously contains a growth factor (at least for erythroid progenitor cells) that is not derived from epithelial, nerve, fibroblast, or platelet growth factors, since these are inactive in the BFU-E bioassay under the conditions employed. It is from the study of such patients and the extraction and purification of the appropriate "growth factor" that therapeutic strategies can be developed for short children and probably for those needing an anabolic but nonandrogenic agent (eg, patients with severe burns or debilitating nutritional disorders).

## Adrenarche and Skeletal Maturation During Luteinizing-Hormone-Releasing Hormone Analogue-Suppression of Gonadarche

The increased secretion of adrenal androgens and the associated early signs of sexual maturation are called "adrenarche." However, during puberty the effects of adrenal androgens upon skeletal maturation are masked by the influence of the more potent gonadal steroid hormones. The investigators have employed a human model to examine the role of adrenal androgen secretion on skeletal maturation. They studied 29 children with central precocious puberty whose gonads were suppressed with an analogue of gonadotropin-releasing hormone factor (GnRH<sub>a</sub>).

Dehydroepiandrosterone sulfate (DHEAS) levels, an index of adrenal maturation, were constant or increased in an age-expected manner. Ten of the 29 children had DHEAS levels above the normal range for their age. However, pre-

mature activation of the adrenal androgen axis did not always correlate with gonadarche; nor did the presence of pubic hair necessarily correlate with DHEAS levels. The increment in bone age over the increment in chronologic age decreased from 1.7 to 0.5, indicating that the GnRH<sub>a</sub> induced a return to a prepubertal gonadal steroid environment; this was associated with a slowing of skeletal maturation. DHEAS levels correlated roughly with skeletal maturation rate before and during therapy.

Thus, the data suggest that adrenal androgens may contribute importantly to epiphyseal maturation, although there does not appear to be a strict correlation among bone age maturation, adrenal androgen secretion, and onset of gonadal activity, at least in these patients with premature puberty.

Wierman ME, Beardsworth DE, Crawford JD, et al: *J Clin Invest* 1986;77:121-126.

**Editor's comment**—These data confirm and extend previous studies that have suggested the independent control of adrenal and gonadal steroid hormone secretion and the physical signs of pubarche and gonadarche. The data in the younger subjects show that premature activation of the gonadal axis does not necessarily imply premature activation of the adrenal axis. Moreover, the association between increasing levels of adrenal androgens and accelerated rates of skeletal maturation during childhood was confirmed. Those who remained pre-adrenarchial during therapy exhibited the greatest slowing of their skeletal advancement. Thus, adrenal androgens may play an important role in skeletal maturation.

## Testing With GRF (1-29) NH<sub>2</sub> and Somatomedin-C Measurement for the Evaluation of GH Deficiency

A large number of provocation tests are available for evaluating the somatotrophic function of the adenohipophysis. The tests are performed with substances and doses that exhibit pharmacologic, but not physiologic, effects. With most tests, it remains unclear whether they directly stimulate the pituitary or the hypothalamus. The detection and synthetic production of growth-hormone-releasing factor (GRF) (1-40) and GRF (1-44) have enabled us to estimate the secretion of growth hormone (GH) in a physiologic and specific way.

Recently, Ranke et al performed similar GRF tests in a large series of patients with growth disorders, administering the fragment (1-29) NH<sub>2</sub> as an intravenous bolus in a dose of one  $\mu$ g/kg. In addition, serum somatomedin-C (Sm-C) levels were determined by radio-

immunoassay (RIA). Thirty-eight children with familial short stature, familial tall stature, early normal puberty, or premature thelarche and pubarche served as controls. The usual arginine and insulin tests were normal in an additional 48 children with intrauterine growth retardation (IUGR), constitutional delay of growth and adolescence, dysmorphic dwarfism, or Turner's syndrome. These children were compared to 45 children with growth hormone deficiency (GHD) and abnormal insulin and arginine tests. Prior to these investigations, comparative measurements with GRF (1-40) and GRF (1-29) NH<sub>2</sub> were performed in 11 healthy volunteers. The results of both studies were indistinguishable from each other.

In the control group, the median maximal concentration that was

reached was 45.3 ng/ml. The values were distributed logarithmically:  $\ln x \pm \ln SD$  was  $3.81 \pm 0.67$ . The lowest normal GH value was 10.0 ng/ml. The median maximal values for the other groups were: IUGR, 67.2; constitutional delay, 28.0; dysmorphic short stature, 85.9; and Turner's syndrome, 25.8. Statistically, no difference could be established between and among the various groups. Correlations with age, sex, relative height, and pubertal development were not statistically significant.

In the pituitary dwarfs, the median maximal value was 5.1 ng/ml, but the individual levels varied considerably. In 11 patients, maximal GH levels exceeded 10 ng/ml, but all levels fell below 40 ng/ml. There was no significant correlation between the maximal GH levels after GRF and the peak values after arginine and insulin. However, the correlation between the peaks after GRF and the max-

imal levels reached during deep sleep was positive.

Sm-C levels above 0.4 U/ml in healthy prepubertal children and above 0.6 U/ml in pubertal children were considered normal. Sixteen of 22 prepubertal patients with GHD had both subnormal GH peaks after GRF and low Sm-C levels.

In 12 of 38 controls, the Sm-C concentration was  $<0.4$  U/ml, with normal peak values seen after GRF administration. In 19 of 23 pubertal patients with GHD, Sm-C and GH determinations were subnormal. One of the remaining four patients had a low Sm-C level with a normal peak of GH after GRF; the other three had normal Sm-C and GH levels. In these latter children, the previously established diagnosis of hypopituitarism certainly should be questioned. Nevertheless, 11 (25%) of 45 GH-deficient patients had GH increases in response to GRF that were within the normal range. Consequently, one has to assume a normal adenohypophysis in these patients and hypothalamic GRF deficiency as the primary cause of the dwarfism in this group. This is in accordance with earlier findings.

Ranke MB, Gruhler M, et al: *Eur J Pediatr* 1986;146.

**Editor's comment**—This is one of the largest published series evaluating the GRF test. The authors, among others, confirm the usefulness of the GRF (1-29)  $\text{NH}_2$ , which appears equally as potent as GRF (1-40) and GRF (1-44). With regard to the pathogenesis of pituitary dwarfism, it appears that a large number (25%) of patients have GHD due to a primary hypothalamic defect. However, it is probable that many more patients might actually have primarily impaired GRF production if the GRF test were to be repeated after several days of priming with GRF. One would not necessarily expect a pituitary gland that has been at rest

for many years to respond fully to one dose of GRF. With regard to the diagnostic value of the GRF test, investigation with GRF alone may produce a rather high incidence of false-negative results in patients with GHD. Thus, the combination of GRF and arginine and/or another test, along with the determination of Sm-C concentration, appears helpful. On the other

hand, the use of Sm-C values alone entails the danger of obtaining too many false-positive results in patients who may have GHD. Thus, the Sm-C level can be complemented by the GRF test; these tests plus another test for GH sufficiency using a pharmacologic agent, such as insulin or L-dopa, are important in evaluating patients with suspected GHD.

## Role of GH-Releasing Factor and Somatostatin on Somatic Growth in Rats

The investigator studied the role of growth-hormone-releasing hormone (GHRH) and somatostatin (somatostatin - release - inhibiting factor [SRIF]) in affecting growth hormone (GH) secretion and long-term growth in the rat by passively immunizing animals with antisera raised against GHRH and SRIF. GHRH antiserum administration significantly inhibited the normal increase in body weight observed in both young male and female rats as well as in newborn rats. The effects of GHRH and somatostatin antisera administration on serum GH concentrations were studied in neonatal rats. In animals between 1 and 20 days old, GHRH-antiserum administration significantly decreased serum GH concentrations compared with levels in control animals. In animals between 1 and 10 days of age, SRIF-antiserum treatment had no effect on GH concentrations, whereas SRIF-antiserum treatment significantly increased GH concentration in 15-day-old and 20-day-old animals.

Wehrenberg WB: *Endocrinology* 1986;118:489-495.

**Editor's comment**—These results confirm that the control of pulsatile GH secretion is through the episodic release of GHRH. Thus, it is not unexpected that those rats treated with GHRH antiserum would grow at a reduced rate; however, no data were pres-

ented to determine what organ systems were affected. Both male and female rats showed similar 25% to 30% decrements in weight gain, implying that GHRH is not involved in regulating the sexually dimorphic growth rates. In addition, the antiserum to GHRH was effective from birth, suggesting that neonatal, as well as later, growth is dependent on GHRH secretion.

In contrast, the passive immunization of neonatal rats with an antiserum to SRIF indicated that it is not until sometime after the tenth day of age that endogenous SRIF can actively regulate GH secretion. Previous investigators have not been able to show biologic effects of SRIF in animals under 5 days of age, so this finding in the present study is not unexpected. Thus, the results suggest that the elevated GH concentrations in neonatal rats are due to hypothalamic GHRH release.

That the rats treated with GHRH antiserum grew at all implies that the pituitary may release GH by a non-GHRH-dependent mechanism, or that some other growth factor(s) is (are) responsible for part of the complex process called growth.

One cannot necessarily transfer results obtained in rats to humans. It would be interesting to ask, however, if the human neonate has the same mechanism for GH release since human neonates have elevated GH determinations during the first few days of life.



## MEETING CALENDAR

**October 5-10** Fall Meeting. American Physiological Society. Clarion Hotel, New Orleans, Louisiana. Contact: Federation of Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-7010)

**October 9-10** 9th Annual Current Concerns in Adolescent Medicine. Warwick Hotel, New York, New York. Contact: M.J. Boehme, Associate Director, Continuing Education, Long Island Jewish Medical Center, New Hyde Park, NY 11042 (718-470-8650)

**October 9-11** 15th Annual Meeting. Child Neurology Society. Boston, Massachusetts. Contact: Child Neurology Society, National Office, Box 486 Mayo, 420 Delaware Street SE, Minneapolis, MN 55455 (612-376-3692)

**October 16-18** Pediatric Nutrition Update. San Francisco, California. Contact: Extended Programs in Medical Education, University of California, Room U-569, San Francisco, CA 94143-0742 (415-476-4251)

**October 29-30** Update on Endocrinology. The Cleveland Clinic, Cleveland, Ohio. Contact: Center for Continuing Medical Education, the Cleveland Clinic Foundation, 9500 Euclid Avenue, Room TT3-301, Cleveland, OH 44106 (800-762-8137) (800-762-8172 in Ohio)

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**November 2-5** 37th Annual Meeting, American Society of Human Genetics. Philadelphia, Pennsylvania. Contact: Gerry Gurvitch, Administrative Director, American Society of Human Genetics, 15501 B Monona Drive, Derwood, MD 20855 (301-424-4120)

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# GROWTH

## Genetics & Hormones

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## Diarrhea and Its Effect on Growth

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diseases of the GI tract and their resultant inhibition of good nutrition and normal growth. Many children in Guatemala, Bangladesh, and northeastern Brazil experience from six to nine episodes of diarrhea per year during their first three years of life.<sup>1-3</sup> Most episodes last for a few days and resolve without serious conse-

quences. Other incidents result in considerable losses of fluids and electrolytes or are accompanied by fever, anorexia, and considerable damage to the intestinal mucosa. The worst episodes yield sequelae and permanent damage, such as growth retardation, or result in death.

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The predominant etiology of diarrhea in the general population of underdeveloped countries is infection by viruses and bacteria. Studies in rural areas clearly suggest an infectious cause. For example, diarrhea initially affects one individual in the family (index case) and then spreads to other family and community members. Infants and toddlers are affected more frequently than older children, adolescents, and adults. The high prevalence of diarrhea in populations with poor personal hygiene and deficient environmental sanitation also points to an infectious cause and is supported by the identification of rotaviruses, *Campylobacter*, enterotoxigenic enteric bacteria, *Cryptosporidium*, *Shigella*, *Vibrio cholerae*, *Salmonella*, *Giardia*, and other parasites in most patients with diarrhea.

Longitudinal studies of children in deprived ecosystems have documented the significance of diarrheal disease in respect to poor nutrition and growth.<sup>1-3</sup> These studies reveal not only the frequency of diarrhea in infants and young children but also the severity of damage from infectious

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## Perspectives on Intrauterine Growth Retardation

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### Introduction

Fetal growth can be defined in terms of changes in newborn size, organ growth, and maturation, and by the many biochemical adaptations that prepare the fetus for extrauterine existence. Intrauterine growth retardation (IUGR) can result from environmental and genetic influences that limit the intrinsic potential of the fetus to grow, or that restrict growth because of decreases in the amount of available

nutrients. IUGR is most commonly defined as a birth weight of less than the tenth percentile at a given gestational age. It is only within the past 20 to 25 years that clear distinctions have been made between low birth weight caused by IUGR and that due to preterm labor. There may be a considerable overlap between IUGR and preterm delivery, which refers to birth at less than 38 weeks' gestation, largely because some of the same risk factors are common to both conditions. Teenage pregnancy, for example, may result in high risk for both prematurity and IUGR. Prognosis of IUGR depends on the underlying condition to a major degree.

### Influences on Fetal Growth

A variety of genetic and environmental factors affect fetal growth.<sup>1,2</sup> Genetic factors may be responsible for species or popu-

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### Effect of Diarrhea on Growth

Upon detection of growth deficiency or failure to thrive, pediatricians in industrial nations rarely consider infection as the first diagnostic possibility. In these nations, most problems of growth failure due to gastrointestinal disturbances are related to physiologic, enzymatic, immunologic, or metabolic alterations of a noninfectious nature<sup>4</sup> rather than infectious causes.

In developing countries, however, the situation is quite different—particularly in infants who are not breast-fed. Interestingly, most breast-fed children grow very well, even under extreme poverty.<sup>1</sup> By contrast, most children in poor rural and urban areas who are not breast-fed suffer several diarrheal episodes each year, usually resulting in weight loss. Diarrhea-induced weight loss is difficult to correct without prompt and adequate nutritional dietary therapy. Diarrhea often persists in children, even after correction of the infection. This persistence and recurrence of diarrheal episodes generally do not permit catch-up growth. This sequence of events is exemplified by the typical Guatemalan village child whose growth is illustrated in Figure 1.<sup>5</sup> In many rural areas, from 5% to 20% of the diarrheas persist for several days or weeks because of *Shigella* infections. Unfortunately, appropriate antibiotic therapy required for resolution of these infections is not available in most poor rural areas.

Another possible factor in persistent diarrhea is lactose intolerance—a frequent finding, particularly in viral diarrhea during the first year of life. For these infants, diarrhea persists for as long as they are fed cows' milk.

The Guatemalan boy whose growth curve is diagrammed in Figure 1 grew well during the period of exclusive breast-feeding and his growth parameters fell along the 50th percentile of the

growth chart of the National Center for Health Statistics (NCHS). When food supplementation was begun at approximately 6 months of age, however, a continuum of diarrheal episodes and weight loss was observed in connection with recurring gastrointestinal and upper respiratory infections. By 1 year of age, the child had experienced several bacterial and viral infections, and altered physical growth was apparent.<sup>6</sup> In addition, infection with parasitic organisms, such as round worms, may also disturb normal growth<sup>6</sup> and may have contributed to alterations in this child.

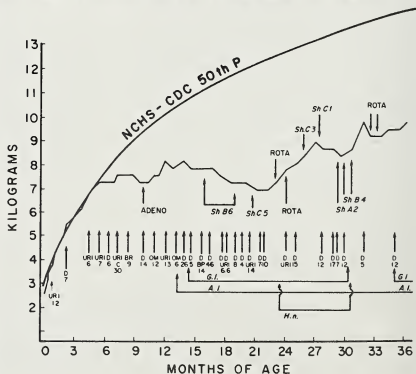
By age 1, the child was distinctly wasted. The encounter with a variety of viruses, bacteria, and parasites continued, and for one year the child remained wasted and at risk of developing severe protein-energy malnutrition or of dying. The possible metabolic and hormonal disturbances in children under such circumstances—who represent the majority of cases in deprived villages and slums—have not been established.

Field studies show that diarrhea adversely affects nutrition and physical growth.<sup>7-9</sup> Figure 2 illustrates

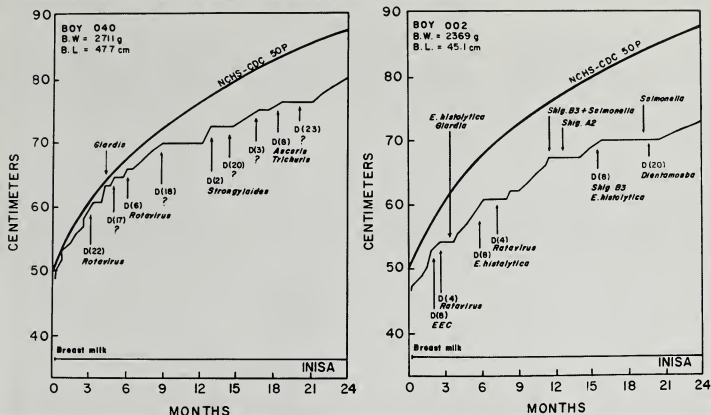
the relationship of growth retardation to diarrheal episodes in two Cauque children. Each recorded episode of diarrhea of known or unknown etiology coincided with an arrest in linear growth. These arrests were of shorter duration and negligible consequence during the first months of exclusive, intensive breast-feeding; upon weaning, however, the magnitude of arrested growth was more marked, often extending for several weeks or months. The effect was even more pronounced in the child with severe fetal growth retardation, as seen in the right side of Figure 2.

These cases demonstrate the prolonged effects of inadequately treated diarrhea and the lack of rapid catch-up growth because the child is repeatedly stricken with infectious episodes. Eventually, the cumulative effects of these episodes (with the additive effects of otitis media, acute respiratory infections, or exanthemas of early childhood) result in a markedly diminished growth rate. In the study village, virtually all children showed some degree of stunting by age 2 years. Cohorts defined by birth weight or by fetal maturity

Figure 1. Typical growth pattern of a Guatemalan village child. Note the drop-off in growth at about five months when the child was weaned from breast milk.



**Figure 2.** The relationship of drop-offs in growth to infectious diseases is clearly illustrated in these two children. The effect is more pronounced in the pattern on the right of a child with severe fetal retardation.



exhibited a positive correlation between intrauterine growth and poor postnatal physical growth.<sup>1,7</sup> The greatest impact is delivered by the adverse microbial environment of underdeveloped countries.<sup>1,5</sup> Overall growth deficit and much of the wasting reported in children throughout the world is probably the result of repetitive diarrheal diseases and other infections, which severely aggravate an already marginal or poor nutritional intake.

A long-term study, conducted in the poor, rural population of Costa Rica, a country in transition, demonstrated that growth was significantly improved as better sanitation was developed.<sup>9</sup> Although food supplementation during weaning frequently was not improved, the less intense infectious environment resulted in very low rates of enteric infection and diarrheal disease.<sup>10</sup> While growth failure was occasionally observed, it was the exception and was attributable to organic disorders or child neglect, as is the case in industrial nations.<sup>9</sup>

### Infectious Diarrhea Induces Malnutrition

Diarrheal diseases are the most important inducers of malnutrition worldwide, because they alter nutrition—and growth—through reduced food intake, disturbed digestion and absorption, impaired use of nutrients, and other metabolic alterations. Each episode has a varying impact on the host economy and nutrition, even when there is no limitation in food availability.<sup>7</sup> Diminished intake of food during diarrheal episodes is often substantial, especially among infants and toddlers. There are two predominant causes: anorexia and restriction dictated by traditions, beliefs, and taboos. In the latter case, the mother or other caretaker suppresses the food intake for days or even weeks, in the belief that food perpetuates the diarrhea.

Anorexia appears to be the most significant reason for decreased food consumption. It is triggered by interleukin 1 (previously known as leukocyte endogenous mediator) and by cachectin (tumor

necrosis factor), hormone-like substances released by macrophages and monocytes under the stimulus of infections or other stress. A manifestation of the "generalized acute-phase metabolic response," anorexia occurs regardless of the type, severity, and localization of infection.<sup>11</sup> Most foods are rejected, although breast milk is least so. The intensity of anorexia does not always correlate with the kind or severity of illness, and a child may become anorectic even with a common cold or mild diarrhea. The effect may last a few hours or extend for days or weeks. As much as 20% to 70% of the available food may be wasted or uneaten during bouts of diarrhea.<sup>7</sup>

Microbial action also increases intestinal secretion and lysis of cells in villous tips by rotaviruses, for example, or by stimulation of cyclic AMP and cyclic GMP by bacterial enterotoxins such as *Escherichia coli*. If repetitive losses are not corrected by rehydration and other therapies—

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## Diarrhea and Growth

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often unavailable in villages in poor countries—they contribute to malnutrition. Hypersecretion also can be induced by bile and fatty acids, hormones, and neurotransmitters, and by greater calcium cell permeability induced by mediators.<sup>12</sup> Agents such as *Giardia* adhere to the surface of enterocytes, while others such as *Cryptosporidium* lodge under the microcalyx but outside the cytoplasm. Some parasites multiply within epithelial cells and in the lamina propria, causing inflammation and bleeding (*Shigella*), or burrow in tissue, eliciting a granulomatous response (*Entamoeba*), or they reach lymph and blood vessels, resulting in sepsis (*Salmonella*).

These infections may generate profuse loss of water, electrolytes, cells, and nutrients, reducing the host to a state of acute malnutrition. Patients, especially infants and young children, may lose 10% or more of their body weight within hours, and may die if shock and dehydration are not promptly corrected. Cells, plasma, amino acids, lipids, vitamins, and hormones may be lost with injury to intestinal mucosa. The dysentery diarrheas are more damaging because they often are accompanied by a protein-losing enteropathy,<sup>13</sup> and exhibit toxic manifestations with weakness and prostration and high mortality.

As with other infections, diarrhea is accompanied by anorexia and fever, breakdown of muscle protein, discharge of insulin and glucagon, mobilization of leukocytes, and sequestration of zinc and iron. Vasoactive intestinal polypeptide (VIP), which inhibits the peristaltic reflex, and other gut hormones (motilin, enteroglucagon, and neurotensin) are increased or decreased during diarrhea. Prostaglandins are increased in diarrhea, including the mild forms seen in toddlers.<sup>14</sup>

### Conclusion

Infectious diseases, and diarrhea

in particular, are the main determinants of wastage and stunting of growth in children in underdeveloped countries. Nations that are able to diminish the incidence of diarrhea and other infections clearly exhibit a secular change in growth and height of children, as observed in Chile, Costa Rica, and other countries in rapid transition.<sup>5,15,16</sup> Children with no or fewer infections have better appetites, and their healthy parents provide better care. In turn, society benefits because of better use of available resources and increases in production. This might explain, in part, why certain very poor areas, which remain basically poor and consume minimal food, exhibit a remarkably good health condition. One example is the State of Kerala in India.<sup>16</sup>

Equally interesting is the observation that in some undeveloped countries, provision of food supplementation or food distribution centers has been unsuccessful in combating malnutrition. In particular, this is especially noticeable in the continuing presence of poor sanitation, which leads to diarrhea and other infectious diseases. As part of any major policy to prevent malnutrition in underdeveloped countries, attention must be directed toward the control of infectious diarrhea. Only in this way can malnutrition and growth failure be prevented.<sup>5,17</sup>

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## Perspectives on Intrauterine Growth Retardation

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lation differences in size at birth. Mean birth weight in human populations can range from 2,400 g in New Guinea to 3,880 g in American Indian populations. Although some of these differences can be explained by factors such as nutrition and maternal size, it is likely that ethnic differences in birth weight occur regardless of socioeconomic status. Males weigh an average of 150 to 200 g more than females at birth. This difference occurs in late gestation and may be related to the testosterone produced by the male gonad, but this has not been proven.

Hormones are important for fetal maturation and for many of the adaptive events that prepare the fetus for extrauterine existence. Insulin appears to be the principal growth hormone for the fetus. Other classic hormones appear to influence specific organ development rather than fetal size. For example, testosterone induces virilization of the genitalia, glucocorticoid influences lung maturation, and thyroxine modulates central nervous system development. With the exception of somatomedins, other putative growth factors, including epidermal growth factor, nerve growth factor, and transforming growth factors, are of likely importance in regulating organ growth and differentiation without greatly influencing newborn size. Somatomedins are present in and synthesized by a variety of fetal tissues, and umbilical cord levels of somatomedin-C have been correlated with birth weight.

Genetic and/or chromosomal disorders can profoundly alter fetal growth, with the degree of growth failure reflecting the specific defect. Growth retardation is a major feature of Down's syndrome, trisomies 13 and 18, and Turner's syndrome. Intrauterine infections may be responsible for as many as 10% of cases of IUGR and should always be considered in the evaluation. IUGR is commonly seen as

part of the symptom complex caused by toxoplasmosis, congenital syphilis, rubella, cytomegalovirus, and herpes simplex (the TORCH organisms). Recently, IUGR-associated malformations, including microcephaly and craniofacial abnormalities, have been described in newborns with an AIDS-related embryopathy.<sup>3</sup>

Drugs and chemicals causing IUGR include classic teratogens, such as antimetabolites, as well as common therapeutic agents such as phenytoin, trimethadione, and warfarin. Heroin addiction, cigarette smoking, and heavy alcohol use are also commonly associated with IUGR. More than 50% of infants born to mothers who drink heavily will be abnormal. In one study, the incidence of IUGR was 7% in babies whose mothers were light-to-moderate drinkers and 27% in those whose mothers were heavy drinkers.<sup>4</sup> Cigarette smoking is a powerful determinant of IUGR and results in a birth weight deficiency of 150 to 250 g.<sup>5</sup> This is most likely related to the combined effects of smoking on maternal appetite, uteroplacental blood flow, and maternal blood levels of carbon monoxide that further impair oxygen delivery to the fetus.

The terms "proportionate" and "disproportionate" have been used to distinguish IUGR newborns with decreased growth potential from those with restricted growth due to impairment of maternal nutrient delivery. Fetuses and newborns with decreased nutrient supplies exhibit disproportionate growth because of a relative sparing of brain growth, whereas congenital infections or genetic diseases that restrict growth potential result in proportionate or symmetrical growth retardation. These patterns of growth can be detected in utero and may indicate the underlying condition that ultimately results in IUGR. Poor maternal weight gain and fundal growth should alert the obstetrician to the likelihood of IUGR so that ultrasonography can be utilized to follow fetal growth parameters such as the biparietal diameter or the relationship of the

head size to the body size (which can be used to identify proportionate or disproportionate fetal growth in utero).

### Fetal Malnutrition

Fetal malnutrition is the most common cause of low birth weight. It can result from maternal malnutrition or from failure of the fetal circulation to deliver adequate substrates to the fetus generally because of maternal diseases that restrict uteroplacental blood flow. Conditions resulting in decreased uteroplacental blood flow include toxemia of pregnancy and maternal hypertension secondary to chronic renal disease. Fetuses in multiple pregnancies may exhibit restricted growth because of failure of the uteroplacental unit to provide optimal nutrition to more than one fetus in the uterus. The smaller twin of a monozygotic pair frequently exhibits IUGR because of arteriovenous communications within the chorionic plate that can severely compromise blood flow to one twin. IUGR is also observed in infants born in high-altitude regions and in those born to mothers with cyanotic congenital heart disease, presumably because less oxygen is available in both instances.

### Maternal Regulation of Growth

Walton and Hammond<sup>6</sup> reported that foals of Shire horses bred with Shetland ponies reflected the size of the mother. Shetland/Shire crosses born to a Shire mare were the size of normal Shire foals, whereas the foals born to the Shetland dam and the Shire cross were the size of the normal Shetland foal. Similar data in other species, including humans, suggest that constraints on fetal growth are imposed by the maternal uterine environment. In human pregnancies, fetal growth is generally not affected by the number of fetuses prior to the 26th week of gestation. After 27 weeks of gestation, however, the growth rate is slowed for triplets; the rate slows after 30 weeks for twins. Uteroplacental constraints may even become op-

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## Perspectives on Intrauterine Growth Retardation

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erative in singleton pregnancies when a weight of about 3,000 g is achieved regardless of the number of fetuses.

The Dutch famine of 1944-45 resulted in a mean birth weight reduction of about 300 g.<sup>7</sup> This effect was observed primarily when the period of starvation occurred within the last trimester of pregnancy. Behavioral testing and IQ performance data did not reveal any deficiencies when the population at risk was studied more than 20 years later.

IUGR caused by malnutrition may be multigenerational. In a marginally nourished rat colony maintained over nine generations, maternal weights and newborn sizes were markedly reduced when compared with those in normally nourished controls.<sup>8</sup> With re-institution of normal nutrition after five generations of marginal nutrition, it appeared that more than one generation of good feeding was necessary to correct the deficits.

### Clinical Evaluation of IUGR

Evaluation of the newborn with IUGR begins with measuring length, weight, and head circumference and plotting the results on standard growth charts to determine if the pattern of growth is disproportionate or proportionate. The Lubchenco charts are most commonly used although they may underestimate IUGR as compared with other standards. A careful assessment of gestational age should be made for all infants. Accurate dates can be confirmed by ultrasound examination of the fetus in early pregnancy or estimated less precisely by the Dubowitz exam immediately after birth.

Infants with nutritional IUGR have a scrawny, wasted appearance because they have so little subcutaneous fat. Many of their problems are associated with decreased metabolic reserves. These infants are at increased risk

for asphyxia and meconium aspiration; therefore, when IUGR is detected antenatally, there should be appropriate monitoring and careful planning concerning the mode of delivery.

Newborns with IUGR are also at increased risk for hypoglycemia and polycythemia. Hypoglycemia is probably due to low fuel reserves and a decreased capacity to carry out gluconeogenesis. Polycythemia occurs in response to the increased erythropoietin levels secondary to relative intrauterine hypoxia. Chronic intrauterine hypoxia may also result in persistent pulmonary hypertension with marked right-to-left shunting because of abnormal thickening of the small pulmonary arterioles in the hypoxic fetus. These are primarily problems of the nutritionally growth-retarded newborn. Those with IUGR secondary to congenital infection and/or genetic disorders are less likely to develop these complications.

### Fetal Adaptation in IUGR

While serious pathology may clearly be the consequence of markedly reduced uteroplacental blood flow, the majority of infants with nutritionally based IUGR have normal development and do not show significant differences in IQ or neurological scores when compared with normal newborns.<sup>9</sup> A strong case can be made that many of the features of nutritional IUGR represent fetal adaptation to a restricted nutrient environment rather than a pathologic condition.<sup>10</sup> Those fetuses with sufficient time to adapt to compromised nutrition may maximize their prospects for a favorable outcome.

In such infants, brain growth is spared because of a redistribution of fetal blood flow. A smaller overall fetal size may reduce substrate and oxygen needs to what can be provided by an impaired uteroplacental circulation. A redistribution of blood flow to the head supports brain growth and head circumference at the expense of both weight and linear growth. Increased blood flow to the brain associated with decreased blood

flow to the viscera increases the ratio of head circumference to abdominal circumference; this ratio can be measured in utero with ultrasound and thus identifies disproportionate IUGR antenatally. Vasopressin released in response to oxygen and/or nutrient deficiency is a likely mediator of increased blood flow to the brain. Polycythemia exhibited by these infants can also be viewed as an adaptation that results in an increase in the capacity of the blood to carry oxygen to the organs and tissues of the growth-restricted fetus.

Finally, severe nutrient restriction appears to be associated with accelerated maturation. Data from experimental animals and in humans suggest a lower incidence of hyaline membrane disease in fetuses with IUGR, which may increase survival if the fetus with IUGR is born prematurely. The infant with IUGR may therefore represent a successful adaptation to a substrate-deficient intrauterine environment. Those infants with IUGR who have sufficient time to adapt to a substrate-deficient intrauterine environment may be at lower risk for serious hypoxic injury that may occur in large fetuses born at term but subjected to acute uteroplacental compromise at the time of delivery. In other words, smaller may be better.

If maternal constraints on fetal growth can be viewed as an adaptation in the IUGR pregnancy, questions can be raised about the effects of intervention programs designed to increase fetal weights. This may potentially cause an adverse outcome in chronically malnourished populations that have already adapted to malnutrition and a constrained uterine environment. Careful evaluation and follow-up of such intervention programs are necessary.

In summary, adverse genetic and environmental influences can impose severe constraints on growth. IUGR resulting from congenital infection, genetic or chromosomal defects, and/or drugs and other environmental insults is likely to be associated with long-

term developmental disability. Fetuses with IUGR secondary to intrauterine nutritional deprivation may have more favorable outcomes due in large part to adaptations such as decreased fetal size with sparing of brain growth, mild polycythemia, and enhancement of pulmonary maturation. In many such infants, IUGR is an advantageous adaptation rather than a pathologic condition.

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### IN FUTURE ISSUES

The Concepts and Mechanisms of Genetic Linkage  
by Thaddeus Kelly, M.D.

Genetic Linkage and Endocrine Disease  
by Thaddeus Kelly, M.D.

Turner's Syndrome  
by Judith G. Hall, M.D.

Directory of Resource Groups for Patients with Endocrine and Genetic Disorders

## Letter to the Editor

### Russell-Silver Syndrome

In Vol. 2, No. 2 of *Growth, Genetics, and Hormones*, an article by Saal et al entitled "Reevaluation of Russell-Silver Syndrome" was abstracted. The Editor's Comment on that abstract prompts this letter.

One of the reasons that the Russell-Silver syndrome is heterogeneous is that *there is no such thing as the Russell-Silver syndrome*. Dr. Russell and Dr. Silver, in their original reports, described two entirely different syndromes. It is a mistake to combine the two and perpetuate the combination. I have mentioned this to Dr. Alex Russell, who agrees. Dr. Silver even described increased urinary gonadotropins in his patients. I point this out to our house staff when they refer such a patient to our clinic. In my experience, most of the patients referred to me for dwarfism, triangular facies, and intrauterine growth retardation fall into the "Russell" category. I have yet to see a patient with increased gonadotropins at a young age in the hemihypertrophy syndrome described by Silver. I continue to be a splitter instead of a lump.

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### Dr. Blizzard's Comments

Dr. Green's letter is in accord with the article written by Saal et al and published in the *Journal of Pediatrics* 1985;107:733. These authors stated that the Russell-Silver syndrome is a heterogeneous entity. Dr. Green would say it is not an entity at all. Undoubtedly, many would agree with Dr. Green. I have asked Dr. Silver to respond and his comments are listed below.

### Dr. Silver's Comments

The confusion about the Silver-Russell syndrome will un-

doubtedly continue until the specific etiology(s) of the syndrome has (have) been defined and/or a specific diagnostic laboratory test is available. Although the heterogeneity of findings suggests that multiple etiologies may be involved, there is no concrete evidence that this is so.

The Silver-Russell syndrome certainly fits the definition of a syndrome: "the sum of signs of any morbid state; a set of symptoms occurring together" (Dorland). As with most other syndromes, not every child with the Silver-Russell syndrome has every finding. However, the combination of all or most of the findings of congenital short stature continuing into childhood, asymmetry involving various parts of the body, triangular facies, clinodactyly, café-au-lait areas of the skin, syndactyly of the toes, and elevated gonadotropins (as first described by me in 1953, and in subsequent publications, and by Russell in 1954) occurs with sufficient frequency to be considered a specific syndrome with one or more etiologies.

Originally, the syndrome was known as the Silver syndrome in this country and the Russell syndrome in Europe. More recently, it has been termed the Silver-Russell syndrome or the Russell-Silver syndrome. Hopefully, Drs. Green, Saal, and others will soon provide us with the information that will permit us to make etiology-based diagnoses. Until then, I believe it is reasonable to continue using the names that have historically been assigned to what appears to be a single clinical syndrome with a characteristic phenotype.

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The address given for the Prader-Willi Syndrome Association in Volume 2, Number 3 was in error. The correct address is: 5515 Malibu Drive, Edina, Minnesota 55436. The phone number is 612-933-0113. Marge A. Wett is the Executive Director.



## Short Stature in Anorexia Nervosa Patients

In following 104 patients with anorexia nervosa, the authors found 85 suitable for comparison with 85 age-matched controls. As seen in the Table, a large percentage of the anorexic patients were short.

Information was available regarding parental heights for 35 patients. The mean actual height was at the 34th percentile, compared to a mean expected height at the 48th percentile, based on calculations of parental heights. Twenty-six females were postmenarchal, permitting comparison with the adjusted mid-parental height (Tanner scale). Nine had evidence of growth impairment and could not be classified under "familial short stature" by this method.

The patients' age at onset of anorexia ranged between 10 and 22 years. Symptoms first appeared an average of 12.9 months before seeking therapy, and the mean weight loss was 29 pounds (25% of total body weight). Of great importance in considering the etiology of the short stature is the fact that 80% developed anorexia after menarche, with symptoms of onset

**Table** Height-Related Statistics in Study Participants

| Height percentiles | Anorexic patients |    | Controls |    | Expected, % |
|--------------------|-------------------|----|----------|----|-------------|
|                    | n                 | %  | n        | %  |             |
| <5                 | 12                | 14 | 1        | 1  | 4           |
| 5-9                | 3                 | 4  | 4        | 5  | 5           |
| 10-24              | 28                | 33 | 16       | 19 | 15          |
| 24-49              | 22                | 26 | 28       | 33 | 25          |
| >50                | 20                | 23 | 36       | 42 | 50          |

occurring more than one year postmenarche in 61%.

The conclusion is that some factor(s) other than malnutrition may account for the fairly high incidence of short stature. Possibly, there is a pathophysiologic factor producing short stature and, subsequently, anorexia. Patients with anorexia sometimes exhibit several indications of a hypothalamic abnormality affecting thyroid, gonadal, and adrenal function. The authors state that excessive somatostatin production cannot be excluded.

Nussbaum M, Baird D, Sonnenblick M, et al. *J Adolesc Health Care* 1985;6:453-455.

**Editor's comment**—These data are not only important but also provocative, since they are unexplained within the context of cur-

rent knowledge. Most patients with anorexia might be expected to have growth failure secondary to malnutrition. In the majority of these patients, growth retardation preceded malnutrition. Growth hormone levels are increased in most patients with anorexia, although IGF-I values are low, as is expected with starvation. We do not know whether GH and IGF-I levels are normal before the onset of anorexia. If available, these data might provide insight regarding the etiology of anorexia nervosa.

Furthermore, could these patients have hypercortisolism long before the anorexia begins? (See the review of the endocrine symposium on neuropsychiatric disorders, reported by Dr. Lifshitz in this issue.) If present, hypercortisolism could account for the growth retardation.

## Hypercalciuria, Hyperphosphaturia, and Growth Retardation in Children With Diabetes Mellitus

The authors evaluated 157 diabetic children, 6 to 16 years of age, with insulin-dependent diabetes mellitus (IDDM) from 0.2 to 14 years. Eleven percent of the 157 subjects were shorter than would be anticipated, as assessed by comparison with the controls. Increments in height became smaller with the duration of IDDM and differed significantly from controls when IDDM had been present for more than seven years.

Growth retardation correlated with increased calcium and phosphorus excretion (as reflected by increased Ca/Cr and P/Cr ratios) and with poor control of IDDM (as evidenced by glycosylated hemo-

globin assays). Hypercalciuria was not correlated with increased serum calcium or other evidence of bone calcium mobilization. Hypercalciuria is reportedly caused by hypophosphatemia, and there was an inverse relationship between serum phosphorus and an increase of urinary P/Cr and Ca/Cr. Renal disease could not be demonstrated as a cause of increased Ca and P excretion when it occurred. The urinary loss of Ca also correlated inversely with plasma glucose at the time of urine collection. The increased urinary phosphorus appears to result from competition between glucose and both Ca and P for renal tubular reabsorption. There was some evidence of hypercalciuria as a renal response to functional phosphorus deficiency.

The authors conclude that the higher incidence of short children with IDDM is primarily associated with poor metabolic control, but the specific mechanism(s) of impaired growth is (are) not well defined and may not be due to a single cause.

Malone JI, Lowitt S, Duncan JA, et al. *Pediatrics* 1986;78:298.

**Editor's comment**—This study is very well done and carefully analyzed. The authors speculate that phosphorus supplementation might be beneficial. Further studies are certainly indicated to elucidate the causes and results of hypercalciuria and hyperphosphaturia, which are frequently seen in patients with poorly controlled IDDM. (See Harrison's article in Growth, Genetics, and Hormones, vol. 2, no. 2.)

## First Trimester Prenatal Diagnosis: Three Reports

Prenatal diagnosis of severe congenital diseases and malformations, which permits selective termination or altered management of affected pregnancies, has become an accepted part of modern medical practice. In the 1970s, amniocentesis and real-time ultrasound evaluation of the fetus during the second trimester were introduced for prenatal diagnosis. In the early 1980s, first trimester sampling of the chorionic villus (the fetal part of the placenta) was developed as an alternative modality for prenatal diagnosis. By the end of 1985, sampling procedures of more than 1,000 chorionic villi had been performed for prenatal diagnosis during the first trimester in ongoing pregnancies.

The article by Jackson in *Seminars in Perinatology*<sup>1</sup> reviews the technique and the indications for first trimester chorionic villus sampling. The technique involves localization of the placenta with ultrasound, and the vaginal removal (by suction under ultrasonic supervision). The test is most easily and safely done between the beginning of the 9th week and the end of the 11th week of gestation. Chromosomal, DNA, and most biochemical assays can be done on chorionic villus material, and the results of such testing are usually available within the first trimester.

The safety and accuracy of chorionic villus sampling have been established by the Internal Chorionic Villus Sampling (CVS) Registry, which was established by Jackson et al two years ago.<sup>2</sup> It is now clear from these data that the incidence of significant complications after CVS is less than 5%. In institutions with experience in the technique, the miscarriage rate after CVS is between 2% and 4%. The background spontaneous abortion rate is approximately 2% or 3%. Thus, additional risk of CVS-caused miscarriage seems small and is probably in the range of 1%.

Separation of fetal from maternal tissue is extremely important for accurate CVS results. One complication that has been observed is a higher rate of chromosomal mosaicism in chorionic tissue than in amniotic tissue.

Transabdominal CVS has recently been described by Smidt-Jensen et al.<sup>3</sup> It may be that this technique will avoid or minimize occurrence of infection, which has occasionally been seen in vaginal sampling.

1. Jackson L. *Semin Perinatol* 1985;9(3):209-218.
2. Jackson LG, Wapner RA, Barr MA. *Lancet* 1986;i:674-675.
3. Smidt-Jensen S, Hahnenmann N,

## Influences in Child Growth Associated With Poverty in the 1970s: An Examination of Hanes I and Hanes II, Cross-Sectional U.S. National Surveys

The association between poverty and growth deficits in children has been reported in developing countries as well as in the United States. In this study, a sample population of 13,750 black and white children aged 1 to 17 years was taken from the Health and Nutrition Examination Surveys, HANES I (1971-1975) and HANES II (1976-1980). These were employed to examine the associations between height, weight, triceps skinfold thickness, subscapular skinfold thickness, and dietary intake measures. The poverty index ratio (PIR) was used to define the poverty threshold. This index represents a more specific measure of poverty than income by including family size and composition, sex of head of household, farm/nonfarm residence, and the current Consumer Price Index. The PIR is widely used by the U.S. Government.

Overall, children above the poverty threshold were taller, heavier, and fatter than children in families living below the poverty level. Specifically, on the average, poor children were 1.3 to 1.9 cm

Hariri J, et al. *Prenat Diagn* 1986; 6:125-132.

**Editor's comment**—There are several advantages to first trimester prenatal diagnosis. These include safety for the mother if termination of pregnancy is deemed necessary and a chance to confirm results by second trimester amniocentesis, if appropriate. Earlier testing is also easier to handle psychologically for most families.

Since prenatal diagnosis is available and since it can be applied to detect many types of growth problems, physicians should be aware of these new advances and the availability of first trimester diagnostic techniques.

shorter, 2% to 3% lighter in weight, and 3% to 8% leaner (by skinfold measurements) than children above the poverty level. An interesting finding was that there were no reported differences in energy consumption and macronutrient intakes between the two groups. However, a trend toward improved growth among the poor children was noted between the time of the HANES I (1971-1975) and HANES II (1976-1980) surveys.

Jones DY, Nesheim MC, Habicht JP. *Am J Clin Nutr* 1985;32: 714-724.

**Editor's comment**—This study suggests that caloric intake does not appear to play a role in the growth failure reported among poor children. Both groups of children consumed equal diets, yet children who were below the poverty threshold were smaller in both weight and height, and had less reserve fat as measured by skinfold thickness than children above the poverty threshold. Other factors that may be associated with poverty, such as more frequent infections, insufficient medical care, and poor sanitation, may have had a negative influence on the growth of the children below the poverty threshold. The authors, however, do not discuss these concerns as they relate to growth.

## Special Report: The Endocrine Society Symposium on Endocrinology of Neuropsychiatric Disorders—June 25-27, 1986, Anaheim, California

Fima Lifshitz, M.D.

Associate Editor—*Growth, Genetics, and Hormones*

The symposium dealt primarily with the interrelationship of nutrition, neuropsychiatric disorders, and endocrinology. Dr. John E. Morley of the University of California at Los Angeles pointed out that many peptide hormones are involved in the control of human eating behavior. For example, cholecystokinin-8 has been called a satiety factor because of its ability to decrease feeding and delay gastric emptying through vagal activity. Dr. Morley also noted that glucagons, somatostatin, bombesin, calcitonin, naloxone, and other opioid antagonists act centrally as satiety factors. Corticotropin-releasing factor is also a potent anorectic agent. Peptides that enhance feeding behavior include the endogenous opioids, pancreatic polypeptide, galinin, growth-hormone-releasing hormone, and neuropeptide Y (bulimin).

Dr. Michelle P. Warren of St. Luke's-Roosevelt Hospital, New York City, discussed endocrine changes associated with anorexia nervosa. Dr. Warren stated that the incidence of anorexia nervosa appears to be increasing. It afflicts between 0.5% and 1.0% of white adolescents who are in the mid-socioeconomic group. There is a 6% concordance in incidence among monozygotic twins although the reasons for this are poorly understood. The peak age of onset is at about 12 to 13 years of age. For some unexplained reason, anorexia occurs more often in girls with scoliosis. The disorder is very rare among blacks and among men (the male-female ratio is 1:9). However, anorexia nervosa occurs in males who are training for competitive athletic activities and are restricting their food intake. Between 5% and 20% of professional ballet dancers can be

classified as patients with anorexia nervosa.

The endocrine changes seen in anorexia nervosa appear to be adaptive phenomena and are similar to those seen in starvation. These include lower levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and decreased pulsatility of LH over a 24-hour period. The pulsatility pattern reverts to that seen in prepubertal subjects. There is also increased secretion of endogenous opioids, but administration of naloxone restores normal LH secretion in only a small number of patients. Thyroid function resembles that in the "euthyroid sick syndrome," with increased 3,3', 5' triiodothyronine concentrations and decreased 3, 5, 3' triiodothyronine secretion. This reduces the metabolic rate and decreases muscle catabolism. Hypercortisolism often occurs because of

## Special Report: National Foundation-March of Dimes Clinical Genetics Conference on Muscle and Its Disorders—June 8-11, 1986, Philadelphia

Judith G. Hall, M.D.

Associate Editor—*Growth, Genetics, and Hormones*

The National Foundation-March of Dimes has reinstituted the clinical genetics conferences that were so successful in the 1960s and 1970s. The earlier conferences focused on the delineation of birth defects. However, because of advances in molecular genetics, developmental genetics, and clinical genetics, a new format became desirable. The new March of Dimes clinical genetics conferences are aimed at providing a better understanding of a particular organ system. At this year's conference, the subject was muscle. Clinical and basic research dealing with normal and abnormal muscle differentiation, muscle biochemistry, and muscle function was presented, allowing clinicians and researchers to learn from each other's work.

Sir Andrew Huxley convened the conference with a historical overview of muscle disorders. Several presentations on molecular research related to the actin and myosin genes followed. Not only have these genes been mapped and their differences described, but the progressive switching on and off during development and in different tissues is becoming well defined. The mapping of specific genes that are tightly regulated during embryologic and fetal development was clearly outlined at the meeting. Much of this work has been done in culture of muscle cells, but there seemed to be correlations in different animal model systems and in muscle from various sites of the body.

The clinical aspects of well-

defined muscle disease, both dystrophies and metabolic disorders, were reviewed. However, a whole new set of specific disorders, many of which can now be understood on a molecular level, were reported by various investigators. Various aspects of myogenesis—both in normal and abnormal cells, and during development and in regeneration—were discussed, as were the interaction of nerve and muscle and the biochemistry related to those interactions.

Experiments of nature—in which individuals with muscular dystrophy have also been growth-hormone-deficient or have had denervation, as by polio, but have not developed the usual muscle deterioration—indicate that many environmental factors can affect genetically determined muscle

decreased clearance of free cortisol, and it is presumed that there is increased secretion of corticotropin-releasing factor (CRF). Growth hormone is increased, but somatomedin-C (IGF-I) levels are decreased; this may conserve nitrogen. There is increased sensitivity to insulin, and norepinephrine secretion is reduced. Vasopressin also appears to be reduced and this may cause difficulty in handling water loads.

Consequences of the amenorrhea induced by starvation may be osteoporosis, stress fractures, and aseptic hip necrosis. All of these conditions are much more common in patients with anorexia than in normal females. Osteoporosis may result from scoliosis, but scoliosis may actually precede anorexia, an interesting observation.

Dr. George F. Koob of the Scripps Clinic and Research Foundation in La Jolla, California,

discussed behavioral and endocrine effects of CRF on the central nervous system (CNS). CRF is a potent stimulus for both adrenocorticotrophic hormone (ACTH) and beta-endorphin release. It has also been shown to increase CNS activity in a manner much like that of caffeine, and it potentiates the acoustic startle response. CRF also affects the limbic system, with its primary effects on learning and behavioral pathology, aggression, and changes in sexual behavior.

Another presentation at the symposium dealt with the pathophysiology of hypothalamic-pituitary-adrenal dysfunction in depression and anorexia nervosa. Dr. Philip W. Gold of the National Institute of Mental Health of the National Institutes of Health in Bethesda, Maryland, reported that hypothalamic dysfunction has been shown to be present in anorexia nervosa and depression.

Moreover, the hypercortisolism present in both disorders appears similar in pathophysiology, but different from that observed in Cushing's disease. Dr. Gold stated that in both depression and anorexia nervosa, there is probable increased secretion of endogenous CRF, attenuated ACTH responses to CRF, and adrenal hyperresponsiveness to ACTH. These abnormalities resolve when the patients gain weight. The hypercortisolism in depression and anorexia nervosa represents a central defect, whereas the hypercortisolism of Cushing's disease is believed to be caused by a defect of excessive ACTH secretion that seems to be localized in the pituitary. Dr. Gold and his co-workers believe that endogenous CRF secretion in patients with depression and anorexia nervosa may be significant in the symptom complexes of these illnesses.

function and deterioration. It appears that the size of muscle cells in Duchenne's muscular dystrophy may be critical in the dystrophic process. Growth hormone deficiency can slow the rate of progression of muscular dystrophy, possibly by limiting the size of the muscle cell. This and other observations give hope that new approaches to symptomatic therapy can be found. Fortunately, new techniques for studying muscle size, composition, and function, such as nuclear magnetic resonance, are beginning to yield clues about normal muscle physiology at the molecular level and about the distribution of abnormalities within the muscle cells.

Many well-known syndromes in which the etiology has not been defined—such as Marfan,

Schwartz-Jampel, and Marinesco-Sjögren syndromes—were examined as possible muscular dystrophies.

Perhaps the most exciting recent advance has been the molecular analysis of the Duchenne's muscular dystrophy gene locus. Two approaches have been used: that of "walking" along the X chromosome and the use of DNA from girls with Duchenne's muscular dystrophy who have X-autosome translocations that can be studied on a molecular level. The area of the Duchenne gene is now starting to be "peppered" with probes that allow prenatal diagnosis and carrier detection. It is now considered likely that the gene locus for Becker's muscular dystrophy is either within or very close to that for Duchenne's muscular dystrophy.

Linkage analysis of other myopathies and muscle problems is improving as well. For example, the linkage of myotonic dystrophy, by using more closely linked genes, now enables much more accurate prenatal diagnosis and premorbid recognition.

In general, the conference was exciting and stimulating, because it encouraged interaction between basic research scientists and clinicians. It is exciting to see how much progress has been made in an area in which new findings and techniques can be rapidly applied to clinical conditions. We look forward to seeing this same approach being used in future March of Dimes conferences to elucidate other organ systems. In this way, birth defects and genetic diseases will be further delineated.



## MEETING CALENDAR

**January 25-28** 34th Postgraduate Course, American Diabetes Association. Marriott's Orlando, Florida. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22320 (800-232-3472)

**March 15-24** International Postgraduate Course in Endocrinology. Siena and Assisi, Italy. Contact: Loretta Giacomello, Washington University School of Medicine, P.O. Box 8063, 600 South Euclid Street, St. Louis, MO 63110 (800-352-9862)

**March 23-27** 14th Training Course on Hormonal Assay Techniques. Bethesda, Maryland. Contact: Nettie Karpin, Executive Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

**April 27-30** Annual Meeting of the American Pediatric Society/Society for Pediatric Research. Disneyland Hotel, Anaheim, California. Contact: Debbie Wogenrich, Department of Pediatrics, University of New Mexico, Albuquerque, NM 87131 (505-277-6628)

**May 1** Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Disneyland Hotel, Anaheim, California. Contact: Dr. Gilbert August, Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue NW, Washington, DC 20010 (202-745-2121)

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**June 4-12** 47th Annual Meeting and Scientific Sessions, American Diabetes Association. Hyatt Re-

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**June 10-12** 69th Annual Meeting, The Endocrine Society. Indianapolis Convention Center, Indianapolis, Indiana. Contact: Nettie Karpin, Executive Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

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# GROWTH

## Genetics & Hormones

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# Lawson Wilkins—Pioneer in Pediatric Endocrinology and Growth Disorders

Robert M. Blizzard, M.D.  
*Chairman, Editorial Board  
Growth, Genetics, and Hormones*

Twenty-four years have passed since 1963, when Dr. Lawson Wilkins died at the age of 69. His demeanor, his accomplishments, and the esteem in which he was held by his peers and his extended family of pediatric endocrine fellows whom he trained are not known to the third and fourth generations of pediatric endocrinologists who are members of the Lawson Wilkins Pediatric Endocrine Society. Since volumes could be written about each aspect of Dr. Wilkins's life, an abbreviated biography is inadequate. Nevertheless, a brief history of Dr. Wilkins's life presents the opportunity to enhance the image of a man who should not be forgotten by pediatric endocrinologists, pediatricians, or geneticists.

Lawson Wilkins was born in 1894 in Baltimore. His father, Dr. George Wilkins, was probably the most highly respected family practitioner in the city. Historical accounts indicate that George Wilkins was intellectually curious, dedicated to his patients, and attentive to detail. His son exhibited the same characteristics. Mrs. Wilkins's death, when Lawson was five years of age, significantly strengthened the already close

bond between father and son.

After receiving a baccalaureate degree from Johns Hopkins University in 1914, Lawson Wilkins began medical school there; in 1917, along with many other medical students, he volunteered to go to Europe and serve as an orderly in a medical unit during World War I. After the war, he was accepted as an intern in internal medicine at Yale for a year. He then returned to Baltimore to serve a pediatric internship at Johns Hopkins, where the influence of Drs. Blackfan, Park, Kramer, and the other giants of pediatric medicine of the period further whetted his keen intellectual appetite.

But it was most likely his desire to follow in his father's footsteps that prompted him to enter pediatric practice in Baltimore in the early 1920s. Until the time he accepted a full-time academic position in 1946, Dr. Wilkins had practiced pediatrics for 25 years with intense intellectual curiosity and great compassion for his patients. This author has on several occasions met adults in Baltimore who re-

membered Dr. Wilkins fondly as their pediatrician. These individuals had no idea that Dr. Wilkins had made major contributions to medicine as an endocrinologist and a geneticist.

In 1935, Dr. Edward Parks, who was instrumental in the development of various subspecialties in pediatrics, invited Lawson Wilkins to establish an endocrine clinic in the Harriet Lane Home of the Johns Hopkins Hospital. Dr. Wilkins was reluctant, since endocrinology at that time was the trade of quacks and charlatans. He accepted the position, however, and with Drs. Fuller Albright, John Eager Howard, George Thorn, Robert Williams, and a few others, he transformed endocrinology into a respectable subspecialty.

Wilkins focused on the problems in pediatric endocrinology—particularly problems of growth and genetics—while his confreres tended to the accumulation of knowledge about endocrinology in adults. Although he was intensely interested in the metabolism and control of carbohydrate and fat metabolism, he assiduously avoided a clinical interest in diabetes. Possibly this was because Dr. Harriet Guild of the Harriet Lane staff had established a diabetes clinic and, characteristically, Dr. Wilkins would not intrude on the

*continued on page 2*

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## Lawson Wilkins

*continued from page 1*

work of others unless invited. Interestingly, he never considered diabetes a disease of the endocrine system, although he believed hypoglycemia was.

### Scientific Contributions

Lawson Wilkins greatly expanded our knowledge of endocrine physiology and pathophysiology. Some of us have been fortunate enough to have shared in his experiences in establishing pediatric endocrinology as a subspecialty. Drs. Bongiovanni, Migeon, and Eberlein shared his interest in adrenal steroid metabolism and the pathophysiology produced by deficiencies of various enzymes for cortisol synthesis, including defects in 21 hydroxylation and 11 hydroxylation that produce congenital virilizing adrenal hyperplasia. In 1950, Drs. Crigler, Klein, Gardner, Migeon, and Rosemberg joined Dr. Wilkins in successfully treating the first patients with congenital virilizing adrenal hyperplasia with cortisone. As always, Dr. Wilkins applied the knowledge he gained from his physiologic studies to therapy.

Drs. Grumbach and Van Wyk worked with Dr. Wilkins in his studies of sexual differentiation. In this area, Dr. Wilkins applied what had been learned from the animal experiments of Alfred Jost to postulate and prove that the anatomy in gonadal agenesis and pseudohermaphroditism in human beings could be explained by the presence or absence of androgens and Mullerian inhibiting factor.

It was with Dr. Wilkins that Gardner developed his interest in genetics and cytogenetics. It was Dr. Wilkins and his students who were among the first to apply the cytological techniques of Dr. Murray Barr to identify the inactivated X chromosomes (Barr bodies) in the nuclei of patients with Klinefelter's syndrome and in female pseudohermaphrodites. These diagnostic aids facilitated the diagnosis and therapy of patients with abnormal-

ities of sexual development.

With Dr. Wilkins, Clayton demonstrated that enzyme defects in the synthesis of thyroid hormone metabolism produce pathologic changes in the thyroid that simulate thyroid carcinoma. Dr. Wilkins had previously demonstrated during his years in practice the effect of thyroid hormone on cholesterol and creatinine metabolism.

These were classic physiologic studies, in which the effects of a hormone were investigated clinically. He had demonstrated during this same period that the epiphyses in patients with thyroid deficiency were misshapen as they calcified (epiphyseal dysgenesis) and delayed in appearance, and that epiphyseal dysgenesis was a frequent finding in the untreated cretin. With treatment, the epiphyses that had not appeared because of thyroid hormone deficiency were often dysgenetic when they did appear, but the epiphyses that were expected to appear after treatment was begun were always intact in their

development.

David Smith and this author benefitted from Dr. Wilkins's astute recordkeeping; he was a master in maintaining growth charts and other documents. With him, we published the effect of thyroxin treatment on the mental development of cretins.

### The Second Generation and Beyond

Other pediatric endocrinologists from the United States who trained with Dr. Wilkins between 1946 and 1960 were Drs. Shepard, Holman, Cara, Mosier, Cleveland, David, Green, Martin, Silverman, and Stempel. Many students from abroad who are now professors also trained with Dr. Wilkins. These include Drs. Bertrand, Eckert, Gerard, Bergada, Papadatos, and Prader. These endocrinologists and professors have trained the third generation of pediatric endocrinologists, who in turn have trained the fourth generation.

Dr. Wilkins wanted to be called "Lawson" by "his boys" as he



Lawson Wilkins plotting metabolic data from a patient with endocrine disease. It was Dr. Wilkins's compulsive accumulation, plotting, and analyses of such data that transformed the subspecialty of pediatric endocrinology into a highly respected scientific discipline.

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**Turner Parents Group**  
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**Turner's Syndrome Support Group**  
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**Association for Research into**  
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**Down's Syndrome Congress**  
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**Dystonia Medical Research**  
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**Human Growth Foundation**  
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**Little People's Association of**  
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c/o Robert Wood, President  
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Elizabeth Bay, NSW 2011  
Australia

**Osteogenesis Imperfecta**  
Foundation, Inc.  
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632 Center Street  
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**Support Organization for Trisomy**  
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**TARSA (Thrombocytopenia Absent**  
Radius Syndrome Association)  
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called those who trained under him, but esteem for him was so great that he remained "Dr. Wilkins" to most for many years.

It is not by chance, however, that there was only one female fellow, Dr. Eugenia Rosenberg, prior to 1960. It was simply Dr. Wilkins's policy not to accept women as fellows. He respected the intellect of female physicians, but he was reluctant to let them examine the male teenagers who came to him for consultation. With the acceptance of Drs. JoAnne Brasel, Virginia Weldon, and Irene Solomon as pediatric endocrine fellows at Johns Hopkins in the early 1960s (when he was professor emeritus but still active), he relented and realized that he had been unduly restrictive.

Lawson Wilkins was more than a scientific giant. He was a man of great magnetism and personality. Few who knew him could forget his bass voice, which he put to good use singing ballads and bawdy songs long into the night. He loved

to sail his boat on the Chesapeake Bay and tell jokes, which he masterfully embellished. He also adored—and was adored by—Lucile Mahool, his first wife, and Teence Anderson, to whom he was married after Lucile died in 1959.

At a meeting in Baltimore of the Lawson Wilkins Pediatric Endocrine Society in the mid-1960s, Dr. John Eager Howard related the following about Dr. Wilkins: "When I first met Wilkins, which was at a time I had heard about his studies. In response to my knock on the door, the rafters fairly reverberated to the booming voice that urged us to come in. His whispers in a conference could cause consternation, for his 'That fellow is putting out pure hogwash' might have been heard all over the room. But I should hasten to say that his comments were rarely uncomplimentary, for an immense generosity toward others was one of his

most endearing qualities." In accord with Dr. Howard's observations, this author found Dr. Wilkins to be a paradox in that he was gruff but gentle. And while he always dominated the situation, he never exhibited dominating behavior toward individuals.

Another mark of the quality of Dr. Wilkins's personality was the grace with which he relinquished his pediatric endocrine clinic and training program to Dr. Claude Migeon and this author in 1960. During the next three years, before he died in 1963, he was present much of the time, he remained intellectually curious, and he continued to contribute in all respects.

We in pediatric endocrinology and genetics are indeed blessed to have had such a man to lead us. The history of Lawson Wilkins is well worth passing along to the third and fourth generations of pediatric endocrinologists, and it is to be hoped that they will pass it along to the fellows who train with them.

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## Special Report: International Symposium: Growth Hormone and Growth—October 8-10, 1986, Buenos Aires, Argentina

Robert M. Blizzard, M.D.  
*Chairman, Editorial Board  
Growth, Genetics, and Hormones*

The conference began with three presentations on normal growth. Dr. Prader (Switzerland) emphasized that growth, skeletal maturation, the timing of puberty, and adult height are genetically programmed. Thus, we can predict adult height and compare it with target height, which is estimated from midparental height. The tempo of maturation and the final height are independent multi-

factorial variables that allow us to understand the classic variations. According to Dr. Prader, maturational variations may possibly be mediated by physiologic variations in the hypothalamic control of growth hormone (GH) secretion.

Dr. Prader also noted that although the sex hormones have little influence on adult height, they do accelerate growth and decrease adult height when they are present too early and in large amounts.

Dr. Tanner (England) discussed the prediction of adult height in

normal and pathologic conditions. He emphasized that the predicted height of patients is probably the best single index of the success or failure of treatment. Dr. Tanner pointed out that the Bailey-Pinneau (BP) method of predicting height, using the Gruelich-Pyle atlas, is not as accurate as other methods and should not be used. The Tanner-Whitehouse (TW-2) method is the method of choice.

Dr. Lejarraga (Argentina) reported on a comprehensive multicenter study to determine normal growth curves for children in Argentina. His data indicate that during the past 50 years, the ultimate height has increased by 10 cm in boys and 8 cm in girls.

The second major topic discussed was the normal hormonal relationships of the "hypothalamic-pituitary-peripheral" axis for GH secretion and action. Dr. Illig (Switzerland) explained that testosterone priming, which enhances the release of GH, can be

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## Special Report: International Symposium

*continued from page 3*

used to diagnose boys with constitutional delay of adolescence (CDA). GH peaks are measured following the administration of the stimulating agents, arginine and insulin. These data suggest that GH production may be increased with adolescence. Dr. Bierich's data regarding the integrated concentrations of growth hormone (ICGH) in normal boys at various stages of adolescence also indicated that GH production goes up at adolescence under the stimulus of sex steroids. These data also showed that ICGH in patients with CDA are lower than those in children of the same chronological age who are not delayed.

Dr. Van Wyk (USA) emphasized that somatomedin-C (Sm-C) or insulin-like growth factor I (IGF-I) is a modulator whose major role is to amplify other hormonal signals for a wide variety of growth processes. These include ovarian function, in which IGF-I potentiates the actions of follicle-stimulating hormone (FSH) in rat granulosa cells, and the synthesis of estrogens and luteinizing hormone (LH) receptors. Dr. Van Wyk also discussed the need for IGF-I and epidermal growth factor to work together to produce mitogenesis in mouse fibroblasts. He emphasized that somatomedins have autocrine and paracrine as well as endocrine functions. Nanomolar and, in some cases, picomolar concentrations of IGF-I stimulate a wide variety of physiologic processes, which—together with other growth factors—control growth in a quite different manner than has been thought in the past.

Dr. Blizzard (USA) presented data from the University of Virginia, which showed that growth-releasing factor (GRF) can stimulate GH release, even when given over 14 days with no down-regulation of GRF on GH release from the pituitary.

The third major topic concerned the diagnostic tests used in children with short stature. Dr. Bliz-

zard has found that the two-site immunoradiometric assay (IRMA) has distinct advantages in measuring GH in serum. Although measuring ICGH can assist in diagnosing GHD, some authors believe that a rise in ICGH is often superfluous.

ICGH tests done at night, preferably over 12 hours, are often adequate when comparing patients with short stature. The IM9 receptor cell assay is the only practical receptor assay at this time.

Dr. Van Wyk stated that the bioassays for IGF-I are expensive, tedious, not specific, and may provide misleading results because of inhibitors. In contrast, the radioimmunoassay for IGF-I has the potential for high specificity. However, it does not accurately measure IGF-I when unextracted plasma is used because of the presence of binding proteins. Nonetheless, Dr. Van Wyk believes that this assay has diagnostic value if we remember that values depend on factors other than GH, eg, starvation, thyroid, prolactin, and estrogen levels.

Dr. Jasper (Argentina) presented data showing that patients receiving GH often grow, even in the absence of increased IGF-I levels. Many discussants at the conference agreed with these findings, but all remained perplexed by these observations.

Other speakers discussed abnormal GH production and action in children. Dr. Illig reviewed the various types of genetic GHD and the gene cluster that is responsible for the synthesis of GH and human placenta lactogen or human chorionic somatomammotropin. She pointed out that to date the only gene defect shown to account for GHD is found in type 1A GHD patients. Surprisingly, not all of these patients have had cessation of growth with GH treatment, even though antibodies to GH have developed consistently.

Dr. Laron (Israel) discussed the

syndrome named after him. He emphasized that GHD may possibly lead to "less than expected" mental development. A lively discussion ensued on whether GH could have such an effect in utero or immediately after birth.

Dr. Eshkol (Switzerland) of Serono Laboratories in Geneva reviewed the preparation of GH by means of mammalian mouse tumor cells (currently being tested at Serono Laboratories). She also discussed additional purification procedures for native pituitary GH, which is still available in South America, Europe, and elsewhere.

The use of GH and other growth-promoting agents in both GHD and non-GHD patients was also a major topic. Dr. Jasper reported on the use of very small doses of ethinyl estradiol to increase growth rates in patients with Turner's syndrome. This result is comparable with the findings reported by Dr. Levine-Ross at the September 1986 NICHD workshop, *Advances in Research in Human Growth*.

On the other hand, several speakers commented that estrogen may not be preferable to other agents in treating patients with

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### Address for Correspondence

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Turner's syndrome. Dr. Stahnke (West Germany) reported the use of oxandrolone (Anavar) in 37 patients treated over 10 years. In these patients, height increased over the first year and, in some, persisted during the second year. The height age to bone age ratio was not adversely affected.

The use of GH in Turner's syndrome was discussed by Dr. Raiti, who directed a collaborative project through the NIH's National Hormone Distribution Program. Although, in general, growth rates increased, the effect of GH on ultimate heights could not be determined, since the program was disrupted when GH was withdrawn from distribution because of the Creutzfeldt-Jakob disease incident. In his study, Dr. Raiti noted that GH did not always increase growth rates while Dr. Stahnke reported that six of eight of his Turner's syndrome patients treated with GH had increased growth rates.

Dr. Raiti presented results from a collaborative study in Germany using Somatomorm® Kabivitrin in GHD patients. The

study patients had a very positive growth response and when recent preparations were used, they exhibited only a minimal antibody response. The growth responses were inversely related to the initial height and the degree of bone age delay, and were directly related to the genetic tendency for tall stature and to a diagnosis of isolated GHD (when compared with multiple hormone deficiencies).

Dr. Bierich also has demonstrated that GH treatment increases growth velocities in patients with constitutional delay. However, he feels that treatment should be reserved for the few patients in whom this agent may be preferable.

Drs. Tanner and Bierich discussed the desirability of increasing the dosage of GH when puberty begins, since GH production very possibly may be increased during normal puberty. Dr. Larón commented that GH may have an additional action on growth of the penis, which is another possible reason for increasing the dosage of GH in males at puberty.

Dr. Poskus and Retegui (Ar-

gentina) presented papers on antibodies to GH and the use of monoclonal antibodies to identify the sites on the GH molecule that allow for binding to receptors. These types of studies someday could allow us to identify patients with immunologically active, but biologically inactive, hormone.

Dr. Raiti discussed some of the problems that may arise during treatment with GH. He emphasized that to his knowledge no patients have developed diabetes while receiving GH. Reports indicate that there may be an increased incidence of slipped capital femoral epiphyses in patients treated with GH. With the possible exception of one case in New Zealand, there have been no additional cases of Creutzfeldt-Jakob disease in patients who have received GH.

Dr. Larón emphasized the need for team counseling of GHD patients. If GHD has been diagnosed and treated in early infancy or childhood so that the child grows at a relatively normal rate, many psychological difficulties can be avoided.

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## Special Report: Laurentian Hormone Conference— August 24-29, 1986, Montebello, Quebec

Alan D. Rogol, M.D., Ph.D.  
*Associate Editor  
Growth, Genetics, and Hormones*

At this conference on hormones, Dr. Anthony Cerami reviewed the status of cachectin—a macrophage protein that induces the catabolic state. This new hormone-like activity is increased in the circulation under conditions in which the predominant mode of metabolism is catabolic. Whether different types of stress—eg, starvation, cancer, or burns—induce a

similar hormone is not yet determined. Because the activity measured is very similar to that of the tumor necrosis factor, cachectin may be a member of a family of hormones with activities opposite to those of the somatomedins.

Dr. Robert Ryan reviewed the structural requirements for the binding of the gonadotropins to their receptors. Not only are there peptide-binding domains, but there are also specific sites of the receptor complex that bind the complex carbohydrate chain. These are separate membrane

constituents called lectins. This complex interaction leads to the microaggregation of the receptor complexes and is absolutely required for activation of the adenylyl cyclase second messenger system.

Specific domains within the primary sequence of the alpha (and beta) subunits have been described; these subunits are crucial to the tightly constrained, folded structure of the alpha-beta dimer. Significant structural changes do not allow subunit binding and thus produce biologically inactive molecules.

Dr. Patricia Donahoe reviewed new data on the molecular biology of mullerian-inhibiting substance (MIS)—the fetal regressor for the mullerian system in the male fetus. This substance is very closely related to tumor necrosis factor and

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## Hormone Conference

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may have important therapeutic value in cancers of the reproductive tract in females as well as in males. The biology of the material is influenced by the ambient steroid hormone levels, with androgens increasing and estrogens decreasing the MIS activity. This is probably why there is no expression of MIS activity in the female fetus. In the ovary, its probable mechanism of action is that of meiosis inhibition.

In a cultured cell line, MIS is able to inhibit the phosphorylation of the epidermal growth factor (EGF) re-

ceptor by inhibiting the tyrosine kinase activity of the liganded receptor. This may be the fundamental mechanism of action—inhibiting the transmembrane signalling of liganded growth factor (and growth-inhibiting) receptors.

Dr. Robert Lefkowitz discussed his continuing work on the mechanism of homologous desensitization of the beta-adrenergic receptor. He described a new enzyme, beta-adrenergic-receptor kinase (BARK). As desensitization to agonists occurs, this enzyme moves from the cytosol to the membrane.

There are striking similarities to the rhodopsin kinase of the visual system that is "homologously desensitized" by light. BARK is able to phosphorylate only the desensitized receptor. An agonist-induced change in membrane protein allows phosphorylation in the carboxyterminal serine and threonine-rich region. This leads to the uncoupling of the receptor from the cyclase enzyme, sequestration of the receptor, breakdown by phosphatase activity, and regeneration of functional activity as the receptor returns to the membrane. The enzyme can affect a number of receptors so that the *specificity* of this process is built into the specificity of the liganded receptor.

## Special Report: The David Smith Workshop on Malformations and Morphogenesis—August 1986, Burlington, Vermont

Judith G. Hall, M.D.

*Associate Editor*

*Growth, Genetics, and Hormones*

A variety of new clinical syndromes were described at this meeting and a number of well-known conditions were revisited. The hypertelorism hypospadias (BBB) syndrome and the hypertelorism dysphagia (G) syndrome were discussed in some detail; the suggestion that they may actually be alleles or the same condition was supported by much of the discussion.

A number of the papers presented suggested that pigmentary abnormalities in the presence of mental retardation with or without additional congenital anomalies may be an indicator of chromosome mosaicism. This finding has been well demonstrated in tetrasomy 12p mosaicism (Pallister-Killian syndrome); pigmentary

streaking is also seen in diploid/triploid/mixoploidy. A number of unusual patients were presented in detail at the meeting. Many of these patients had normal leukocyte chromosome studies, but chromosomal mosaicism was apparent in fibroblast chromosome studies. Hypomelanosis of Ito (streaky areas of decreased pigmentation with asymmetry in the size of the two sides of the body) is a syndrome deserving further study; it was suggested that it may represent chromosomal mosaicism. The take-home message was that fibroblast cultures should be strongly considered for patients who have normal peripheral blood chromosomes, pigmentary abnormalities, mental retardation, and short stature with or without additional anomalies.

Several new observations were made concerning neural tube defects. Careful autopsies of children

who had spina bifida with no additional congenital anomalies showed marked abnormalities of blood vessels to the affected part of the spinal cord and vertebral area. It is not clear whether this change is a primary or secondary disturbance. A distinction can be made between high neural tube defects (primary neurulation defects) and lower neural tube defects (canalization defects) in terms of the recurrence risk for having another child with a neural tube defect. Lower neural tube defects appear to have much less risk of recurrence for the family. Brain stem auditory evoked potentials may be a useful way of determining whether or not a particular patient with meningocele has abnormal brain development.

The recent film and play about John Merrick, the "Elephant Man," focused a great deal of attention on neurofibromatosis, which was addressed in an interesting presentation. The "Elephant Man" may not actually have had neurofibromatosis. Review of the autopsy findings on John Merrick and the historical information that is available today strongly suggest that it is much more likely that he actually had the Proteus syndrome, a condition described by Wiedemann and characterized by ham-

artomatous overgrowth.

Several presenters reviewed the accuracy of prenatal ultrasonic diagnosis for congenital anomalies and suggested that the technique may be only 50% accurate unless one focuses on a particular body area or knows what to suspect in a particular area. Thus, the physician should alert the ultrasonographer if there is an area of concern when careful examination is indicated. It was strongly recommended that fetal karyotyping of amniocytes be done when a structural abnormality is found on ultrasound during the third trimester. Indeed, a chromosomal abnormality is present in as many as one third of cases in which congenital anomalies are detected during the third trimester. Knowing that a chromosomal abnormality is present may alter management.

A new syndrome that may be quite common was described. The urofacial syndrome consists of obstructive urologic problems seen in association with abnormal facial muscular movement, so the child's smile looks more like a grimace. Intelligence is normal, and the condition probably has an autosomal recessive inheritance.

It would appear that Pena Shokeir syndrome (an autosomal recessive syndrome characterized by congenital contractures and hypoplastic lungs) is not a diagnosis but rather a phenotype that results from decreased intrauterine movement of the fetus and is attributable to many different causes. Thus, when any of the features of the Pena Shokeir phenotype (intrauterine growth retardation, congenital contractures of limbs, craniofacial anomalies, hypoplastic lungs, short umbilical cord, or polyhydramnios with short gut syndrome) is present, physicians should look for the other signs in the newborn infant. A variety of pathologic findings have been seen in babies diagnosed as having Pena Shokeir syndrome. However, the reported familial cases are all probably autosomal recessive disorders, and prenatal diagnosis should be offered for any future pregnancies.

## Special Report: 25th Annual Meeting of the European Society for Pediatric Endocrinology—August 31-September 3, 1986, Zurich, Switzerland

Jürgen R. Bierich, M.D.

*Associate Editor*

*Growth, Genetics, and Hormones*

In a round table discussion that opened the meeting, Drs. Raiti and Kaplan reported on the use of biosynthetic human growth hormone (hGH) preparations in the United States, while Drs. Job (France) and Preece (England) reported on their use in Europe in patients with classic pituitary dwarfism. In all studies, the results were comparable with those obtained with native pituitary hGH. Initially, patients developed high titers of antibodies to growth hormone. Subsequent improvements in purification techniques significantly reduced antibody incidence to that seen with the highly purified native pituitary preparations. Low titers are clinically meaningless and do not inhibit growth.

Drs. Albertson-Wiklund (Sweden), Bierich (Germany), and Brook (England) discussed the use of hGH in nonclassic hypopituitary short stature. The results were excellent in patients with constitutional delay of growth and adolescence and in those with partial growth hormone deficiency (GHD). Spontaneous growth hormone (GH) secretion was diminished in both types of patients. Thus, hormone treatment serves as replacement therapy. Girls with Turner's syndrome also were successfully treated in many cases.

Dr. Prader (Switzerland) reported the results of two large longitudinal growth studies in Zurich. According to the data, the secular acceleration of growth and maturation has been constantly positive

for decades in young men, but this appears to be no longer true for infants. Prior to puberty, body height and growth velocity are identical for both sexes. Differences appear with the onset of the pubertal growth spurt, which starts at age 10 in girls and at age 12 in boys. This growth spurt is much more rapid in boys than in girls, and explains the difference in height between adult men and women. Sex hormones are responsible for the pubertal growth spurt but not the final height. In contrast, the midgrowth spurt, which occurs at age 7, is independent of sex and gonads and corresponds to the adrenarche.

Stanhope et al (England) reported on the mechanism of the pubertal growth spurt induced by pulsatile gonadotropin-releasing hormone (GnRH) treatment. Twenty-six normal short patients received GnRH subcutaneously for 90 minutes each night for ten to 16 months. The girls immediately began to secrete increased amounts of GH. The pulse amplitude was increased but not the number of pulses. In contrast, boys first demonstrated a growth deceleration and diminution of GH secretion. During maturation and coinciding with a testicular volume of 10 ml, GH secretion increased and the pubertal growth spurt occurred. Hindmarsh et al, who are in the same investigative group, found a highly significant positive correlation between growth velocity and circadian GH secretion. This led these researchers to administer hGH subcutaneously, 2 IU each night for six-month peri-

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## Special Report: ESPE Meeting

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ods, to 17 children with short stature. A gain in growth velocity (SDS) from  $-0.49$  to  $+2.86$  was observed.

Most idiopathic GHD is attributed to perinatal lesions. However, the pathogenesis of these processes is still unclear. Charlesworth et al (England) studied high-resolution computed tomographic scans of the pituitary and hypothalamus of five GHD patients. Definitive enhancing lesions were found in the anterior hypothalamus in each case. Obviously, most cases of pituitary dwarfism arise from hypothalamic damage. These findings are in accord with numerous reports concerning growth-releasing factor (GRF) tests. In the majority of cases, the patient's own GH secretion can be stimulated by exogenous GRF.

Argente et al (France) presented interesting correlations between plasma levels of GRF (by radioimmunoassay) and sexual maturation. At midpuberty, plasma GRF levels increased fivefold in girls but only twofold in boys over prepubertal values. Patients with idiopathic delayed puberty had markedly lower values.

Garnier et al (France) explored the continuing difficulties in determining the cause of short stature. This group investigated hGH secretion in 54 children with growth failure by evaluating nocturnal sleep and GH release to GRF and by performing various pharmacological tests. They concluded that the GRF test does not differentiate among atypical growth disorders.

The final height of 22 patients with hormone deficiencies treated with long-term GH correlated significantly with the midparental height and inversely with the height at onset of therapy, according to a study by Frisch et al (Australia). Eight children had isolated GHD, and 14 suffered from multiple hormone deficiencies. The duration of treatment was  $6.6 \pm 3$  years, and the average GH dose

was 9 IU/week. No correlations were found between final height and the standard deviations of chronological age, the chronological age itself, or bone age. Also, no correlation was found with insulin-like growth factor I levels or with GH levels obtained following provocative tests. Patients who had gonadotropin deficiency had a better prognosis with respect to height than those with idiopathic GHD.

The Henning Andersen Prize for the best paper was awarded to Drs. Maes, Amand, and Ketelslegers (Belgium). They fed rats a protein-poor diet for one week. The capacity of the liver membrane to bind GH, the affinity constants, and the basal somatomedin-C (Sm-C) levels were similar in this group of rats and controls. However, in the protein-deprived rats the increase in Sm-C levels after GH stimulation was only one third that of controls. These investigators concluded that in rats, protein malnutrition induces a GH postreceptor defect.

### Letter From the Editor

Dear Colleague:

Welcome to the third year of publication of *Growth, Genetics, and Hormones*. The Editorial Board has worked industriously to provide you with updated summaries of topics of interest to pediatric endocrinologists and geneticists and to provide particularly pertinent abstracts from the literature. We have especially enjoyed providing editorial comments on the abstracts and summaries of meetings we have attended.

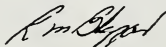
The first issue of Volume 3 has departed somewhat from our usual format. We thought that you might enjoy a historical perspective of Dr. Lawson Wilkins, the pioneer in pediatric endocrinology and, in many respects, in genetics as well. Should your response to this presentation be positive, we will consider presenting further historical perspectives on Dr. David Smith, the Lawson Wilkins Pediatric Endocrine Society, and others in the future.

The second issue of Volume 3 will be devoted in large part to Turner's syndrome. Dr. Judith Hall will contribute a major review article and discuss many aspects of Turner's syndrome in both children and adults. There will also be an update on constitutional delayed growth and adolescence by Dr. Jürgen Bierich. An article regarding antibodies against growth hormone will be included. The author will be Dr. Louis Underwood.

The third issue will include an article on basic genetic concepts and chromosome linkage. This superlative presentation by Dr. Thaddeus Kelly provides a wealth of information for those who do not specialize in genetics but wish to deepen their understanding of the subject. We encourage you to set aside a few hours for studious review of Dr. Kelly's article; it will be well worth your time and professional interest.

The articles for the fourth issue of Volume 3 have not yet been chosen. The members of the Editorial Board encourage you to send to us your suggestions for future issues. Our goal this year is to highlight the most recent information available and to address your educational needs in the fields of growth, genetics, and hormones. We extend our best wishes for the coming year.

For the Editorial Board,



Robert M. Blizzard, M.D.  
Chairman

## Contiguous Gene Syndromes: A Component of Recognizable Syndromes

There are now seven disorders in humans in which some patients with the disorder have visible chromosomal abnormalities and others do not. The conditions in which visible chromosomal deletions are sometimes seen include Prader-Willi syndrome, in which approximately half of the patients have deletions at 15q11; DiGeorge's syndrome, in which about 5% of patients have deletions at 22q11; Langer-Giedion syndrome (trichorhino-phalangeal syndrome type II), in which 80% of the patients have deletion at 8q24; Miller-Dieker syndrome, in which approximately 90% of patients have deletion at 17p13; retinoblastoma, in which 5% of patients have deletion at 13q14; the triad of Wilms' tumor, aniridia, and genitourinary tract malformation, in which about 95% of patients have deletion at 11p13; and the Beckwith-Wiedemann syndrome, in which 5% of patients have duplication of distal 11p. Patients with any of these conditions should have chromosome studies done to establish whether or not the cytogenetic abnormality is present. Frequently, both blood lymphocytes and fibroblasts need to be studied.

These syndromes are particularly interesting, since it is not at all clear whether a specific gene has been deleted or duplicated by the chromosomal abnormalities associated with the syndrome or whether the syndrome is produced by abnormalities in and interactions between a set of genes. The sizes of chromosomal abnormalities are quite variable among patients who are clinically very similar. These seven conditions represent a new category of disorders in which visible chromosomal changes may be seen.

Schmickel RD. *J Pediatr* 1986;109:231-241.

**Editor's comment**—As we learn more about molecular genetics and single gene mutations, we realize that many mutations are actually deletions of genes or parts of genes. Thus, it is not surprising that the larger the deletion, the more likely that more than one gene is involved in the deletion. However, we are only beginning to realize that specific syndromes may actually be the products of multiple gene deletions. The interesting point among the cases reported so far is that in no condition is there uniformity as to the absence of visible chromosome material in all cases of the condition.

## The McCune-Albright Syndrome: A Lethal Gene Surviving by Mosaicism

The etiology of this disease is unknown. There is no evidence of a hereditary basis, since there is no convincing report of a family incidence except one report involving monozygotic twins. Dr. R. Happle of Germany theorizes that the syndrome is caused by a gene that is dominant and lethal, unless the effect is diluted through mosaicism. He postulates that patients with this syndrome are mosaics for the gene.

Happle states that pigmented lesions often show a unilateral arrangement, strictly respecting the ventral midline. He also states that one important fact has been overlooked previously: Based on his observation of a patient, plus a review of the literature, pigmentation in this syndrome follows the lines of Blaschko. As a general rule, nevoid skin lesions following the lines of Blaschko result from the dorso-ventral outgrowth of two different populations of cells during early embryogenesis, thus reflecting mosaicism. Since patients suffering from the McCune-Albright syndrome have this cutaneous pattern for their pigmentation,

Thus, we cannot equate the syndrome per se to chromosome deletion. It is not at all clear at this time what the relationship of the deletion is to the production of the abnormality. Also interesting is that two of the conditions, the Beckwith-Wiedemann and Prader-Willi syndromes, have overgrowth, while three of the conditions have cancerous overgrowth. Thus, the gene(s) involved is (are) altered in such a way as to upset normal growth mechanisms. This certainly is an interesting group of diseases, and the etiologies will become clearer as progress is made in molecular genetics.

Happle believes it is likely that these patients have two different clones of cells.

Happle postulates that if the gene for McCune-Albright syndrome were merely functional, one would expect that the syndrome would be inherited. However, all cases are sporadic. This can best be explained by the presence and action of a "dominant" lethal gene that kills the embryo during its development. Patients with this gene could survive only if they were mosaics. If this thesis is correct, the mosaic state could be produced either by a gametic half chromatid mutation or by an early somatic mutation. Unilateral or even more circumscribed involvement would result from a mutation occurring at a later time in embryogenesis.

This theory could explain the scattered and asymmetric distribution of bone lesions. It could explain the protean variability of endocrine disturbances. It could also explain the occurrence of incomplete forms of the syndrome, which would be attributed to a minor proportion of mutant cells within the total cell population. The mosaicism resulting from a gametic half chromatid mutation could also explain the simultaneous occurrence observed in a set of monozygotic twins.

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## McCune-Albright Syndrome

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Happle concluded that both males and females with the McCune-Albright syndrome are able to produce offspring. For the practical purpose of genetic counseling, the action of a lethal gene would explain why the risk of recurrence is not increased for the patient's siblings and children. The concept would imply that affected women should have an increased rate of spontaneous abortions. The loss of the zygote,

however, might occur at the time of implantation and thus remain unnoticed. Special attention will be given to this question in further clinical studies.

Happle R. *Clinical Genetics* 1986; 29:321-324.

**Editor's comment**—This is a fascinating postulate. Happle has previously written about mosaicism and the occurrence of certain dermatological lesions that follow the lines of Blaschko. In an article in *Human Genetics* (1985; 70:200-206) which is entitled "Lyonization and the lines of Blaschko," Happle writes that the lines of

Blaschko represent a nonrandom developmental pattern of the skin fundamentally differing from the system of dermatomes. He found a causal relationship between lyonization and the lines of Blaschko to be quite obvious. Apparently, in women affected with X-linked skin disorders, the lines of Blaschko visualize the clonal proliferation of two functionally different populations of cells during embryogenesis. The lesions arise probably from cells in which the X chromosome that bears the mutation is the active one, whereas the normal skin develops from cells in which the normal cell is active.

## The Origin of 45,XO Males

Maleness in association with a 45,XO karyotype is a very rare and hitherto unexplained condition, previously described in fewer than ten patients. Most individuals with this karyotype develop as phenotypic females with Turner's syndrome. How maleness arises in the XO males, who have invariably been sterile, has been unclear until the study of De la Chapelle et al.<sup>1</sup>

Two 45,XO males were studied. Both had third-degree hypospadias and cryptorchidism, but two testes were found in each. One testis, which was examined in the first patient at 6 to 7 years of age, was "normal." In the second patient, both testes were on the left side and shared a common vas deferens. Both patients were below the second percentile in height; there was no significant mental retardation. There were characteristics suggestive of Turner's syndrome in the first patient, including a mild pterygium colli, highly arched palate, shield-shaped chest, laterally located mamillae, clinodactyly of the fifth fingers, deep-set nails, and coarctation of the aorta.

Both parents of both patients were cytogenetically normal. Four blood cultures and one fibroblast

culture from the first patient had only 45,XO mitotic cells. However, a buccal smear revealed 15 of 1,000 cells had fluorescent spots that were believed to reflect the presence of a Y chromosome or a Y chromatin body. A repeat buccal smear several years later showed that 5% of the cells had a fluorescent-staining body. A repeat skin fibroblast culture showed five of 186 cells with a 46,XY karyotype. Another repeat culture yielded similar findings, and these cultures were used for studies to identify Y-DNA sequences. Repeat cultures in the second patient were negative and repeat buccal smears were negative for fluorescent-staining material.

By using restriction digestion, agarose electrophoresis, gel transfer, and hybridization with radiolabeled, cloned DNA probes, it was possible to demonstrate a small amount of Y-DNA material (3%) from the cells of the first patient. There was no demonstrable Y-DNA material from the cultures of the second patient. Using refined techniques, it was possible to show that the X chromosomes of both patients originated from their mothers.

A 45,XO male might be a 45,X/46,XY mosaic, in whom the XY line is rare or has been eliminated altogether, at least in some tissues. The first patient appears to

fall into this category. The Y chromosome present in 3% of fibroblasts was structurally normal. Extensive cytogenetic and DNA studies in the second patient produced no evidence of Y chromosomal material, even in a minority of cells. Current techniques used in this study permit identification of a normal Y chromosome in as few as one in 10,000 cells. Therefore, mosaicism of a normally structured Y chromosome is unlikely in this patient. However, some of the identifiable fragments of the Y are located principally in the distal Yq and, thus, would be of little use in detecting mosaicism involving an abnormal Y chromosome lacking that region. The DNA hybridization studies alone, then, cannot argue against low-grade mosaicism for a structurally abnormal Y chromosome in the second patient. There is also the possibility that a Y-bearing cell line existed in tissues other than those that were sampled or existed in the fetal stage, but later eliminated.

Maleness in 46,XX males may be explained by the X-Y interchange hypothesis, which states that the Xq-bearing position of the father's X chromosome can be replaced by a testicular-determining portion of his Y chromosome, which hypothetically might occur as a result of interchange of genetic material between the X and Y

chromosomes at paternal meiosis. Consistent with this hypothesis is the identification of certain single-copy, Y-specific DNA sequences that were detected in 12 of 19 XX males who were tested by Page et al.<sup>2</sup> Thus, it appears that X-Y interchange can account for many cases of XX maleness. In both cases of XO maleness reported here, however, there is no evidence for genetic transfer of Y material. The X chromosome in both patients was of maternal origin. Mosaicism may have accounted for the differentiation of the genitalia along male lines in the first patient. In the second patient, the absence of a certain single-copy Y-DNA sequence argues against, but cannot exclude, the presence of the testis-determining portion of the Y chromosome.

1. De la Chapelle A, Page C, Brown L, et al. *Am J Hum Genet* 1986;38:330-340.
2. Page C et al. *Am J Hum Genet* 1986;38:109.

**Editor's comment**—These patients, although extremely rare, remind us how much we do not know about normal sexual differentiation. For several years, attention focused on the presence of H-Y antigen to explain differentiation of the normal male fetus along male lines. We now have learned that H-Y antigen is probably of no consequence, even if it exists. The concept that Y chromosomal material is necessary for differentiation of the gonad along male lines has been defied by the second patient, although mosaicism may have been present at

some time early in his life.

Of importance to clinicians and investigators is the concept that cultures of fibroblasts from the gonads are exceedingly important when karyotypes from lymphocytes or skin fibroblasts confuse our interpretation of what has taken place. Several years ago, Goldstein et al (*J Pediatr* 1977;90:604) described a short female with stigmata suggestive of Turner's syndrome and gonadal agenesis. Cultures of skin fibroblasts and lymphocytes revealed a 46,XX karyotype; when grown from biopsies of the gonadal streaks, fibroblasts had a karyotype of 45,XO. Possibly, if fibroblasts had been grown from the testes of the two 45,XO males, Y chromosomal material would have been more readily identifiable.

## Treatment of Duchenne's Muscular Dystrophy With Growth Hormone Inhibitors

Although major advances are being made in isolating the gene for Duchenne's muscular dystrophy, the basic mechanism of this disorder is still unknown. It may be several years before the function of the gene is understood. In the meantime, the disease continues its relentless deterioration process in affected males.

An interesting clinical observation reported by Zatz et al five years ago has led to some very important therapeutic implications. The report was about a boy with Duchenne's muscular dystrophy who also had growth hormone deficiency (GHD) and a relatively benign course. When compared with other affected individuals in his family, he was very much less severely affected. For this reason, Zatz et al undertook to utilize growth hormone (GH) antagonists in the treatment of Duchenne's muscular dystrophy. Specifically, she treated one of two identical

twins with the disease in a double-blind controlled study. The treatment involved the use of the GH antagonist mazindol. After one year of the therapeutic trial, the code was broken and the identical twin boys were compared. The twin being treated by GH antagonist was significantly less severely affected after a year of therapy than his brother, who had typical progression of his disease.

In the same issue of the *American Journal of Medical Genetics*, Zatz et al report the follow-up on the patient observed five years ago. The boy is still alive and functional at 18 years of age, while the other affected members of his family had already died or been non-ambulatory by the same age.

Zatz M, Betti RTB, Frota-Pessoa O. *Am J Med Gen* 1986;24:549-566, 567-572.

**Editor's comment**—Duchenne's muscular dystrophy is one of the most common and distressing of single gene disorders. The basic mechanism of the disease has eluded definition, and until this report of Zatz et al, there has been no

real hope for interrupting the progressive deterioration of affected boys. Advances in genetics are frequently made by experiments of nature and observation; the insight to appreciate that the boy who was affected with GHD was actually doing "well" with regard to his Duchenne's muscular dystrophy was extremely important. The observation that the identical twin who was treated with GH antagonist is significantly better than his non-treated twin raises the possibility that cell growth may have some role in Duchenne's muscular dystrophy. It may well be that smaller cells somehow do better and survive longer in the presence of the Duchenne's muscular dystrophy gene. It may be that boys with Duchenne's muscular dystrophy begin to do poorly with the increased turnover of cells and that GHs stimulate this turnover. Whatever the case, this important clinical observation may well lead to immediate treatment for Duchenne's muscular dystrophy. In addition, it is interesting to speculate whether GH antagonist may offer potential therapy in other degenerative diseases.

## Retinoic Acid Embryopathy

Retinoic acid is a derivative of vitamin A and is presently used effectively to treat a variety of skin disorders, including serious acne. It has long been recognized from animal studies that retinoic acid and isotretinoic acid are teratogens, but when isotretinoin came into general use for cystic acne in human patients, many pregnant women were exposed to it. A "retinoic acid embryopathy syndrome" is now recognized among babies whose mothers took vitamin A derivative during pregnancy. Features of the syndrome include abnormalities of the cranium and face, central nervous system, heart, and thymus. The authors point out that it is difficult to conduct a proper prospective study because most women on treatment elect termination of pregnancy. However, among 36 pregnancies studied prospectively, eight resulted in spontaneous abortions, 23 in normal infants at birth, and five in malformed infants.

This paper reports 21 malformed infants with a characteristic pattern of malformations. Cranial and facial

abnormalities include microtia and anotia, micrognathia, and cleft palate; cardiac anomalies include conotruncal heart defects and aortic arch abnormalities; central nervous system abnormalities include hydrocephalus, fourth-ventricle cyst, holoprosencephaly and microcephaly, as well as major errors in cortical and cerebellar neuronal migration; and thymus abnormalities include ectopia, hypoplasia, and aplasia.

The exact timing and mechanism of teratogenesis are unknown. The authors speculate that exposure to isotretinoin may produce abnormal cephalic-neural-crest-cell activity around the 28th day of gestation. This would imply that early exposure is of greatest concern. Isotretinoin has a short half-life of between 16 to 20 hours. Thus, it would appear that there is no long-term effect of isotretinoin ingestion, and nonpregnant women need not worry about an effect after discontinuing this medication. The exact dose that may produce anomalies has not yet been determined.

The data in this report suggest an increased risk for spontaneous abortions and a risk of about 20%

for having a child with obvious congenital anomalies at birth among women who have taken isotretinoic acid early in pregnancy.

Lammer EJ, Chen DT, Hoar RM, et al. *N Engl J Med* 1985;313:837-841.

**Editor's comment**—It is quite clear from animal and now human studies that vitamin A and its analogues are teratogenic in humans and cause a characteristic pattern of anomalies. It is extremely important that physicians make this potential side effect clear to women of childbearing age when prescribing isotretinoin or other vitamin A preparations. If a woman has been inadvertently exposed, she must be offered the option of prenatal diagnosis because many of the structural defects can be identified prenatally. Many fetuses exposed to vitamin A derivatives will spontaneously abort. To date, over half the reported children who have been exposed to isotretinoin during the early stages of gestation appear normal at birth. However, long-term follow-up of their intellectual development has not yet been possible.

## Effects of Cyproterone Acetate on Statural Growth in Children With Precocious Puberty

For the treatment of precocious puberty (PP), drugs with three different actions are available: progestational steroids with antigonadotropic activity (eg, medroxyprogesterone acetate); progestins with additional antiandrogenic effects (eg, cyproterone acetate or CPA); and luteinizing-hormone-releasing hormone (LHRH) analogues, which block the pulsatile secretion of the gonadotropins from the hypophysis. With all three types of drugs, it is possible to suppress the sexual

development of patients.

Less satisfactory is the effect on longitudinal growth and skeletal maturation. Untreated girls with idiopathic PP usually attain an adult height of between 146 and 154 cm (Thamdrup [1961]; Sigurjonsdottir and Hayles [1968]). Treatment with medroxyprogesterone acetate does not produce an increase in adult height. Opinions concerning CPA are contradictory. In fact, outcome of therapy has been difficult to evaluate, since only predicted heights and no measured heights have been available. LHRH analogues have not been tested for a long enough period for reliable judgment.

In a multicenter study, Sorgo et al recently ascertained the suc-

cess of long-term administration of CPA in 44 patients with PP, 31 of whom had idiopathic PP. The investigators measured adult height. Twenty patients had received CPA in a dosage of  $117 \pm 6$  mg/m<sup>2</sup>/day (group A) and 24 in a dosage of  $60.8 \pm 2.4$  mg/m<sup>2</sup>/day (group B); the duration of treatment averaged 4.8 years. The chronologic age (CA) of female patients was 5.45 years at start of treatment and the bone age (BA) was 8.57 years. Thus, BA exceeded CA by 3.12 years. During therapy, height standard deviation scores for chronologic age (SDS<sub>CA</sub>) dropped from 2.54 to 1.28 in group A, whereas group B showed no significant change. Height SDS<sub>BA</sub> did not change in either group. The height velocity

scores for CA and BA quickly decreased and reached subnormal values by the second or third year of treatment. At the same time, skeletal maturation expressed by BA/CA fell from very high (2 to 3) to normal values (around 1.0). Thus, after initiation of treatment, no further deterioration or relative height loss occurred. No significant differences between the two groups were found. Also, selecting only the idiopathic cases of PP, no difference in adult height was encountered in the groups: group A measured 153.3 cm, group B 153.4 cm.

Sorgo W, Kiraly E, et al. *Eur J Pediatr* 1985;145.

**Editor's comment**—This large study confirms that high-dose and low-dose treatment with CPA does not increase statural growth in patients with idiopathic PP. The results are particularly important because the parameter used was measured, not predicted, final height. This makes a great difference, when compared with the findings of most earlier publications.

It is critical to mention that investigators should not mix children with idiopathic PP with those who have McCune-Albright syndrome, since most patients with the latter condition do not have increased secretion of gonadotropin. It is not surprising that patients with McCune-Albright syndrome have significantly diminishing relative height during CPA treatment—which is of minor value in this condition.

Patients with idiopathic PP, however, did not have deteriorating growth (final height) prognosis. Predicted and final height did not significantly differ. In our opinion, this may represent a therapeutic success, although a limited one. Observations by Bierich (1980) and Murram et al (1984) predicted that final height gradually diminishes in young children with idiopathic PP who remain untreated.

## Levels of Growth-Hormone-Releasing Factor During Growth Hormone Stimulation Tests and During Puberty: Two Reports

Donnadieu and co-workers<sup>1</sup> established an assay for growth-hormone-releasing factor (GRF) and evaluated the concentration in the plasma both in children receiving various stimulation tests for growth hormone (GH) release and in children at various stages of puberty.

This assay measured GRF-40 and GRF-44 equally and required the extraction of 2 ml of plasma. Eight samples collected over a two-hour period from each of three boys varied from 43-73 pg/ml in the first, to 8-22 pg/ml in the second, and to 41-95 pg/ml in the third.

In the first report, these authors found that L-dopa stimulation of GH release is preceded by a significant rise in GRF. In contrast, when ornithine infusion is used as a pharmacological agent to cause GH release, GRF falls. The authors conclude that different mechanisms account for GH release by these two agents.

In the report by Argente and colleagues,<sup>2</sup> basal GRF concentrations were measured in samples from 180 children. These were collected between 8 AM and 10 AM after an overnight fast. Correlations between basal GRF values of children in various stages of puberty and steroid and insulin-like growth factor I (IGF-1) levels were

sought by the investigators.

As shown in the Table, basal levels in girls increased progressively during the first four stages of puberty and fell in stage V. Basal levels in boys increased from stage I to stage II and to stage III progressively. The values plateaued during stage IV and then fell in boys with stage V sexual development. The pubertal values in girls were significantly higher than in boys and increased progressively until stage IV, after which they fell markedly. There was no correlation between plasma GRF levels and sex steroids or growth velocity. Positive correlation was found between basal GRF values and IGF-1 values in both sexes.

Fourteen boys with delayed puberty had values of  $30.8 \pm 7.5$  pg/ml. These were comparable to the values found in boys in stage I of puberty.

1. Donnadieu M, Evain-Brion D, Tonon MC, et al. *J Clin Endocrinol Metab* 1985;60:1132.

2. Argente J, Evain-Brion D, Munoz-Villa A, et al. *J Clin Endocrinol Metab* 1986;63:680.

**Editor's comment**—These authors are to be commended for developing an assay that permits evaluation of the physiologic role that GRF plays in the secretion of GH. Readers probably will want to follow the literature closely to observe the reporting of further observations pertaining to the role that GRF plays (and does not play) in the normal and abnormal physiology of GH secretion.

Table: GRF Levels During the Stages of Puberty

|       | Prepubertal | Early Pubertal | Midpubertal  | Late Pubertal |            |
|-------|-------------|----------------|--------------|---------------|------------|
|       | I           | II             | III          | IV            | V + Menses |
| Girls | 30.3 ± 4.3  | 56.6 ± 6.1     | 143.7 ± 21.3 | 176.6 ± 35.7  | 60.5 ± 6.0 |
| Boys  | 48.1 ± 5.2  | 75.9 ± 4.3     | 103.5 ± 13.8 | 99.3 ± 9.3    | 60.6 ± 5.7 |



## Prenatal Diagnosis of Autosomal Dominant Polycystic Kidney Disease With a DNA Probe

Polycystic kidney disease is one of the most common dominantly inherited disorders in man, occurring in about one in 1,000 individuals. Approximately 10% of all cases requiring renal dialysis and kidney transplant have autosomal dominantly inherited polycystic kidney disease of the adult type. Recently, the disorder had been localized to an abnormality of the short arm of chromosome 6, and

genetic linkage has been demonstrated to the alpha chain of human hemoglobin and phosphoglycolate phosphatase. With this knowledge, it is possible to diagnose "presymptomatic" carriers; most recently, prenatal diagnosis has been accomplished by DNA analysis of chorionic villus sampling.

The report, which is very straightforward, describes techniques that have been developed in the last few years. It is important, however, for the practitioner to be aware that these sorts of techniques have been developed and that analysis of family members is possible. Linkage can be determined and presymptomatic car-

riers can be detected, thus permitting prenatal diagnosis.

Reeders ST et al. *Lancet* 1986;7:6.

**Editor's comment**—The remarkable advances that have been made in the last few years are very dramatic. Only three years ago, presymptomatic detection of individuals with polycystic kidney disease was not thought to be possible. Now it is possible to diagnose the entity before symptoms occur. Equally important is the fact that prenatal diagnosis during the first trimester using chorionic villus sampling is also possible. This allows prospective parents to make alternative decisions.

## Peroxisomal Disorders: Three Reports

A recent review of peroxisomal disorders has focused attention on them and has allowed their features to be summarized. The list of peroxisomal disorders is increasing with the increased index of suspicion. Thus far, all of the peroxisomal disorders show accumulation of bile acid precursors and very long chain fatty acids, with impaired biosynthesis of plasmalogens. In some cases, abnormal peroxisomes can be seen on electron microscopy.

Interestingly, all cases of peroxisomal disorders also have minor congenital anomalies. These include structural abnormalities of the brain due to malformation of neurons, dysplastic cystic kidneys, retinitis pigmentosa due to dysplasia of the retina, hepatomegaly, deafness, stippled epiphyses, and abnormal facies with high forehead and myopathy. Mental retardation and hypotonia are usually present as well. None of these clinical features are pathognomonic.

The biochemistry of peroxiso-

mal disorders is poorly understood at this time. However, good screening tests of urine, serum, and fibroblasts are beginning to be developed. Presently, Zellweger's syndrome, adrenoleukodystrophy, infantile Refsum's syndrome, infantile Conradi's syndrome, and Leber's disease have been defined as peroxisomal disorders. The index of suspicion for these disorders should be raised when evaluating any child with minor anomalies of the type described above.

The report of pseudo-Zellweger's syndrome by Goldfischer et al<sup>1</sup> indicates that one can have abnormal peroxisomal function in the presence of abundant peroxisomes. The report of Leber's disease by Ek et al<sup>2</sup> described specific changes of macular hyperpigmentation and absence of electroretinographic responses without the other features of Zellweger's syndrome. This finding suggests that any disorder with dysplasia of the retina needs to be considered as a potential peroxisomal disorder. The description of "infantile" Refsum's syndrome by Sargini et al<sup>3</sup> reminds us that the presence of neurosensory deafness and retini-

tis pigmentosa indicate the possibility of the diagnosis of a peroxisomal disorder. This is particularly true in children with hypotonia and hepatomegaly. Sargini et al found four patients within six months when they began to look for the disorder. This suggests that this may be a relatively common condition.

1. Goldfischer S, Collins J, Rapin I, et al. *J Pediatr* 1986;108:25-32.
2. Ek J, Kase BF, Reith A, et al. *J Pediatr* 1986;108:19-24.
3. Sargini S, Budden MD, Kennaway NG, et al. *J Pediatr* 1986;108:34-39.

**Editor's comment**—Peroxisomal disorders are a whole new class of inborn errors of metabolism in which the combination of long chain fatty acid metabolism and congenital developmental anomalies is seen. Although the definition of peroxisomal metabolism is in its infancy, peroxisomal disorders appear to be inborn errors of metabolism in which morphogenesis is affected. This is an exciting new area that will surely allow the description of a whole new set of specific single gene disorders.

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- The Endocrine Society Symposium on Endocrinology of Neuropsychiatric Disorders—June 25-27, 1986, Anaheim, California
- National Foundation-March of Dimes Clinical Genetics Conference on Muscle and its Disorders—June 8-11, 1986, Philadelphia

## MEETING CALENDAR

**April 27-30** Annual Meeting of the American Pediatric Society/Society for Pediatric Research. Disneyland Hotel, Anaheim, California. Contact: Debbie Wogenrich, Department of Pediatrics, University of New Mexico, Albuquerque, NM 87131 (505-277-6628)

**May 1** Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Disneyland Hotel, Anaheim, California. Contact: Dr. Gilbert August, Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue NW, Washington, DC 20010 (202-745-2121)

**June 4-12** 47th Annual Meeting and Scientific Sessions, American Diabetes Association. Hyatt Regency Hotel, Indianapolis, Indiana. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22320 (800-232-3472)

**June 10-12** 69th Annual Meeting, The Endocrine Society. Indianapolis Convention Center, Indianapolis, Indiana. Contact: Nettie Karpin, Executive Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-9660)

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# GROWTH

## Genetics & Hormones

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## Antibodies to Growth Hormone: Measurement and Meaning

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Some patients develop serum antibodies to growth hormone (GH) during GH therapy. Although these antibodies do not produce immune complex disease, their occurrence causes concern among endocrinologists because they may attenuate the growth-promoting effect of GH therapy in a small number of patients. In this presentation, we will review the factors involved in the production of antibodies to GH, the methods for detecting and quantitating serum GH antibodies, and the relationship between the amount of antibodies measured and the occurrence of growth attenuation.

### Causes of GH Antibodies

With insulin and other peptides of animal origin, the lack of identity between the structure of the peptide used therapeutically and the corresponding peptide of the host is one cause for stimulation of the immune response and production of serum antibodies. Intuitively, this should not be the cause for GH antibodies when authentic human growth hormone (hGH) is given to humans. GH preparations used therapeutically (including biosynthetic GH), however, contain some GH with modified molecular structure. These modifications include deamidation, dimerization, pro-

teolytic cleavage, and deletions, which produce forms that may not be secreted normally. In addition to exposure to a foreign peptide, the immune system may be excited to form antibodies when the peptide is presented by an unusual route, the peptide preparation is contaminated with substances that act as adjuvants, or the secondary and tertiary structure of the peptide is altered.

Historically, the occurrence of antibodies to pituitary-derived GH correlated with the quality of the preparation injected. Antibodies were observed in as many as 60% of the hypopituitary children treated with early preparations that contained aggregates of GH and an abundance of contaminating peptides. As purer preparations of monomeric GH came into use, the incidence of antibodies fell to below 10% with some preparations, and growth attenuation due to antibodies was rare. Unlike insulin antibodies that have been shown to be more prone to form in insulin-treated diabetic patients who have certain HLA types, the host factors that predispose to the formation of antibodies to injected GH have not been defined. Exceptions to this are the rare patients who are GH-deficient (type 1A) because of a deletion in the gene

encoding for GH. In these patients, formation of a massive quantity of antibodies to GH is common.

Before recombinant DNA-derived human insulin was available, it was widely believed that its use would obviate the formation of antibodies to insulin in diabetics. This, however, was quickly proven *not* to be the case. Likewise, it came as a surprise when treatment with the first preparations of recombinant methionyl GH caused antibodies to form in a significant percentage of patients. Production of GH antibodies declined sharply, however, after improvements in purification procedures for methionyl GH were made and since the introduction of recombinant hGH with no N-terminus methionine. If antibodies are to occur, they usually can be detected early in therapy (by three to six months), regardless of the

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## Antibodies To Growth Hormone: Measure and Meaning

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hGH preparation used. Evidence from one laboratory indicates that antibodies may be transient, especially in patients with low levels of binding. Therefore, the frequency of occurrence of antibodies may vary, depending on the frequency and timing of their measurement.

### What Needs to Be Done Before Screening for Antibodies

The question of whether a child has GH antibodies arises when he/she fails to grow in response to GH therapy. However, in the list of possible causes of poor responses to GH therapy, antibodies must rank near the bottom. The following questions should be answered before one screens for antibodies: Is the diagnosis of GH deficiency correct? Are the measurements of linear growth and the calculation of growth rate accurate? Has the patient had significant illness during the interval in question, and, if so, could such an illness have impaired the response to treatment? Has the child's nutritional intake been sufficient to support growth? Is the child receiving the prescribed GH dose, and is the hormone being administered properly? Most of these questions can be answered readily. Should they provide no clues, serum should be drawn for assessment of antibodies.

### Screening for GH Antibodies

Screening for GH antibodies is accomplished by incubating an aliquot of the patient's serum (the potential source of antibodies) with a small quantity of radio-labeled GH (~10,000 cpm). The incubation is ended by separating the labelled GH that is bound by antibody from the unbound labelled hormone. Binding in patient serum that is more than twice the mean of sera from subjects not treated with GH is usually taken as evidence of the presence of antibodies. It is advisable to confirm that the binding observed is spe-

cific for GH by assessing the inhibition of binding of labelled GH by excess unlabelled GH. When antibodies are present, the addition of an excess of unlabelled GH will reduce the binding to values equal to those of control sera.

In the incubation procedure, two features are crucial for adequate screening. First, sufficient serum must be added to detect small amounts of antibody. Second, a reliable method for separating bound from free hormone must be available. The final dilution of serum in the reaction mixture, which contains radioimmunoassay (RIA) buffer, should be 1:20 or less. Of the separation methods available, we prefer to use a gamma globulin precipitant such as polyethylene glycol (PEG) or an anti-human gamma globulin second antibody that is known to precipitate gamma globulin efficiently. We prefer to separate bound from free hormone by precipitating the former with a second antibody, because precipitation with PEG is subject to variability from a number of factors. Less reliable separation methods include chromatoelectrophoresis and dextran-coated charcoal binding of labelled hormone not bound to antibody. With the latter, spurious information is obtained when the antibody-bound labelled hormone separates in the charcoal fraction along with the free hormone.

### Measurement of Binding Capacity, Affinity, and Titer

High binding capacity seems to correlate best with the growth-inhibiting effect of GH antibodies. Measurement of binding capacity is accomplished in much the same way one would set up a standard curve in an RIA. Fixed amounts of the test serum and labelled GH are added to a series of tubes containing increased amounts of unlabelled GH (concentration range of 1 to 1000 ng/ml). Following incubation and separation of bound from free hormone, a curve of competition can be derived. From this, the binding capacity can be determined using a Scatchard plot.

While growth attenuation from GH antibodies appears to correlate best with binding capacity, affinity of binding also can be determined from the Scatchard plot and may also be a determinant of the biological importance of GH antibodies. However, the use of affinity measurements has been limited, since these figures are difficult to quantify precisely.

In many of the studies of GH antibodies in GH-treated patients, laboratory assessment has focused on measurements of titers. We believe that determining the highest dilution of serum at which binding can be detected is of minimal value unless it is followed by a measurement of binding capacity. However, clues to the possible importance of GH antibodies in inhibiting growth can be obtained by noting the percentage of tracer bound to antibody in the original screening test. Binding in the range of 20% or less at a serum dilution of 1:10 is not usually associated with high binding capacity or inhibition of growth. On the other hand, if binding is above 50% on the initial screen, the binding capacity and the titers probably will be high, and one should be concerned that the antibodies might interfere with the bioavailability of injected GH. In our experience, binding capacities of less than 5 mg/l of bound GH in the patient's serum are not associated with growth attenuation. Values of 10 mg/l or greater are usually found in patients who have impaired responses to exogenous GH. The relationship between the time that the serum sample is drawn for antibodies and GH therapy must be considered in the interpretation of binding capacity. Lack of antigenic stimulation, as during cessation of therapy, results in a decrease in binding capacity. Also, a recent injection of GH may saturate the antibody, thereby lowering apparent binding capacity.

### GH Antibodies and Attenuation of Growth

Serum GH antibodies almost certainly exert their biological effect by binding injected hormone,

thereby limiting the hormone's availability to tissues. Whether binding is great enough to have biological significance is probably determined by the abundance of antibody in the serum, the affinity of binding of the hormone by antibody, and the competition between binding to antibody and to GH receptors on cells. Supporting this is the observation that large amounts of antibody (more than 10 mg/l) slow the removal of GH from the circulation during the phase of disappearance that is ordinarily determined by the uptake of GH by tissues.

Detection of GH antibodies in the serum of a patient being treated with GH does not require that the therapeutic plan be changed unless the growth response is attenuated. In the case of attenuated growth in association with a high serum binding capacity for GH, it may be advisable to change from one GH preparation to another. In the past, when a variety of pituitary GH preparations were available, changing from one preparation to another often resulted in a resumption of growth and a fall in antibody binding capacity. Alternatively, it may be possible to overcome the GH resistance induced by antibodies by increasing the dose of GH. The latter approach, however, has not been attempted to date. Hopefully, as recombinant hGH preparations are improved, inhibition of the growth response by antibodies will not occur except in those patients with hGH deficiency secondary to gene deletion.

### Additional Reading

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## Letter From the Editor

Dear Reader:

This issue contains three excellent reviews of pertinent topics to geneticists and endocrinologists. The article by Drs. Underwood and Moore provides a fresh perspective for endocrinologists and geneticists who have not yet had the chance to become acquainted with the techniques used to detect serum antibodies to hormonal peptides. The article is also very timely, since there has been much discussion recently regarding the importance of antibodies to growth hormone (GH) in patients treated with native GH, methionyl-GH, and met-less GH. As Drs. Underwood and Moore point out, there is little significance to antibodies that are present *unless* there is significantly high binding capacity ( $>5$  mg/l).

Dr. Hall's review of Turner syndrome is comprehensive in all respects. The variability of the pathology of this syndrome never ceases to amaze me. We see patients with an XO karyotype who have extensive dysmorphism but who, on occasion, have normal estrogen production. We see others with the same karyotype who have minimal dysmorphism, but who may have complete absence of ovarian tissue. The etiology of the short stature is still an enigma. Many of us postulate that the short stature is attributable to a presumed chondrodystrophy but, if so, what kind? There are no consistent radiological findings of the skeleton, and no abnormality of body proportions to permit us to deduce this. Turner syndrome should challenge also the molecular geneticists. How do the genes on the X and Y chromosomes affect growth?

Constitutional delay of growth and adolescence is the most frequent type of short stature seen by clinical endocrinologists in boys. Dr. Bierich reviews the explicit signs and the vagaries of making this diagnosis. We would all agree that it is often difficult to differentiate between partial growth hormone deficiency, constitutional delayed growth and adolescence, and even psychosocial short stature. In his article, Dr. Bierich presents his current thoughts on constitutional delay of growth, an entity to which he has devoted considerable time, effort, and concern.

The Editorial Board hopes you will find these articles informative, helpful, and provocative. We invite you to write us about them. We are pleased to offer *Growth, Genetics, and Hormones* as a forum of communication between the Board and our readers.

For the Editorial Board,

Robert M. Blizzard, M.D.  
Chairman of the Editorial Board

# Turner Syndrome: An Update

Judith G. Hall, M.D.

Associate Editor

Growth, Genetics, and Hormones

In 1938, Henry Turner described a syndrome of sexual infantilism, webbed neck, cubitus valgus, and short stature in a group of females.<sup>1</sup> Subsequently, gonadal dysgenesis was recognized as a part of this syndrome. It was not until 1959, however, that the chromosomal abnormalities of the syndrome described by Turner were defined.<sup>2</sup> The presence of only one normal functioning X chromosome is characteristic; the other sex chromosome may be missing or have been deleted, or it may be present in a mosaic form. Isochromosomes, which are duplications of the short or long arm of one of the X chromosomes, are also seen. Although, in the past, the buccal smear sometimes was helpful in establishing the diagnosis of Turner syndrome, cytogenetic studies are necessary to confirm it.<sup>3</sup>

## Diagnosis and Karyotype

The minimal diagnostic criterion for Turner syndrome is an abnormal karyotype in at least one tissue in which a portion or all of the X chromosomes is missing. There have been reports of patients with 46,XX karyotypes of lymphocytes and fibroblasts from skin, but who have abnormal karyotypes of cells in the gonads. In contrast, there are patients who have abnormal karyotypes in the lymphocytes and fibroblasts, but who probably have normal karyotypes in the ovarian tissue. These are the patients who may be able to become pregnant.

Individuals with 45,X/46,XY karyotypes may present with the typical stigmata of Turner syndrome, or they may present as males with insignificant hypospadias. Identifying 45,X/46,XY, if present, is essential because malignant degeneration (gonadoblastomas) may occur in the streak gonads. Any patient diagnosed as having Turner syndrome

who has not been karyotyped in the past should have karyotype analysis performed to rule out the presence of a Y chromosome. In the future, the presence of Y-bearing cells in 45,X Turner syndrome patients may also be detected by monoclonal antibodies to the Y chromosome or by DNA probes to the Y chromosome.

Turner syndrome should be considered in a female infant with lymphedema or in any female with short stature, primary or secondary amenorrhea, or both. Many patients have minimal dysmorphic features, and there is no single pathognomonic clinical feature that clinches the diagnosis.

Attempts to correlate the types of X chromosome anomalies in specific individuals or in groups of individuals according to their phenotypic findings has been of limited success.<sup>4</sup> Genes involved in gonadal function in both the proximal part of the short arm of the X and distal part of the long arm of the X are believed to exist, while the genes for other somatic features must be distributed along the length of the short arm and the middle section of the long arm of the X. There is a rough correlation between the absence of the short arm of the X chromosome and the typical clinical features of Turner syndrome. These features include broad chest, hypoplastic nipples, prominent ears, webbing of the neck, low hairline, high palate, short fourth metacarpal, cutaneous valgus, hypoplastic nails, multiple pigmented nevi, structural anomalies of the kidneys, coarctation of the aorta, and hearing impairment.

One in 2,500 live-born females has Turner syndrome. However, 98% of fetuses with a missing sex chromosome are aborted spontaneously. Typically, 45,X abortuses have a huge cystic hygroma and

generalized edema. It is important to do a karyotype on cells from abortuses suspected of having Turner syndrome, since this syndrome does not carry a recurrent risk (of affecting future offspring), but many other causes of fetal hydrops do.

Infants born to the few women with Turner syndrome who are capable of conceiving are more likely to have a chromosomal abnormality (sex chromosomal and autosomal aneuploidies).<sup>5</sup> Advanced maternal age does not appear to be a risk factor for having an infant with Turner syndrome. The paternally derived X chromosome is more likely to be the absent X, but the reasons for this are obscure.

Prior to 12 weeks of gestation, the ovaries in a 45,X fetus appear normal histologically. At that time, the number of primordial germ cells is normal, but the number of follicle cells per oocyte decreases thereafter. The oocytes continue to degenerate; by birth there are few, if any, left, and fibrous streaks replace the ovarian tissue. Two normal X chromosomes are necessary for the follicles to be maintained.

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## Structural Abnormalities and Other Malformations

Structural or positional abnormalities of the kidneys occur in approximately 60% of patients with Turner syndrome. These abnormalities rarely cause renal malfunction, although recurrent urinary tract infections may occur. Double collecting systems or the absence of a kidney occurs in 20% and malrotation in 15%. An ultrasound of the abdomen is recommended to determine the structure of the kidneys and collecting system. Regular screening for urinary tract infection is indicated in those patients with anomalies.

Lymphedema occurs in approximately one third of the patients. It is caused by congenital hypoplasia, late maturation, and delayed canalization of the lymph channels *in utero*. Persistence of embryonic lymph sacs *in utero* results in severe lymphedema, particularly in the neck area. By birth or shortly thereafter, the cystic hygroma usually recedes, leaving folds of skin and webbing in the neck and a low nuchal hairline. Peripheral edema also decreases during childhood. However, edema may again become a problem when estrogen therapy is begun. Salt restriction and "support" hose may be helpful.

Congenital heart malformations are frequent and all patients with Turner syndrome should have ultrasound and echocardiogram studies of their hearts performed. Coarctation of the aorta occurs in 15% to 30% of patients, but it is often mild. Ectopia cordis and hypoplastic left heart occur rarely. Whenever these two rare cardiovascular anomalies are seen in females, chromosome studies are indicated. Pulmonary stenosis occurs, but it is seen more often in the chromosomally normal Noonan's syndrome, which has a somewhat similar phenotype. Bicuspid aortic valves are seen in approximately one third of patients.<sup>6</sup> Such patients should receive prophylactic antibiotics prior to dental work or surgery to avoid endocarditis. Aneurysm and dissection of the aorta also have been reported in Turner syndrome.<sup>7</sup>

Various other vascular malformations, including intestinal telangiectasia, hemangiomas, lymphangiectasia, and venous ectasias, are occasionally seen in patients with Turner syndrome. Although the malformations are relatively infrequent, they can cause considerable distress. Multiple renal arteries are seen in approximately 90% of patients. No correlation has been made between the extrarenal arteries and hypertension, renal artery fibromuscular hypoplasia, or structural renal anomalies.

## Other Conditions Associated With Turner Syndrome

Hypertension occurs frequently in Turner syndrome, even when patients with coarctation and renal anomalies are treated or excluded. Hypertension in Turner syndrome is often idiopathic, is often associated with obesity, and responds favorably to weight reduction.

Autoimmune disease of the thyroid and of the bowel occur. There is a significantly increased incidence of thyroid antibodies, and hyperthyroidism or hypothyroidism can occur. Patients with Turner syndrome need to be screened annually for thyroid disease and examined for autoantibodies or elevated levels of thyroid-stimulating hormone or both.<sup>8</sup>

Regional enteritis and ulcerative colitis are also increased in incidence. Patients who have an isochromosome of the long arm seem to be particularly susceptible to inflammatory bowel disease.<sup>9</sup>

Diabetes mellitus in women with Turner syndrome occurs more frequently than usual. An impaired serum glucose response to an oral glucose load is seen in 25% to 60% of adults with Turner syndrome, but only a few have overt clinical diabetes.<sup>10</sup> Islet cell antibodies are absent in this group of patients. These women have type II diabetes resulting from glucose intolerance, not an autoimmune type of diabetes.

Patients with Turner syndrome run a high risk for tumors of the

reproductive system. The risk for gonadoblastoma in 45,X/46,XY individuals increases substantially after the age of 10, with the incidence reaching 25% or more after the age of 30.<sup>11</sup> Ideally, 45,X/46,XY patients should have their streak gonads removed prior to starting school. Both streaks should be removed, since the risk for bilateral tumors is high. Isolated case reports of dysgerminomas, seminomas, and teratomas in these individuals have been recorded.

A variety of other tumors has been described, including chronic myelogenous leukemia, virilizing hilus cell tumors, basal cell carcinomas, eosinophilic adenomas, thymomas, neural crest-derived tumors, and pancreatic growth-hormone-releasing hormone tumors. Theoretically, the risk for malignant degeneration secondary to ulcerative colitis is also increased, although this has not yet been reported.

## Skeletal Abnormalities

The skeletal changes associated with Turner syndrome are diverse. These changes, which are sometimes helpful in making a diagnosis, include short fourth metacarpal bones, cubitus valgus, midfacial hypoplasia, Madelung's deformity, increasing angulation of the carpal bones, pes cavus, irregular tibial metaphyses, Schmorl's nodules of the vertebrae, and a somewhat android configuration of the pelvis.

Scoliosis and lack of lumbar lordosis occur fairly frequently. Patients should be observed carefully to detect early curvature of the spine when estrogen therapy is begun. Congenital dislocation of the knees and hips is also seen.

Osteoporosis is said to occur with increased frequency and at an early age because of the radio-lucency of the bones, the coarse trabecular patterns, the decreased cortical width, and the decreased bone mineral content. These findings, which are common, do not appear to worsen with age or change significantly with

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## Turner Syndrome: An Update

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estrogen therapy. Patients with Turner syndrome do not have an increased number of fractures or collapsed vertebrae. Thus, it is not clear whether or not osteoporosis occurs more frequently in older women with Turner syndrome than it does in the general population.

A triangular face, down-slanting palpebral fissures, epicanthal folds, ptosis, a broad and short neck often with webbing and a low hairline, mid-facial hypoplasia with undergrowth of the maxilla, deepening of the posterior cranial fossa, and a small mandible with widely spaced mandibular rami characterize the craniofacies. High arched palates often cause feeding problems during the first year of life. Recurrent otitis media is a problem in infancy and childhood and is related to anatomic alterations at the base of the skull that change the angle of the eustachian tube, with the result that drainage from the middle ear is poor. The incidence of hearing impairment in adults with Turner syndrome is high, and chronic middle ear disease appears to be the etiology.

Keloid formation occurs frequently in patients with Turner syndrome. Other dermatological features include abnormal dermatoglyphics, with a large number of whorls on the tips of the fingers and a high percentage of distal triradii. These changes seem to be related to the edema that occurs early in embryonic development. Small, hypoplastic convex or concave fingernails and upturned toenails are also seen frequently.

Pigmented nevi are common and tend to appear late in childhood. Malignant degeneration of these nevi is not a problem.

### Mental Function

Although early reports indicated a high incidence of mental retardation among patients with Turner syndrome, this has not turned out to be the case. If a patient proves to have true mental retardation,

then the possibility of autosomal chromosome abnormalities should be evaluated and a careful investigation conducted for other causes of the mental retardation.

Girls with Turner syndrome are more likely to have specific problems with conceptualization of spatial relations and numerical identification.<sup>12</sup> Full-scale I.Q. testing reveals that the total I.Q. of Turner syndrome patients is usually average or above average. However, there is often a deficiency in perceptual motor organization or in fine motor execution. Therefore, the nonverbal I.Q. is significantly lower than the verbal I.Q. Learning disabilities in girls with Turner syndrome are currently being studied in greater detail. Arrangements for tutoring and special training programs may have to be made with the child's school if she is having difficulty with mathematics, history, or geography.

The personalities of girls with Turner syndrome are quite varied. Inertia to emotional arousal, high capacity to deal with stress, and strong traditional femininity are described. These girls tend to be isolated from others as they proceed through adolescence. Every effort should be made to enhance growth and sexual development before these girls "isolate" themselves because of sexual infantilism and short stature.

### Growth Retardation

Growth curves for patients with Turner syndrome are now readily available through Genentech, Inc. The average adult height achieved by patients with the syndrome is 145 cm, which is the 50th percentile. The heights at the 10th and 90th percentiles are 138 cm and 152 cm, respectively. It should be noted that a significant number of these patients do not fall behind in their growth curves until they are 4, 5, or 6 years of age. Thus, a positive diagnosis of Turner syndrome cannot be ruled out on the basis of a normal growth rate in early childhood.

A report by Ross et al<sup>13</sup> suggested that patients with Turner syndrome may have growth hor-

mone deficiency (GHD). The mean integrated concentrations of growth hormone (GH) over a 24 hour period were similar in Turner syndrome patients and normal girls 8 years of age and younger ( $4.6 \pm 0.7$  ng/ml v  $2.9 \pm 0.2$  ng/ml) and not statistically different from the 2.5 ng/ml mean value observed in Turner syndrome patients 9 to 17 years of age. However, normal subjects between 9 and 17 years of age had values of  $5.7 \pm 0.8$  ng/ml. The somatomedin-C (Sm-C) determinations were significantly decreased in 6- to 12-year-old girls with Turner syndrome, in comparison with those in normal girls matched for chronological and bone age. Although the authors concluded that a relative GHD in pubertal patients may contribute to their adult short stature, a more simple explanation for the higher integrated GH values during puberty in normal adolescents may be the presence of sex steroids, since these seem to be correlated with the normal increase in Sm-C levels seen at adolescence.

### Treatment for Short Stature

Treatment for the short stature associated with Turner syndrome has included low-dose estrogen, oxandrolone (Anavar), GH, and a combination of GH and oxandrolone.<sup>14</sup> The growth rates after the use of oxandrolone (0.125 mg/kg/day) or GH (0.125 mg/kg 3 times a week) increased from 4.3 cm/year to 7.9 cm/year and 6.6 cm/year, respectively, over a one-year period in one series of patients. The combination of these two agents increased the mean growth rate to 9.8 cm/year. The Food and Drug Administration, however, has not yet approved GH for use in patients with Turner syndrome, and these study findings should be considered preliminary. Also, it is not known whether the use of either of these agents increases growth velocity only or ultimate height as well. Extended studies are required to determine whether ultimate height is increased. The use of oxandrolone is not recommended if the pa-

tient's bone age is less than 9 years because of the possibility of inappropriate acceleration of skeletal maturation in relation to the height acceleration.

The use of low-dose estrogen (100 ng/kg/day) has been advocated on the basis of short-term studies, which reported that this increases growth velocity—at least of the tibia and ulna. These studies have not been carried out long term, and it is still equivocal whether low-dose estrogen therapy has a growth-promoting effect in Turner syndrome.

## Summary

Turner syndrome occurs in variable forms and should be suspected in short females regardless of associations of dysmorphology characteristic of Turner syndrome.<sup>15</sup> Chromosomal karyotypes are essential for the diagno-

sis. Physicians should be aware of the complications that occur in patients with Turner syndrome and should anticipate them, so the consequences that result will be minimal. Treatment should be directed towards increasing the height. Preliminary studies indicate that GH plus oxandrolone may be helpful in increasing growth velocity, although it is unknown whether this treatment increases ultimate height. The action of GH, if favorable, probably reflects a pharmacological effect and does not reflect GHD as such.

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## Special Report: First International Conference on Achondroplasia—November 17-21, 1986, Rome, Italy

Judith Hall, M.D.

Associate Editor

Growth, Genetics, and Hormones

This conference was held to review the international experience with some of the new methods that have become available to treat achondroplasia. These included leg lengthening, enlarging the foramen magnum, and prevention of kyphosis. A number of other areas were also explored, including genetics and natural history, psychosocial adjustment, lay support group organizations, neurologic complications, and basic biological research.

Among the most exciting reports were those describing leg-lengthening operations. Major progress has been made over the last three to five years in reducing the complications of leg-lengthening procedures by using percutaneous surgery with external fixators. Previous attempts at leg lengthening were complicated by infection and nonunion. However, presently available tech-

niques include innovations by Russian, Italian, and Spanish investigators that have led to a marked decrease in severe complications and a marked improvement in the actual amount of lengthening achieved. On average, a remarkable 30 cm of additional growth has been achieved in the lower limbs. The actual incidence of such complications as nerve compression, joint stiffness, and lack of full range of motion are not yet known. However, the advantages of the new technique are short hospitalization and mobility during the procedures. In addition, some of the other problems associated with achondroplasia—such as bowing of the legs, abnormal joint angles, lumbar lordosis, and lack of range of motion of the hips—are significantly alleviated by the procedures.

Very few data from basic science investigations are available

as yet, but the results presented at the conference were dramatic: The leg-lengthening procedure is a potential therapy for patients with disproportionately short stature. If successful, there is no reason to think the procedure would not benefit patients with other types of dwarfing conditions. In addition, it is proposed that leg lengthening might be accomplished after children are fully grown, although the ideal age may be 14 to 16 years. It is quite clear that older individuals can also benefit from this type of procedure.

In recent years, it has been shown that hormonal therapy has been beneficial in patients whose short stature is due to a variety of etiologies. Since the chondrodysplasias are not among these, it is therefore exciting to learn about the development of an orthopedic procedure that may be an appropriate mode of therapy.

## Special Report: The Western Society for Pediatric Research Meetings— February 3-6, 1987, Carmel, California

Judith Hall, M.D.

Associate Editor

*Growth, Genetics, and Hormones*

Many excellent papers of interest to endocrinologists and geneticists were presented at the meetings of the Western Research Society. Among them was one by Dr. Ray White, who reported that the order of linkage on chromosome 7 for cystic fibrosis is met-CF-J3111. Dr. White also pointed out that the crossover ratios for different segments of different parts of different chromosomes are different in males and females. There is no consistent pattern, and each arm and arm segment of different chromosomes is being recognized to give markedly different crossover rates.

Dr. Judson Van Wyk delivered the Stanley Wright Memorial Lecture. He discussed peptide growth factors and indicated that there may be fewer peptide growth factors than previously thought, because any given growth factor may act in many different tissues in different ways and under different stimulation. Interestingly, however, a specific peptide growth factor seems to be effective only on neuroectodermal or mesodermal or endodermally derived tissues.

Dr. Larry Shapiro reported that there is clearly a pseudoautosomal part of the short arms of both the X and Y chromosomes; this portion is shared and has obligatory crossover. Between the

locus on the X chromosome and the centromere there is a steroid sulfatase (STS) locus. On the Y chromosome at the same site there is a pseudo-STS gene, and proximal to that on the Y chromosome is the testes-determining factor. Rarely in human beings is there crossover between the X and Y chromosomes below the pseudoautosomal area; this explains why there are occasional XX males and STS-deficient females.

A memorial symposium in honor of Dr. Joseph St. Geme was established, at which a review of cytomegalic inclusion disease was presented by Dr. Charles Alford. He pointed out that this disease, which leads to extensive teratogenic effects in human beings, is probably now the most serious preventable viral illness in pediatrics. Approximately 35,000 newborns in the United States are affected each year, and at least 20% have significant sequelae.

In his report, Dr. David Rimoin said that the Kniest syndrome, the spondyloepiphyseal dysplasias, and Stickler's syndrome all seem to have linkage to type II collagen. Dr. Hollister reported that Marfan's syndrome seems to be an abnormality of fibrillin. Monoclonal antibodies to fibrillin were used to identify Marfan's patients in a double-blind study. These patients have recognizable fibrillin abnormalities.

Dr. A. Fujimoto described a new autosomal dominant pseudocleft syndrome characterized by a broad nose, colobomas of the eye, and branchial arch involvement.

Dr. Claire Leonard reported several cases of craniosynostosis and facial dysmorphism associated with maternal hyperthyroidism. The thyrotoxic state appeared to trigger early fusion of the cranial sutures.

Dr. Colleen Morris discussed the findings of a survey of 81 patients with Williams' syndrome and defined an evolving natural history that changes from infancy to adulthood.

Dr. Judith Hall reported that gonadal mosaicism appears to be responsible for some autosomal dominant conditions—such as pseudoachondroplasia—seen in siblings with normal parents. As many as 3% of apparent new dominant mutations may actually occur because of gonadal mosaicism for the abnormal gene in one of the parents. That is, during embryologic development of the parent, a somatic mutation occurred, giving rise to some tissues that carry the mutation, but not enough tissues to express the disorder in the parent. However, the gonad or gonads would carry the mutation and therefore the condition can be passed on to more than one child in the family.

### In Future Issues

The Concepts and Mechanisms of Genetic Linkage  
by Thaddeus Kelly, M.D.

Restriction Fragment Length Polymorphism:  
Applications to Linkage Analysis  
by Thaddeus Kelly, M.D.

On July 1, 1987, Dr. James M. Tanner and Dr. William L. Clarke will join the Editorial Board of *Growth, Genetics, and Hormones*. Dr. Tanner, of the Institute of Child Health at the University of London, is noted for his work in anthropometrics. Dr. Clarke, of the University of Virginia Medical Center, Charlottesville, is a pediatrician and pediatric endocrinologist with a special interest in diabetes. Dr. Tanner and Dr. Clarke will be "introduced" more formally in the next issue.

# Constitutional Delay of Growth and Adolescent Development

Jürgen R. Bierich, M.D.

Associate Editor

Growth, Genetics,  
and Hormones

In 1957, Lawson Wilkins first gave a comprehensive description of a syndrome called "constitutional delay of growth and adolescent development (CDGAD)." His definition included retarded linear growth beginning early in childhood, retarded skeletal maturation (often with a two- to four-year delay), retarded sexual development, and a familial incidence. Wilkins stated, "A certain level of general maturity must be reached before the pituitary gonadotropic mechanism can be activated." In this way, the concept emerged that the delayed sexual maturity is a secondary phenomenon—a sequel to the retarded growth.

Numerous alternative names have been used to denote CDGAD. These include constitutional delay of development, idiopathic short stature, simple delay of growth, and essential growth retardation. CDGAD must be differentiated from "genetic short stature," which has been called "constitutional short stature" by some. Genetic short stature and CDGAD may occur coincidentally. Tanner et al coined the term "small/ delay" for these patients. These patients have two common but distinct entities and not either simply CDGAD or simply genetic short stature. Furthermore, some of the children with "normal variant short stature" described by Rudman<sup>1</sup> have CDGAD; some children with what is today called "growth hormone neurosecretory dysfunction (GHNSD)" probably have CDGAD as well. Spiliotis et al<sup>2</sup> in 1984 wrote, "Children with GHNSD represent a substantial number of short children previously diagnosed as having CDGAD." Although one is justified in classifying children with short stature due to acquired cerebral lesions

(particularly those lesions resulting from CNS irradiation) as patients with GHNSD, one would not be justified at this time to use this term to describe patients who may have CDGAD.

## **Incidence and Growth Patterns**

CDGAD is more common than any other type of short stature. Estimates indicate that 60% to 90% of the parents of children with CDGAD themselves also experienced delayed growth and adolescent development. As a rule, this condition is inherited from one parent. Mendelian recessive inheritance, therefore, can be excluded. Simple dominant transmission is possible, but it certainly does not account for all cases.

The auxological features of CDGAD include normal birth length and weight. Growth velocity frequently slows during the first five years of life. When they begin primary school, these children are typically among the smallest. The growth curve then parallels the third percentile, and the velocity may sometimes be less than 3 cm/year.

The pubertal growth spurt commences two to four years after the usual age (mean age) for adolescent development, though this is the appropriate time based on skeletal maturation, and the peak height velocity is lower than average for children who mature at the usual time. Skeletal maturation is often delayed by several years. The high correlation coefficients between bone maturation and other parameters of development are presented in Table I.<sup>3</sup>

## **Endocrine Findings**

The endocrinologic findings are related to alterations in gonadotropin, growth hormone (GH), and somatomedin-C (Sm-C) or insulin-like growth factor-I (IGF-I) secretion. The gonadotropin concentrations correlate with bone age and delayed maturation but not with

the chronological age. In response to stimulation with luteinizing-hormone-releasing hormone (LHRH), the follicle-stimulating hormone (FSH) levels usually rise higher than do the luteinizing hormone (LH) levels. The reverse occurs as pubertal development ensues. It has been found that this test cannot differentiate patients with CDGAD from those with partial GH deficiency (GHD) that may be accompanied by a similar delay in skeletal maturation.

GH release following provocative stimuli (pharmacologic agents) is usually normal.<sup>4-6</sup> In a minority of children, the GH responses are within the hypopituitary range, but retesting these same children during puberty often yields peaks of GH concentrations that are within the normal range. For example, in 1979, Gourmelen et al<sup>7</sup> reported the results of ornithine stimulation of GH secretion in 105 children with retarded growth, the majority of whom had significant delay of bone age. Significant concentrations of GH were present after ornithine and/or insulin stimulation in 74, but not in 31. Of these, seven had confirmed GHD with peak lev-

*continued on page 10*

**Table I. Correlation Between Bone Age (BA) and Parameters of Development (Coefficient of Correlation *r*) in Patients With CDGAD**

|                           |      |
|---------------------------|------|
| Height age/BA             | 0.92 |
| Testicular development/BA | 0.86 |
| Pubic hair/BA             | 0.83 |
| 17 oxosteroids/BA         | 0.71 |
| Testosterone (urine)/BA   | 0.86 |



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els of less than 3 ng/ml. The remaining 24 children were between 11 and 15 years of age and in pubertal stages 1 and 2. Their peak GH levels were 3 to 8 ng/ml. Ten of these 24 were retested after reaching stage 3 of sexual development, and they had peak GH concentrations of between 8 and 45 ng/ml, with a mean of 21 ng/ml. The authors interpreted these observations as indicative of "transient-partial GHD." Other authors<sup>5,8-10</sup> have observed and reported similar instances in patients who appear to have GHD prepubertally but who have normal responses to provocative tests following the onset of puberty.

It is probably incorrect, however, to compare normal values obtained after the onset of puberty with the normal values found in prepubertal children. The mean stimulated values in normal adolescents are approximately twice those of prepubertal children. With stimulation, patients with CDGAD reportedly do not have GH peaks of the same magnitude as do normal children during puberty.

Because of the variability of results obtained after pharmacologic stimuli, several authors<sup>5,11-13</sup> have examined the spontaneous secretion of GH throughout the day or night or around the clock. In only a few instances were abnormally low peaks found during sleep, eg, in two of 14 patients reported by Wise and colleagues.<sup>13</sup> In 1979, and again in 1985, Bierich and co-workers<sup>14,15</sup> investigated sleep-induced GH secretion. The results in children with CDGAD were compared with those in healthy controls with equivalent sexual maturation. These investigators determined the area under the GH-v-time curve to obtain the integrated total secretion over 5.5 hours at night. The highest peak levels were also determined. The results obtained from 124 patients and 28 controls are shown in Table

II. These data indicate that patients with CDGAD secrete less GH at all stages of pubertal development than do control children. Differences between the patients and controls were statistically significant in all groups, except for those in stage 2 of sexual development. The interpretation of these data prompts the hypothesis that there is permanently diminished GH secretion.

A second form of growth delay, which is designated as "short stat-

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At least three groups of investigators have reported increased IGF-I levels in tall children (compared with children of normal stature); others have reported decreased IGF-I values in short children (compared with children of normal size).<sup>19-21</sup> Children with CDGAD have IGF-I values in accord with their skeletal maturation rather than with their chronological age. Link et al<sup>22</sup> demonstrated that

**Table II. Sleep-Induced GH Secretion in 124 Patients With CDGAD and 28 Controls**

|   | Puberty stages   |                   |                |                  |                  |                  |
|---|------------------|-------------------|----------------|------------------|------------------|------------------|
|   | P <sub>1</sub>   |                   | P <sub>2</sub> |                  | P <sub>3</sub>   |                  |
| (Number of cases)                                   | (86)             | (18) <sup>†</sup> | (31)           | (3) <sup>†</sup> | (7)              | (7) <sup>†</sup> |
| Sleep-induced GH secretion 5½ hrs (ng/ml × 330 min) | 2469**<br>± 1068 | 4349<br>± 1134    | 3347<br>± 1619 | 5905<br>—        | 3580**<br>± 1633 | 9794<br>± 1711   |
| Peak GH level (ng/ml)                               | 22.5**<br>± 12.1 | 38.8<br>± 14.0    | 27.2<br>± 16.6 | 49.8<br>—        | 21.2*<br>± 8.1   | 66.2<br>± 27.0   |

\*Statistically significant difference v controls  $P > 0.01$

\*\*Statistically significant difference v controls  $P > 0.001$

<sup>†</sup>Control children

ure due to an immunologically reactive but biologically inactive GH," is sometimes clinically indistinguishable from CDGAD. In this entity, growth retardation is presumed to result from the production of a GH molecule that is biologically inactive, since the GH levels are normal or high and IGF-I is low as in GH-deficient children. However, patients with this entity, which was described by Hayek and co-workers<sup>16</sup> and Kowarski et al,<sup>17</sup> are usually more severely retarded in growth than are patients with CDGAD. Subsequent patients also have been described by Bierich et al,<sup>4</sup> Rudman and co-workers,<sup>1</sup> and Valenta et al.<sup>18</sup> The only attempt to determine the structure of GH was made by Valenta et al,<sup>18</sup> who proposed a circulating GH molecule of abnormal structure. (See abstract in

the administration of testosterone to these patients increased both GH production and IGF-I levels. It is on this basis, as well as upon the observation that IGF-I levels increase early in puberty, that one may hypothesize that testosterone increases the circulating concentrations of GH and the generation of IGF-I in early adolescence.

## The Need for Therapy

The need for therapy and the treatment chosen should be considered for each individual child. The adult stature eventually reached by children with CDGAD varies considerably and is related to the heights of the parents. The majority of patients attain a height within the normal range, unless they are in the small/delay category. Nevertheless, Preece and colleagues<sup>23</sup> first demonstrated

that the adult height of those with CDGAD is below the mean for the general population. This was confirmed by Ranke et al.<sup>24</sup> Whether therapy will increase ultimate height or whether it will affect only the rate of growth during treatment is a question of great importance. If testosterone or androgens are used, one must consider whether adult height may be reduced. Treatment certainly is indicated in many patients and, in particular, in adolescent boys who are psychologically less able to cope with their shortness, their delayed sexual maturation, and their high-pitched voices. At no other time in childhood is the height gap with relation to age mates as great as it is during early adolescence, particularly since the growth rate of children with CDGAD reaches its nadir at the same time that the adolescent growth spurt occurs in normal children of the same age.

Treatment should be seriously considered for patients who are in danger of developing a severe inferiority complex. One of two forms of therapy can be chosen: the use of testosterone with or without anabolic steroids, or the use of GH.

### Therapy With Testosterone and/or Anabolic Steroids

Testosterone and/or anabolic steroids have been employed for many years to treat CDGAD. The anabolic steroids are derived from testosterone. With the possible exception of oxandrolone (Anavar), all anabolic steroids display similar androgenic or virilizing effects—including increased skeletal maturation—in comparison with testosterone. However, oxandrolone has a significant growth-promoting effect in relation to its minimal, modest virilizing action. At a dosage of 0.1 mg/kg/day, oxandrolone has been used successfully in patients between the ages of 11 and 16 who are more concerned about their height than their delayed sexual development. In Europe, Stanhope and Brook<sup>25</sup> reported favorable results with oxandrolone in 24 boys with CDGAD.

The use of testosterone is limited to boys, usually 14 years of age and over, who have significant concern about their delayed sexual development. Martin and co-workers<sup>26</sup> showed that doses of 50 mg of a long-acting testosterone ester (testosterone enanthate) once a month will enhance sexual development without affecting adult height. Doses of 100 mg or greater very possibly reduce the ultimate stature. Treatment is often continued for six to 12 months, at which time the testosterone is discontinued to determine whether spontaneous puberty has occurred. Occasionally, a repeat course of therapy beginning six months after the end of the first course is appropriate.

### Treatment with GH

Studies evaluating GH for the treatment of CDGAD are few in number. The first report of the successful administration of GH to such patients was from Grunt et al in 1972,<sup>27</sup> who stimulated growth in four of ten children with CDGAD. In a subsequent report by Kastrup et al,<sup>8</sup> growth velocity rose from 3.7 to 8.4 cm/year during therapy. Bierich and co-workers<sup>4</sup> in 1983 reported an increased growth rate (from 4.1 cm/year to 8.3 cm/year) with therapy. Gertner and co-workers<sup>28</sup> have reported similar findings. Individual differences are considerable, but the greater the delay in bone age, the better the acceleration of growth velocity. The growth rate in children with CDGAD had been maximal during the first year of treatment and decreased thereafter, a pattern that occurs with GH-deficient patients receiving GH.

Although adequate data are presently unavailable to evaluate the long-term effect of GH treatment on children with CDGAD, this author has the clinical impression that the growth prognosis of most of these children improves. Since none of the patients in our series has reached final height, no definitive statements can be made regarding an increase in ultimate height. At present, treatment with

GH of patients with CDGAD should be reserved for the most severely dwarfed children. Additional studies under controlled conditions are urgently required before GH treatment—outside of centers where the efficacy of GH treatment in patients who are not GH-deficient is being studied—can be recommended for patients with CDGAD.

The use of other agents, such as growth-hormone-releasing hormone (GHRH) and clonidine, an  $\alpha_2$  adrenergic drug that stimulates the release of GH, requires further study. Results evaluating GHRH in the treatment of CDGAD have not been reported. In 1985, Pintor et al<sup>29</sup> administered clonidine to four patients with CDGAD over a period of two months, and they described increased values of circulating GH and IGF-I and accelerated growth. However, this is an exceedingly small series of patients treated for a short period of time. Currently, several investigators are involved in studying the effect of clonidine in CDGAD further.

### Summary

CDGAD is the most common form of short stature. Preliminary studies suggest that children with CDGAD have inadequate GH production, whereas children of the same age who do not have CDGAD, have adequate GH production. Some would call this a physiological state (partial GHD). In this sense, CDGAD is similar to the previously described GHNSD. Testosterone, and probably estrogen as well, stimulates increased production and secretion of GH. This, in turn, stimulates increased IGF-I concentrations in normal early puberty.

The use of GH as a therapeutic agent in CDGAD is under clinical investigation. Currently, oxandrolone (0.1 mg/kg/day) is the treatment of choice in patients under 14 years of age who require a growth-promoting agent for primarily psychological reasons. Depot testosterone (long-acting enanthate or cypionate) is the

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treatment of choice for patients over 14 years of age who are concerned about delayed sexual maturation as well as short stature. GH, GHRH, clonidine, or all three may have therapeutic importance in the future for patients with CDGAD, but are not currently recommended except for patients enrolled in research protocols.

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## Letter to the Editor

### Another Perspective on Intrauterine Growth Retardation

Intrauterine growth retardation (IUGR) is a complex clinical condition that is directly related to maternal health and behavior during pregnancy and is in large measure preventable. The purposes of this communication are to illustrate the complexities in the epidemiology, pathogenesis, and diagnosis of IUGR, and to point out that the Denver fetal growth charts, which apparently are the most widely used in the United States,<sup>1</sup> are inadequate for diagnosing IUGR and consequently underestimate its frequency by a wide margin.<sup>2,3</sup>

**Epidemiology and pathogenesis.** It has been widely recommended that IUGR be diagnosed on the basis of a marked reduction in birth weight at a specific gestational age.<sup>1,4</sup> When IUGR is diagnosed by this method, any condition that lowers birth weight in relation to gestational age must be taken into account. There is an increasing awareness of a link between IUGR and low birth weight (LBW) to the extent that many growth-retarding conditions are common to both outcomes.<sup>1,5,6</sup> Most term infants with LBW have IUGR. The comprehensive report on the prevention of LBW<sup>5</sup> lists 41 principal risk factors associated with LBW; they include genetic, environmental, demographic, and socioeconomic conditions, medical and obstetric complications of pregnancy, fetal infections, and inadequate nutrition of fetuses. Most of these risk factors have been observed in term infants with

IUGR.<sup>7</sup> With so numerous and so varied a list of risk conditions, it should not be surprising that some pregnancies are complicated by multiple risks that are associated with even greater reductions in birth weight that occur with single risks.<sup>8</sup> Single risks occur in some pregnancies, but they are less frequent than generally suspected (except for cigarette smoking, which has occurred in 30% to 40% of gravida in some populations).

There are three main types of IUGR; they differ in their pathogenesis, their frequencies, and in their postnatal courses. The symmetric type of IUGR is diagnosed by a short crown-heel length for gestational age. The asymmetric type, which is the least severe form of IUGR, is diagnosed by a low weight-height ratio or ponderal index. The combined type (symmetric and asymmetric) is the most severe type of IUGR and, fortunately, the least frequent. These infants are small for their dates and markedly deficient in soft-tissue mass.

These types can not be diagnosed from birth weight alone, but require measurements of crown-heel length and calculations of either weight-height ratios or ponderal indices. Accurate measurement of crown-heel length is not easy in infants who are tensed in the flexed position. Crown-heel lengths are more accurate when the infant is quiet and put in the tonic-neck reflex position that allows one leg to be straightened

more readily.

**Diagnosis and frequency.** The use of appropriately constructed fetal growth charts and tables for diagnosing IUGR at birth is critical in determining its frequency. Accurate determinations of weight and body measurements at birth are essential in every infant, not only to diagnose IUGR, but also to provide reliable baseline data for physicians evaluating postnatal growth of infants and children. Ultrasound methods for diagnosing IUGR prenatally have been improved, but the ultimate responsibility for diagnosing IUGR rests with the physician caring for the newborn infant.

The location of "bottom" lines on fetal growth charts used in diagnosing IUGR depends on the investigators who design them and who decide which infants are to be excluded from the charts and tables, how gestational age is to be determined, and whether the bottom lines should be located on the third, fifth, or tenth percentiles or at two standard deviations below the means. The extreme variations in the data and locations of bottom lines of fetal growth charts and tables in the United States have been described.<sup>9</sup> An example of these extreme variations is seen in the Denver charts and Kansas City tables of birth weights.<sup>9</sup> The bottom lines on the Denver charts (tenth percentiles) are 400g to 500g lower for infants than the bottom lines in the Kansas City tables (fifth percentiles). These differences produced a sixfold increase in the frequency of IUGR when the same group of infants was diagnosed using the Kansas City tables as compared with the Denver charts.<sup>7</sup> The explanation for the differences in bottom lines is partly related to Denver's mile-high altitude as compared with Kansas City's altitude (about 800 feet above sea level). But it is also related to a marked difference in the criteria for excluding infants from the charts and tables. Infants were excluded from the Kansas City tables if their mothers had any of a long list of risk conditions.<sup>7</sup> Infants were excluded from the Denver

charts if their mothers had diabetes or if hydrops fetalis or major congenital malformations were present. These exclusions amount to a small percentage of the many risk conditions that have the potential for retarding fetal growth. Untold numbers of infants born to mothers with potential fetal-growth-retarding conditions that were present during their pregnancies were included in the Denver charts.

The diagnosis of IUGR is further complicated by conditions present in all pregnancies that have the potential for lowering birth weight significantly. These are maternal race, height, weight-height ratio at conception, parity, and age, and sex and gestational age of infants.<sup>10,11</sup> These conditions require that either special fetal growth tables be constructed for each condition or that these universally occurring conditions be controlled when diagnosing infants with IUGR. For example, reported data indicate that black infants born at term to low-risk mothers delivered at the University of Kansas Medical Center are smaller and weigh less at birth than white infants of the same gestational age and sex when controlling for mother's socioeconomic status, risk conditions, height, weight-height ratio at conception, gravidity, parity, and weight gain during pregnancy.<sup>12</sup> Another study suggests that black infants born to mothers who smoke heavily (>10 cigarettes per day throughout pregnancy) have lower birth weights than infants of white mothers born under similar circumstances.<sup>13</sup> Cigarette smoking during pregnancy is almost certainly one of the most, if not the most, frequent risk condition associated with IUGR. The Denver charts do not take cigarette smoking into account and do not provide data on fetal growth of blacks.

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## Dr. Blizzard's comments

Dr. Joseph Warshaw's article in *Growth, Genetics, and Hormones* ("Perspectives of on Intrauterine Growth Retardation," Vol. 2, No. 4) prompted Dr. Herbert Miller to respond with his perspective on IUGR. These two presentations complement each other exceedingly well. All readers who are interested in this important topic will benefit from comparing these perspectives.



## Insulin-like Growth Factors I and II: Evaluation of Growth Retardation

Rosenfeld et al measured plasma insulin-like growth factor (IGF-I and IGF-II) levels in 197 normal-sized children, in 44 normal short-statured children (NL-SS) with normal growth hormone (GH) release in response to pharmacologic stimuli, and in 68 GH-deficient children. Because of the variation in IGF-I levels related to age and sex, the results were stratified by sex into six age groups. Normal percentile curves were constructed for each group. The results for all groups are shown in Table I.

Evaluation of the mean IGF levels according to patient status and age groups provided the following analysis. Mean IGF-I levels in GH-deficient children were significantly below the mean levels seen in children of normal stature in each of the six age groups. IGF-II levels were significantly below the mean levels in only 50% of the group with GH deficiency (GHD). There were significant differences between the mean levels of IGF-I and IGF-II in normal and NL-SS children who were younger than 8 years of age, but these differences were not consistent by gender.

Analysis of both assays according to patient status is shown in Table II. When IGF-I and IGF-II levels were used in combination, the distinction between normal and GH-deficient children was more pronounced than when either assay was used alone. Only one (0.5%) normal child and five (11%) short normal children had low plasma levels of both IGF-I and IGF-II. On the other hand, only three (4%) GH-deficient children had normal plasma levels of both somatomedins.

Rosenfeld RC et al. *J Pediatr* 1986; 109:428.

Table I. Percentile Curves

|              | IGF-I   |        | IGF-II  |        |
|--------------|---------|--------|---------|--------|
|              | 5-95th* | < 5th* | 5-95th* | < 5th* |
| Normal (197) | 98%     | 2%     | 95%     | 5%     |
| GHD (68)     | 18%     | 82%    | 58%     | 52%    |
| NL-SS (44)   | 68%     | 32%    | 65%     | 35%    |

\*Percentiles

Table II. Analysis of IGF-I and IGF-II assays

|              | NL/NL* | NL/L† | L/L‡ |
|--------------|--------|-------|------|
| Normal (197) | 93%    | 6%    | 1%   |
| GHD (68)     | 4%     | 59%   | 37%  |
| NL-SS (44)   | 46%    | 43%   | 11%  |

\* Both IGF-I and IGF-II levels are between the 5th and 95th percentiles.

† Either the IGF-I or the IGF-II level is between the 5th and 95th percentiles.

‡ Both IGF-I and IGF-II levels are below the 5th percentile.

**Editor's comment**—The data from this large series of children may serve as a first order attempt at differentiating short normal (but GH-sufficient) children from those with GHD. If only it were so simple! First, IGF-II determinations are not routinely available. Although the children in each of the two short-stature groups were separated by responses to pharmacologic provocative tests for GH secretion, the categorization of children into GH-sufficient and GH-deficient groups is accurate only at the extremes. There are a number of children with a GH-deficient phenotype who respond to pharmacologic stimuli by releasing GH, but who do not grow well. Are they physiologically deficient in GH?

How one answers depends upon the "definitions" used. In fact, it is not so important to label or categorize each patient by GH response to stimuli, but to determine prospectively which children are likely to respond to exogenous hormonal therapy—be it recombinant human growth hormone (hGH) or (in the future) recom-

binant IGF-I. Thus, many short children may have a neurosecretory alteration that does not permit enough GH to be secreted at the proper intervals to maintain liver and tissue growth factor levels, thereby preventing the child from reaching his or her genetically determined growth potential.

The unavoidable implication of GH secretory pattern studies, especially when combined with the observations in this report, is that the diagnosis of GHD based on the results of provocative tests is both arbitrary and nonphysiologic. Suboptimal GH secretion or activity may be reflected in decreased GH pulsatility, reduced integrated GH concentrations, or suboptimal IGF-I and/or IGF-II levels. With the imminent availability of essentially unlimited quantities of hGH (and IGF-I), the ability to determine which short children are most likely to have accelerated growth (catch-up growth) in response to therapy with these growth factors becomes of paramount importance.

Alan D. Rogol, M.D., Ph.D.

## Growth, Bone Maturation, and Biochemical Changes in Brazilian Children From Two Different Socioeconomic Groups

Growth and bone maturation were measured to assess the influence of malnutrition on growth in two groups of children and adolescents (ages 7 to 17 years) in Brazil. The groups (674 from the upper socioeconomic class and 226 from the lower socioeconomic class) were evaluated for weight, height, and bone age. Biochemical measurements, including plasma calcium, phosphorus, alkaline phosphatase, and serum proteins, were also taken.

The growth of children from the upper socioeconomic class was similar to American standards for growth, with the mean weight and height following the 50th percentile curves on the National Center for Health Statistics growth charts. However, the children from the lower socioeconomic class were underweight for their height and growth retarded for their chronological age. Their mean values for weight and height fell below the 25th percentile on the same growth charts. Interestingly, boys were more severely affected, with many having height measurements below the 5th percentile.

Evidence of delayed skeletal maturation was seen in only 9% of the upper socioeconomic class children, while 84% of the lower socioeconomic class children had a delay in bone age of at least two years. Boys were more affected than girls, and bone age delays of greater than three years were seen only in boys. Abnormal bone structure, including evidence of growth arrest and fewer coarse trabeculae, was also found in 13% of the children in the lower socioeconomic class.

Plasma calcium, magnesium, vitamin D, and total protein levels

were similar in both groups of children and no signs of rickets were found. In underprivileged children, albumin levels were significantly lower ( $P < 0.001$ ) and plasma alkaline phosphatase and phosphorus levels remained elevated even after the predicted age of the adolescent growth spurt had been reached. Menarche was delayed by two years in the girls of the lower socioeconomic class.

Linhares EDR et al. *Am J Clin Nutr* 1986;44:552.

**Editor's comment**—The authors describe a serious problem of chronic malnutrition in the lower socioeconomic class that affects as many as 50% of Brazilian children. This study also points out that environmental conditions may be more important than racial fac-

tors in influencing growth. Privileged Brazilian children grew and developed as well as American children.

The gender differences in height and development may also be culturally induced. The more severely impaired growth seen in underprivileged boys may reflect the fact that adolescent boys are forced to find work while the girls remain at home. Long hours and poor working conditions generally affect health and nutritional status adversely, thus resulting in impaired growth and delayed adolescent development. Effective programs to protect the young in underprivileged communities, where malnutrition is prevalent, could alleviate the unfortunate consequences of nutritional dwarfing.

Fima Lifshitz, M.D.

## Growth in Thyrotoxicosis

Buckler and co-workers followed 46 children and adolescents who developed thyrotoxicosis after infancy to determine the effects of this condition on growth velocity, adolescent development, and ultimate height. As expected, girls with thyrotoxicosis outnumbered boys in this study, where 41 of the 46 subjects were female. Diagnosis was based on symptoms, clinical signs, and biochemical measurements of thyroid hormone levels. All but two subjects were adequately controlled by medical treatment, as determined by clinical and biochemical criteria.

At presentation, most of the children had heights above average and some were very tall (average,  $+ 0.75$  SD). The skeletal age was often more advanced than the height age, but to a variable degree. The children were underweight for age (average,  $- 0.32$  SD) and thus quite underweight for height.

Ultimate height for the girls was  $+ 0.54$  SD, which is greater than their target height ( $+ 0.0$  SD) based on the midparental centile values.

Buckler JMH et al. *Arch Dis Child* 1986;61:464.

**Editor's comment**—As expected, these children were tall and had advanced bone ages at presentation. The advanced bone ages were often out of proportion to the increase in height. However, the ultimate height prognosis was good, which is not the case with patients whose increased height and advanced bone age are accompanied by virilizing adrenal hyperplasia or precocious puberty. What cannot be determined is the role of "good control" of thyrotoxicosis on ultimate height, since many patients had periods of mild toxicity or mild hypothyroidism while on therapy.

Alan D. Rogol, M.D., Ph.D.

## MEETING CALENDAR

**June 14-18** 27th Annual Meeting of the Teratology Society, Marriott Rancho Las Palmas Resort, Rancho Mirage, California. Contact: Alexandra Ventura, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1841)

**July 19-22** Clinical Genetics Conference: Neural Crest and Craniofacial Disorders. March of Dimes—Birth Defects Foundation and the University of Minnesota, Twin Cities. Radisson Hotel, Minneapolis, Minnesota. Contact: Dr. Robert Gorlin, University of Minnesota School of Dentistry, 515 Delaware Street SE, Minneapolis, MN 55455

**August 15-19** David Smith Malformations and Morphogenesis Meeting. Furman University, Greenville, South Carolina. Contact: Dr. Roger Stevenson, Greenwood Genetics Center, 1 Gregor Mendel Circle, Greenwood, SC 29646 (803-223-9411)

**September 6-11** 9th International Workshop on Human Gene Mapping. Paris, France. Contact: Prof. Jean Frezal, Hôpital des Enfants Malades, 149 Rue de Sevres, 75743 Paris CÉDEX 15 France. (1-42 73 80 00)

**September 11-12** New Genetics and the Human Gene Map. Paris, France. Contact: Prof. Jean Frezal, address and phone as above.

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**September 28-30** International Congress on Advances in Growth Hormones and Growth Factors Research. Milan, Italy. Contact: Drs. Daniela Cocchi and Vittorio Locatelli, Department of Pharmacology, Chemotherapy, and Toxicology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy

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**October 7-10** 38th Annual Meeting of the American Society of Human Genetics. Town and Country Hotel, San Diego, California. Contact: Administrative Office, American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

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# GROWTH

## Genetics & Hormones

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### Genetic Linkage: Introduction to Basic Concepts

Thaddeus E. Kelly, M.D., Ph.D.  
*Professor of Pediatrics  
Department of Pediatrics  
University of Virginia School of  
Medicine  
Charlottesville, Virginia*

Analysis of the linkage of genes and the construction of gene maps are basic approaches to understanding how genes control both normal and abnormal physical and biochemical differentiation and function of the human organism. During the past 10 years, the development of recombinant DNA technology and its utilization in mapping genes have greatly enhanced our knowledge in these areas and, concomitantly, have increased the application of this information to clinical medicine. This article is presented to clarify concepts about genetic linkage and to sharpen the reader's understanding of gene linkage and its implications.

#### Practical Applications of Gene Linkage and Mapping

If a genetic disorder segregates in families and is consistent with a mutation at a single gene locus, the location of that gene within the entire human genome can potentially be mapped. This can have several practical applications:

1. To determine whether a molecular defect in a clinical condition, such as genetic growth hormone (GH) deficiency, is due to an absence or to a mutation of a specific gene.
2. To determine if clinical conditions with similar characteristics

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#### Letter From the Editor

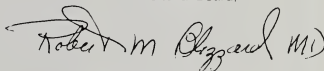
Dear Colleague:

An understanding of the genetic approach to diagnosis and treatment is essential for all pediatricians, regardless of subspecialty. The complexities of this approach are great; indeed, comprehending the language and the tools of the geneticist is a challenge for many of us.

Since a major goal of this publication is to expose our readers to a concise review of important current topics, we invited Dr. Thaddeus Kelly to write two articles to introduce us to the geneticist's current approach to the diagnosis and treatment of inherited diseases. In my letter to you in *Growth, Genetics, and Hormones*, Volume 3, Number 1, I stated, "We encourage you to set aside a few hours for studious review of Dr. Kelly's articles; they will be well worth your time and professional interest." I am now encouraging you to follow my suggestion to curl up in a chair away from the phone and to read and study Dr. Kelly's two contributions published in this issue. The investment of time will be rewarding, and the articles will provide you with a basis for understanding more detailed information regarding our capability to pursue the diagnosis and treatment of inherited diseases.

As an interested reader, you can expand your knowledge by reading an excellent article on this topic published in the *Mayo Clinic Proc* (1987;62:387-404) by Dr. S.S. Sommer and Ms. J.L. Sobell. The title of the review is "Application of DNA-based Diagnosis to Patient Care."

For the Editorial Board,



Robert M. Blizzard, M.D.  
Chairman

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## Genetic Linkage

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occurring among members of unrelated families are actually identical in origin. This can be done by studying the genes of affected individuals for identity or non-identity. For example, patients with types IA and IB of genetically inherited GH deficiency have non-identity, or different etiologies. In type IA, the GH gene is missing. The gene is present in type IB.

3. To diagnose certain clinical conditions by determining gene haplotypes (eg, HLA types), since there is genetic linkage between certain conditions and the HLA loci. Congenital virilizing adrenal hyperplasia can be diagnosed in a fetus by studying the HLA haplotypes of the fetus, its parents, and its affected sibling.

4. To design replacement therapy for a missing gene product, such as GH, by utilizing recombinant DNA techniques.

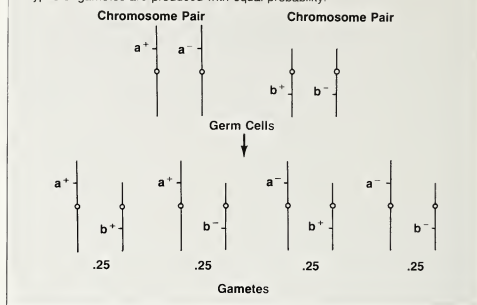
5. To design methods for the insertion of normal genes into an affected fetus or child. A possible application could be in the Lesch-Nyhan syndrome.

### Gene Alleles, Meiotic Segregation, and Gene Crossover

When the genes for a trait are identical in all individuals, the genes are said to be *monoallelic*. When multiple variations occur in gene structure, *polyallelism* is said to be present whether gene function is normal or abnormal. For example, the presence of various alleles for the HLA-A locus (HLA-A1, A2, etc) is termed polyallelism. Similarly, polyallelism also occurs when the variations lead to abnormal gene function.

During the first cell division of meiosis (the reduction division from 46 to 23 chromosomes), the members of each pair of homologous chromosomes separate and each daughter cell ends up with one or the other chromosome of a pair. *Segregation* of alleles at a given gene locus occurs during this stage of meiosis. Alleles at loci on non-homologous chromo-

**Figure 1.** The alleles at two loci on different chromosomes ( $a^+$ ,  $a^-$  on one chromosome and  $b^+$ ,  $b^-$  on the other) assort randomly in meiosis. Four types of gametes are produced with equal probability.



somes will assort randomly, as illustrated in Figure 1. The alleles  $a^+$  and  $a^-$ , one on each chromosome of a homologous pair of chromosomes, and the alleles  $b^+$  and  $b^-$ , also one on each chromosome of another homologous pair, assort randomly in meiosis. With respect to these two gene loci, four types of gametes are produced with equal probability.

Logically, all the genes present on a single chromosome should be transmitted intact as a unit during meiosis. However, a phenomenon of gene transfer called *crossover* often occurs between the chromosomes of each chromosome pair during the first cell division of meiosis, as diagrammed in Figure 2. Crossovers result in new combinations of maternally-derived and paternally-derived alleles in the chromosomes of gametes. Although only one crossover or recombination is diagrammed in Figure 2, a pair of large chromosomes may have three or four crossovers per meiotic cell division.

The result of crossing over is that the alleles initially at two loci on the same chromosome may or may not assort randomly during meiosis. The frequency of crossing over between two loci depends primarily on the physical distance between the two loci. As shown in Figure 3, when the two loci of inter-

est (A and B) are on opposite arms of the chromosomes, crossing over between these loci takes place readily and frequently during meiosis. If, however, the two loci of interest (B and C) are closely associated on the chromosome, crossing over between these loci occurs rarely. This results in *non-random assortment*, which occurs only when the loci are *linked* or very close together.

### Address for Correspondence

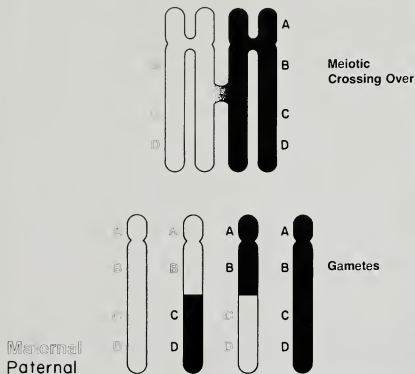
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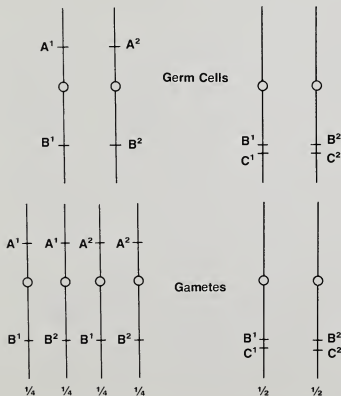
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**Figure 2.** Crossing over between a pair of homologous chromosomes in meiosis results in a new combination of maternally and paternally derived genes in the recombinant chromosomes. The figure shows one crossover; a pair of large chromosomes will typically have three to four crossovers per meiotic cell division.



**Figure 3.** The alleles at two loci (A and B) on the same chromosome may be sufficiently far apart that assortment is random, or the loci (B and C) may be sufficiently close that they segregate as a unit.



## Determination of Informative and Non-Informative Matings

A person may have two homologous (paired) chromosomes but be heterozygous for the alleles at each of two loci (for example, A<sup>1</sup> and A<sup>2</sup> at locus A, and B<sup>1</sup> and B<sup>2</sup> at locus B, as diagrammed in Figure 4). When geneticists speak of *alleles of interest*, they usually mean the mutant or the less frequent alleles that are used to study linkage. When both *alleles of interest* are on the same chromosome, as are A<sup>2</sup> and B<sup>2</sup> in individual X in Figure 4, the alleles of interest are said to be *in coupling*. If the two alleles of interest are on opposite homologous chromosomes, as are A<sup>2</sup> and B<sup>2</sup> in individual Y in Figure 4, the alleles of interest are said to be *in repulsion*. The state of these relationships is referred to as the *coupling phase or state*. Crossing over between the two loci results in a reversal of the coupling phase and is called *recombination*.

Families in which a member has a specific disease (the individual is referred to as the *proband*) can be studied for possible linkage of genes. The study of linkage in a sibship requires that the parents of the siblings be *informative* for linkage analysis. An *informative mating* is one in which the genotypes of the parent will allow for the recognition of genetic recombination or non-recombination in their offspring. The determination of linkage between two loci is based on an analysis of the frequency of recombination among the alleles of interest in the proband and his or her siblings.

A classic example to elucidate whether a *mating is informative* is in the study of the relationship of the gene locus of the ABO blood-type and the locus of a "structural gene" which, when mutated, results in the nail-patella syndrome. To analyze linkage between these two loci, it is essential that the affected individual be heterozygous at both the ABO locus and the locus for the nail-patella syndrome. The ideal mating for linkage analysis exists if the affected individual has type AB blood and is married to an unaffected indi-

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## Genetic Linkage

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vidual with type O blood. If the affected individual has type O blood and the mate also has type O, it is not possible to determine if recombination occurs between the two loci since homozygosity for type O is present at the blood type loci of both mates; this precludes studying linkage and is referred to as a *non-informative mating*.

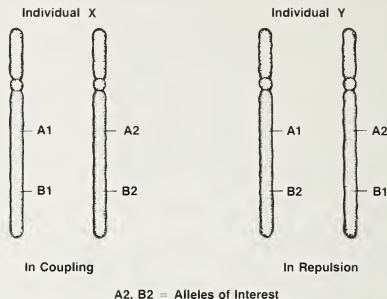
If no linkage exists between two loci, assortment of the genes at these loci is random, and recombination should occur 50% of the time. There is equal likelihood that an offspring will receive either an apparently unchanged parental haplotype or one in which the coupling phase is reversed. On the other hand, if the two loci are physically linked, analysis of the genes of the offspring will show distortion of this segregation ratio with a *recombination frequency of less than 50%, which is "linkage" by definition*.

Restated, a double heterozygote for two *unlinked loci* will produce four types of gametes with equal frequency (25% each). This is shown in Figure 3. The distortion by linkage of the expected 25%:25%:25%:25% segregation of two loci is dependent on the frequency of crossing over between the two loci. With *linkage of two loci* and a *recombination frequency* of 20%, the *segregation ratio* of the gametes becomes 40%:40%:10%:10%, as diagrammed in Figure 5.

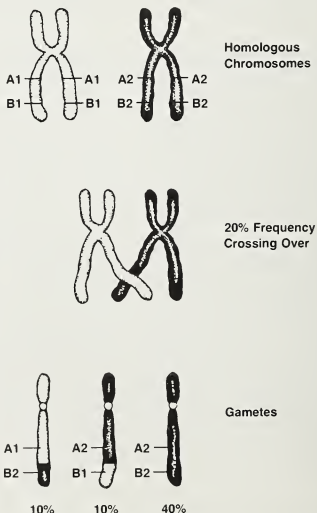
## Studies of Linkage Analyses by Traditional Studies of Families

For years, the analysis of linkage of gene loci was restricted to the construction of pedigrees in which a single gene-determined disorder was segregating and in which the analysis of a limited number of genetic markers might indicate linkage. The available genetic markers included red blood cell antigens, such as ABO, Kell, Rh, Duffy, and polymorphic plasma proteins, such as the amylases, haptoglobins, and peptidases.

**Figure 4.** The coupling phase of linked loci refers to whether the "alleles of interest" are on the same chromosome ("cis" configuration) or on opposite chromosomes ("trans" configuration).



**Figure 5.** The distortion of the expected 25%:25%:25%:25% segregation of two loci by linkage is dependent on the frequency of crossing over between the two loci.



The investigator had no prior knowledge of the likely chromosomal assignment of the gene under study and, often, many of the families studied were not informative for linkage analysis, because of a low order of polymorphism for many genetic markers. Furthermore, the available markers for linkage analysis covered only a small portion of the human genome.

Nevertheless, this proved contributory and clinically helpful in certain studies. Dupont et al used this traditional approach in 1977 to establish linkage between the HLA locus and the structural locus for the steroidal 21-hydroxylase enzyme. This important finding provided a method to identify fetuses affected with congenital virilizing adrenal hyperplasia (CVAH). A pedigree reported by Dupont et al is diagrammed in Figure 6; a, b, c, and d represent specific HLA haplotypes. In the father, the HLA haplotype A24, B35, C4 (designated b) is in coupling with the mutant allele for 21-hydroxylase; in the mother, the mutant allele for 21-hydroxylase is in coupling with A1, B8 (designated d). Two of the children were affected. Note that the unaffected child, having received the mutant allele from the

father and the normal allele from the mother, is a carrier for 21-hydroxylase deficiency. Determination of HLA typing permits the physician to determine if any unaffected person in the family is a carrier for the mutant gene and whether the mutant gene in an unaffected person is derived from the mother or father.

### The Significance of the LOD Score

The *LOD score* or *logarithm of the odds* is a mathematical statement of the probability of linkage between two loci at a specified rate of recombination. The distance between two linked loci is expressed as a function of the frequency of recombination.

Establishment of linkage is determined by generating a LOD score. The probability that the observed segregation of alleles within families represents linkage can be compared with the probability that these same observations would occur if no linkage exists between the two loci under consideration. When the coupling phase in the heterozygous mate can be determined, as with co-dominantly expressed traits such as HLA, and if heterozygous children can be separated from ho-

mozygous normal children, the number of recombinants and non-recombinants can be directly counted among the offspring.

The LOD score is calculated by means of a complex formula to determine if two loci are linked and, if they are linked, to determine the best estimate of the distance between the loci. In the LOD score equation, various estimates of the frequency of recombination ( $\theta$ ) are used to determine the frequency of recombination that gives the highest LOD score. The value for  $\theta$  that gives the highest LOD score is taken as the best estimate of the frequency of recombination between the two loci under study. A LOD score of 3 or greater establishes linkages. A LOD score of 3 (for a given value of  $\theta$ ) means that it is 1,000 times more likely that the observed pattern of non-recombinants and recombinants occurred because of linkage rather than because of chance. (The LOD score equation, along with an explanation for its usage, will be provided to readers upon request.)

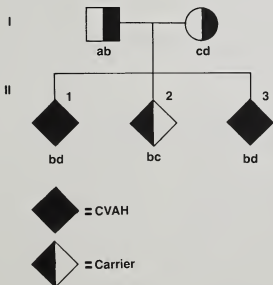
### Limitations in the Use of Linkage for Diagnosis

There are some limitations in the use of genetic linkage for diagnosis, as mentioned previously when discussing the linkage of the ABO blood group and the nail-patella syndrome. In this instance, the affected individual would have to be heterozygous at both gene loci. To say that linkage analysis is possible for 85% of at-risk couples implies that the frequency of polymorphism at the linked genetic marker renders 85% of couples informative and 15% non-informative for analysis.

The second limitation is related to the distance between linked loci. The greater the distance between linked genes, the greater the likelihood that recombination would separate the linked genes and lead to error in the calculations.

Neither of these limitations is significant for the prenatal diagnosis of CVAH by HLA typing. The high rate of polymorphism of

**Figure 6.** The two children with CVAH in this family were concordant at the HLA loci, while the second-born child is a carrier of 21-hydroxylase deficiency (from Dupont et al).





## Genetic Linkage

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alleles at the HLA loci renders most at-risk couples informative. Moreover, the distance between the HLA-B and 21-hydroxylase loci is so minute that no recombination with separation of these two alleles has been observed. Therefore, the potential error is minuscule.

### Summary and Comment

Currently, a major research goal in human genetics is to develop genetic markers that are regularly spaced throughout the human genome at a maximum distance of 30 to 40 cM (centiMorgans). Such a

set of markers currently exists for the human X chromosome. When identified for other chromosomes, it will be possible to map the unique structural genes within the human genome. Precise mapping of a locus will be the first step in determining the molecular basis for many disorders (such as Huntington disease and cystic fibrosis) and is essential for treatment based on genetic engineering. The advent of recombinant DNA techniques and their utilization in gene mapping and linkage analysis have led to great progress toward these goals. These techniques are discussed in greater detail in the accompanying article.

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# Restriction Fragment Length Polymorphism: Applications to Linkage Analysis

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Restriction endonucleases are enzymes that cut DNA at specific nucleotide sequences (called "recognition sites") that are composed of 4 to 6 nucleotides. For a restriction endonuclease to cut both strands of double-stranded DNA, the sites must be mirror images or *palindromes* of each other. For example, the palindrome recognized by Eco RI is GAATTC on one DNA strand and CTTAAG on the other. If this sequence is altered, the enzyme will not cut the DNA at that location. These naturally occurring differences, which are called *polymorphisms* in nucleotide sequence, at restriction endonuclease recognition sites result in the generation of DNA fragments of different lengths; these are referred to as restriction fragment length polymorphisms, or RFLPs. Because the restriction sites (and their variants) are inherited in families as Mendelian

traits, RFLPs can be used for linkage analysis in the same manner as other biochemical or genetic markers.

RFLP analysis can be combined with other methods of gene mapping to allow study of diseases not previously approachable at the molecular level. RFLP analysis is made possible by the availability of a large number of DNA probes and restriction endonucleases, which will be discussed in greater detail. This will be followed by a discussion of the study of genetic heterogeneity by RFLP analysis and how diagnosis by RFLP analysis is accomplished.

In molecular genetics, a probe is defined as a DNA or RNA sequence that has been tagged with a radioactive element and is used to detect the presence of a complementary sequence by molecular hybridization. DNA probes for RFLP analysis are of three types.

1. Complementary or copy DNA (cDNA) probes are manufactured using the messenger RNA (mRNA) for a specific protein as a template. These probes contain the coding DNA sequence of

only the exons of the structural gene; the introns have been excised during the processing to form mature RNA.

2. Genomic DNA (gDNA) probes are generated by first digesting genomic DNA with a restriction nuclease, followed by random insertion of the DNA fragments into a vector. A vector is a plasmid or phage used to carry a DNA segment that is to be cloned. *E. coli* are then infected with the vector, and many copies of the DNA segment are produced. These segments can be separated from the vector and purified. This approach generates a library of DNA fragments and many of these prove to be useful as probes. The gDNA sequences that are useful as probes are those that contain unique DNA sequences that match (are complementary to) a specific location in the human genome. These probes contain both exons and introns.

3. Synthetic oligonucleotide probes are complementary to segments of structural genes and are manufactured using the amino acid sequence that forms a portion

of the protein molecule from which one can then deduce the nucleic acid sequence of the structural gene. Only 16 to 18 nucleotides of the proper sequence are necessary to generate a complementary probe. A complementary probe requires the specific nucleotide sequence of the structural gene.

The concept of linkage analysis with RFLP is illustrated in Figure 1. A Southern blot study is used to evaluate polymorphism or polyallelism for the growth hormone (GH) gene. An endonuclease called Hinc II was used to generate fragments from chromosome 17 where the GH gene is located. A cDNA probe generated from mRNA for hGH is used to study the length (polymorphism) of the gene fragments. Polymorphism occurs normally for the GH gene, as studied by Hinc II endonuclease. The fragments of one allele are 6.7 Kb in length and the other, 4.5 Kb. Individuals may be homozygous for the 6.7 Kb fragment (46% of the population), homozygous for the 4.5 Kb fragment (10% of the population), or heterozygous with each allele present (44% of the population). Linkage analysis or use of linkage for diagnosis requires that the individual be heterozygous. To

increase the capability for linkage study, other restriction enzymes can be used to break the GH gene into different fragments and further heterozygosity can be demonstrated. Two enzymes, Msp I and Bgl II, along with Hinc II, have been particularly useful in rendering most matings informative for linkage analysis with hGH probes.

RFLP analysis of the GH gene has permitted the identification of one type of inherited isolated GH deficiency, IGHD-IA, where the affected individuals are homozygous for absence of the gene. In some instances, the affected individuals have been found to have a deletion of only part of the GH gene. Studies using RFLP analysis in other families with inherited GH deficiency have not identified either absence of the GH gene or concordance for the genotypes of the hGH gene among affected siblings. Consequently, a cause for the GH deficiency other than an abnormality of the GH gene must account for the problem. These patients are currently said to have IGHD-Type IB.

Maury et al carried out similar studies of children having the GH deficient-like phenotype attributed to immunoactive, biologically-

inactive GH. The low concordance rate for RFLP suggested that most children with this phenotype are not homozygous for a mutation of hGH structural genes.

These studies demonstrate that hGH genotyping with an hGH DNA probe and RFLP analysis can separate individuals who are affected with what appears to be the same disease from each other with respect to etiology. Carriers of a trait can also be identified by these techniques.

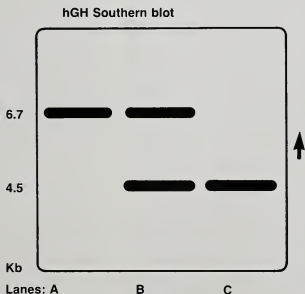
### Application of RFLP Linkage Analysis to Single Gene Disorders

Diagnoses utilizing RFLP linkage analysis can be extended further by comparing genotypes between a known affected member of a family and a member whose status is unknown. The approach is applicable to single gene disorders inherited as autosomal dominant, autosomal recessive, or X-linked traits. Currently there are four instances in which such an approach is useful.

The first occurs when there is clinical ambiguity or indecision as to whether a particular member of a family has the same disease as an affected member of the family. Disorders inherited as autosomal dominant traits, such as Marfan syndrome, often display variable phenotypes that may render the clinical diagnosis difficult in certain members of the family. Genotype analysis by RFLP studies may offer assistance in this situation. However, such analysis is not helpful in the diagnosis of an isolated case, except in rare instances where a gene deletion might be detected.

The second occurs when predictive testing is important to evaluate whether a member of a family with a disorder that makes its appearance late in life, such as Huntington disease, will develop that disease. The issue is important for genetic counseling and life-style planning. Non-insulin dependent diabetes mellitus and autosomal recessive primary hemochromatosis are similar examples. The latter may be diagnosed by link-

**Figure 1.** Autoradiography of fragments resulting from Hinc II endonuclease digest of hGH genes from 3 normal individuals is shown. Lane A has fragments from a homozygote for the 6.7 Kb band only, lane C has fragments from a homozygote for the 4.5 band only, and lane B has fragments from a heterozygote for the 4.5 and 6.7 bands.



## Restriction Fragment Length Polymorphism *continued from page 7*

age analysis using HLA typing and DNA analysis.

The third occurs when detection of carriers is important. This is particularly pertinent for X-linked diseases, since testing by methods other than RFLP analysis has been fraught with many ambiguities because of the peculiar biology of X-linked expression in heterozygous females. RFLP analysis is increasingly providing less ambiguous methods of carrier detection and is applicable currently to detect carriers for Duchenne muscular dystrophy or hemophilia A or B.

The fourth is for prenatal diagnosis of various congenital anomalies or diseases. This permits the option of terminating the pregnancy (if the fetus has an un-

treatable severe disease) or the development of fetal therapy in other conditions such as congenital adrenal hyperplasia.

The progress made in recent years in mapping the human genome has created significant advancement in both diagnosis and therapy of inherited defects and a new era of multidisciplinary research with achievable goals. An immediate goal of this collaborative effort is the construction of a gene map for each chromosome that is adequate to permit linkage analysis and, thus, mapping of any gene locus. The mapping of a gene locus is no longer considered an esoteric exercise, but a crucial step in understanding disease mechanisms. Now that the genes for Huntington disease, cystic fibrosis, and Duchenne muscular dystrophy have been mapped, efforts are underway to

isolate these structural genes and to define their molecular structures. Determination of the molecular structure will enhance therapy through genetic engineering. These research activities will not be limited to single-gene disorders but will expand to enable study of the basic biology of neoplasia and, most excitingly, permit us to understand the evolutionary processes that generated the human species.

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2. Rumsby G, Carroll MC, Porter RR, et al: Deletion of the steroid 21-hydroxylase and complement C4 genes in congenital adrenal hyperplasia. *J Med Genet* 1986;23:204-209.
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## Abstracts From The Literature

### Gender Verification for the 1988 Winter Olympics

In 1968, the International Olympics Committee began to require that individuals taking part in women's sporting events have their female gender confirmed. This has been done by examining a buccal mucosal smear for evidence of X and Y chromatin. If a test is inconclusive, then further testing must be done by the International Olympics Committee Medical Commission. Several recent articles have suggested that there are major technical pitfalls to the use of X and Y chromatin analysis for this purpose. This type of testing for gender is inaccurate and expensive but, just as important, does not deal with phenotypic females, such as those with 45, XO Turner syndrome, XY gonadal dysgenesis, and androgen insensitivity syndromes in which individuals are raised as females but have negative buccal smears. Nor does it deal with XX males who have been raised as phenotypic

males. Although complete genetic studies could address the technical concerns, this approach of gender verification does not deal with the psychological aspects of the individual competitors who, if they have a genetic disorder, have nevertheless been raised during their lives as members of the gender to which they have been assigned. Nor does it deal well with the competitor who intentionally wants to deceive the International Olympics Committee. It has been suggested that a simple physical examination and inspection by a physician would be less expensive and be just as accurate in establishing gender. Presumably, the intent of sex determination by the International Olympics Committee is to prevent unfair competition from a male posing as a female and using his superior muscle strength to unfair advantage. The male impostor could be easily identified by means of physical inspection, and this would appear to be less costly, more efficient, and a simpler method of gender verification.

1. Lowry RB. *Bulletin of the Hereditary Diseases Program of Alberta* 1986;5:9.

2. de la Chapelle A. *JAMA* 1986; 256:1920-1923.

3. Simpson JL. *JAMA* 1986;256: 1983.

**Editor's comment**—The International Olympics Committee's decision to use a screening test must have arisen from an unfortunate experience. However, using an outdated, expensive, and unreliable technique to verify gender seems to be inappropriate.

The Lawson Wilkins Pediatric Endocrine Society has addressed this issue during its annual meeting. A resolution urging appropriate athletic competition sanctioning bodies to discontinue the use of sex chromatin tests for verification of "athletic gender" and to convene an international conference of sports and medical experts to develop more appropriate and sensitive criteria to verify female gender for athletic competition was presented at the meeting; the resolution passed.

## Pulsed-Field Gel Electrophoresis: A New Technique For Fractionating Large DNA Molecules

Pulsed-field gel electrophoresis for fractionating large DNA molecules is a technique that has increased the size of DNA molecules that can be separated by almost 100-fold. The development of this technique opens up the possibility of separating and analyzing pieces of DNA from 10,000 to 1,000,000 base pairs in size. The technique involves the

use of restriction endonucleases that cut the DNA infrequently within the human genome. The electrophoresis is based on the rate at which these large molecules alter their shape to migrate inside the gel matrix. The technique depends on the stiff DNA molecules undergoing distortion or relaxation under the influence of the electrical field. Because there is an alternating electrical impulse, which lasts from one second to five minutes, the DNA molecules migrate in alternating fields and zig-zag their way across the gel. The technique holds promise, both for isolating specific genes and for

mapping the genome of various organisms.

1. Anand R. *TIG* 1986;Nov: 278-283.
2. Chu G, Vollrath D, David RW. *Science* 1986;234:1582-1585.
3. Smith CL, Cantor CR. *Nature* 1986;319:701.

**Editor's comment**—Just as the Southern blot technique revolutionized DNA research 12 years ago, it would appear that this new technique represents a giant step in allowing the separation of large unique sequence pieces of DNA, both from the human genome and from other organisms.

## Transient Increases in Progesterone in Daily and 2-Hourly Saliva Specimens From Adolescent Girls

Detailed information on short-term changes in progesterone concentrations during the peri- and post-menarcheal period has been sparse because of difficulties associated with frequent collection of samples of plasma or urine. With the advent of reliable assays for progesterone concentration in saliva, Truran et al determined progesterone levels in perimenarcheal girls.

Saliva specimens were collected from clinically healthy premenarcheal and adolescent girls either daily throughout the menstrual cycle or at 2-hour intervals throughout a 24-hour period.

The mean age of the adolescents at menarche was  $12 \pm 1.3$  (SD) years. A minority of these cycles were consistent with luteal phase (ovulatory) progesterone increases, especially within the first two years following menarche. The frequency of transient increases of salivary progesterone declined after the menarche and was negatively correlated with the first postmenarcheal year.

A large number of isolated in-

creases (levels exceeding 150 pmol/l and preceded and succeeded by at least one sample in which levels are less than one-third those in the increased sample) were noted in the samples drawn every 2 hours from premenarcheal adolescents.

Truran PL, Leith HM, Read GH. *J Endocrinol* 1986;111:513-518.

**Editor's comment**—Transient increases of salivary progesterone seem to be largely confined to the period of adolescence, with a steady decline in the rate of "spiking" in the years after the menarche. However, detailed studies in adult women (in which plasma samples were withdrawn every 10 to 20 minutes) show a pulsatile pattern of progesterone secretion in the luteal phase of the menstrual cycle. Thus, the ovary (follicle and corpus luteum) can translate the pulsatile pattern of luteinizing hormone secretion to intermittent progesterone (and estrogen) secretion.

Salivary progesterone concentration determination represents a new noninvasive method for determining alterations in ovarian physiology. Most other steroid hormones can be determined in this fluid, and assays have been validated for cortisol, estrogens, and androgens.

## In Future Issues

Catch-up and Catch-down Growth: A Review  
by James M. Tanner, M.D., D.Sc.

Fetal Alcohol Syndrome  
by Kenneth Jones, M.D.

Nutritional Dwarfism in Adolescents  
by Fima Lifshitz, M.D.

Osteogenesis Imperfecta  
by Peter Beyer, M.D.

Anabolic Steroid Hormones in Athletes:  
Efficacy or Fantasy?  
by Alan D. Rogol, M.D., Ph.D.

Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.



## Decrease in Plasma High-Density Lipoprotein Cholesterol Levels at Puberty in Boys With Delayed Adolescence: Correlation With Plasma Testosterone Levels

Kirkland and co-workers performed a three-phase study to test the hypothesis that the decrease in the high-density lipoprotein cholesterol (HDL-C) level that occurs at puberty is related to an increase in the plasma testosterone concentration. HDL-C is the fraction of total cholesterol concentration that is inversely related to the incidence of coronary artery disease.

Plasma HDL-C levels are similar in both sexes during childhood until a decrease in the HDL-C level occurs in boys around the age of puberty, with the major drop occurring between 12 to 13 and 14 to 15 years of age. Subsequently, males maintain lower HDL-C levels than do females throughout adult life.

The first phase of the study in boys (ages 10.1 to 16.9 years) with short stature, delayed adolescence, or both investigated the relationship between plasma testosterone and HDL-C. The boys were classified into four stages of pubertal development by clinical examinations (testis length and penile length). Advancing pubertal stages were associated with increasing levels of testosterone and decreasing levels of HDL-C.

In the second phase of the study, fourteen boys (ages 13.3 to 16.8 years) with constitutional delay of pubertal development were evaluated during and after treatment with testosterone enanthate. Within one to two weeks after injection, a rise in plasma testosterone levels and a concomitant decrease in HDL-C levels were observed.

The third phase of the study was designed to determine the relationship between plasma testos-

terone and HDL-C levels during spontaneous onset of puberty following treatment with testosterone. In thirteen subjects with constitutional delay of sexual development, the spontaneous increase in plasma testosterone levels was accompanied by a decrease in the HDL-C level.

Kirkland RT, Keenan BS, Probstfield JL, et al. *JAMA* 1987; 257:502-507.

**Editor's comment**—This study provides evidence that testosterone, both endogenous and exogenous, directly or indirectly influences HDL-C metabolism during

puberty. The results of the treatment phase suggest a cause-and-effect relationship between the increase in the plasma testosterone level and the decrease in the HDL-C level.

It should be remembered that during spontaneous puberty, testosterone secretion is highly episodic only at night with significantly decreased levels during the day. Thus, the correlations noted are really underestimates of the actual physiologic condition.

These data are convincing and should allow interventional protocols to be devised to attempt to raise HDL-C in young men as "prophylaxis" against coronary artery disease.

## Biosynthetic Somatomedin-C (Sm-C/IGF-I) Increases the Length and Weight of Snell Dwarf Mice

Somatomedins are thought to mediate the effects of growth hormone (GH) on body growth. However, whether Sm-C/IGF-I will also stimulate true skeletal growth (body length) and maintain the harmony of the weight/length relationship and the growth of various organs has not been determined. This study reports the effects of biosynthetic IGF-I on various growth parameters of GH-deficient Snell dwarf mice.

Groups of animals received either buffer (control), human growth hormone (hGH), or one of three doses of IGF-I three times daily for four weeks. Body length and weight were measured once a week. At the end of the study, organs were removed and weighed. Only the highest dose of IGF-I, 7.4 µg per day, was effective in increasing both the length and weight of the mice. In addition, hGH 2.8 µg/day induced significant but similar increases over

controls. The relative weight of the heart of the hGH-treated mice was significantly increased, when compared to the IGF-I treated group and the controls.

van Buul-Offers S, Ueda I, van den Brande JL. *Pediatr Res* 1986; 20:825-827.

**Editor's comment**—These results indicate that circulating IGF-I can lead to increased body length and weight in dwarf mice. However, they do not indicate whether this situation is physiologic, since hGH was more effective than IGF-I molar for molar. Recent results from other investigators indicate that both GH and IGF-I are important to linear growth. According to the dual effector hypothesis, GH leads to differentiation (commitment) of cells and IGF-I to clonal expansion of those differentiated to permit an orderly and efficient process of growth. The data from the present study indicate that IGF-I alone can lead to overgrowth, but they do not indicate whether this is the physiologic or most efficient mechanism of growth.

## The Rapid Ovarian Secretory Response to Pituitary Stimulation by the Gonadotropin-Releasing Hormone Agonist, Nafarelin, in Sexual Precocity

Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations reach similar maximal concentrations following a three- to four-hour high-dose infusion of gonadotropin-releasing hormone (GnRH) itself or a single maximally effective dose of a GnRH agonist. However, circulating estradiol levels in adult women usually increase within 12 to 24 hours only after the agonist injection.

The present investigation was undertaken to determine the steroidogenic response both to nafarelin, a long-acting GnRH agonist, and to an intensive standard GnRH test.

Thirteen girls with central precocious puberty were studied. GnRH was infused intravenously at a rate of  $2 \mu\text{g/kg/hour}$  for 3 hours. Nafarelin was given as a single subcutaneous injection in a dose of  $0.2 \mu\text{g/kg}$ . The serum LH and FSH concentrations were elevated by both GnRH infusion and nafarelin administration to reach a plateau at 3 hours. The initial rises were significantly more rapid with the GnRH analog, but only after nafarelin injection did serum LH and FSH remain significantly elevated. Plasma estradiol levels increased slightly after 3 hours with both agents, but rose 3.6-fold 24 hours after nafarelin administration.

Rosenfeld RL, Garibaldi LR, Moss GW Jr et al. *J Clin Endocrinol Metab* 1986;63:1386-1389.

**Editor's comment**—A single injection of the GnRH agonist, nafarelin, sequentially stimulates pituitary and ovarian secretion in

girls with precocious puberty just as early and promptly as in adult women. The pattern of steroid secretion in response to nafarelin is typical of normal ovarian follicular secretion. The ability of nafarelin to test the integrity of ovarian, as well as pituitary, function makes this compound appear useful for clinical testing.

The gonadotropin and gonadal steroid profiles noted suggest that this compound may be useful in the treatment of precocious puberty and hormonally dependent neoplasms by long-term receptor desensitization (down regulation) of the gonadotropes.

## Organ Procurement for Transplantation in Children

The number of organ transplants in children with fatal childhood diseases is increasing. Many of these disorders are either genetic or have a genetic component in their etiology. In the United States, it is estimated that there are 300 to 500 children with end-stage renal disease who could discontinue dialysis if kidneys for transplantation were available. For an additional 400 to 800 children with liver failure and 400 to 600 children with severe forms of congenital heart disease, organ transplantation is their only hope for survival. These figures do not take into account those children with inborn errors of metabolism who could also benefit from organ transplants. While the technology of transplantation has improved dramatically over the last few years, a source of tissue for organ transplantation has become a major problem. Two recent articles have addressed this problem and the ethical issues involved.

Organs from anencephalic fetuses or infants have become a potential source for transplantation. Although anencephaly is a

hopeless defect of the central nervous system, the other vital organs of anencephalic infants are usually normal. It appears that fetal organs, although somewhat immature, may yield excellent results if they are transplanted, because of their ability to grow and their almost total lack of antigenicity, which makes them less likely to be rejected. However, the ethical questions involved in declaring "personhood" and death in an anencephalic infant or fetus creates some very difficult issues. First, the anencephalic fetus, for legal purposes, must be considered brain dead. Furthermore, most anencephalics are diagnosed prenatally and, therefore, are initially considered as unborn patients. However, diagnosis in the second trimester does not preclude the use of fetal organs. The usefulness of transplanting the organs from a fetal or term anencephalic depends instead on whether the organs can be maintained in a healthy condition prior to transplantation.

By defining the ethical and legal issues involved in the transplantation of organs from anencephalic infants and fetuses, investigators are making progress in an area that may affect children with a variety of genetic disorders. Another important issue is whether the cost of organ transplantation (\$100,000 to \$200,000) for one individual represents an appropriate use of limited health resources.

1. Harrison MR. *Lancet* 1986;ii:1383-1385.
2. Moskop JC. *J Pediatr* 1987;110:175-180.

**Editor's comment**—The use of organ transplantation for children will be increasing over the next few years. Thus, it is important to be aware that anencephalics may serve as one of the important sources of healthy tissue for transplantation.

## Controlled Trial of Zinc Supplementation During Recovery From Malnutrition: Effects on Growth and Immune Function

Deficiencies of such trace minerals as zinc, iron, copper, and magnesium are often associated with protein-calorie malnutrition (PCM). To evaluate the role of zinc supplementation on growth and immune function in malnourished infants during recovery from PCM, 32 marasmic infants were randomly assigned to receive either 2 mg/kg/day elemental zinc supplement or a placebo without zinc. The marasmic condition was defined by birth weight > 1,500 g, weight for age < 80% WHO standard, and history of primary malnutrition without an underlying disease associated with malnutrition. All infants received a milk-based formula which provided 150 to 200 kcal/kg, 4.5 to 5.0 g protein/kg, and 3.0 to 3.5 mg zinc/day. Weight and length were measured daily; arm circumference and triceps skinfold, biweekly.

Plasma zinc and copper determinations and complete blood counts were performed at days 0, 30, 60, and 90 of the study. Function of the immune system was assessed on days 0 and 90 of zinc supplementation by cutaneous delayed hypersensitivity reaction, T-cell blast proliferation, immunoglobulin concentrations, the number of febrile days, and the type/number of intercurrent infections. Plasma zinc and copper levels were measured serially and maintained within normal limits. They were similar in both the supplemented and placebo groups.

At 60 days, the overall gain in weight for length as a percent of standard was 9% in the supplemented group and 3% in the placebo group ( $p < 0.05$ ). Zinc-supplemented infants had significantly fewer infectious illnesses, such as pyoderma, when

compared with the placebo group ( $p < 0.05$ ). A significant negative correlation between the plasma zinc level and the number of febrile days in the placebo group was noted during the 1- to 2-month interval ( $r = 0.66$ ,  $p < 0.05$ ). Following 90 days of zinc supplementation, the percentage of anergic infants had increased more than in the placebo group ( $p < 0.05$ ). Serum IgA concentrations were greater in the zinc-supplemented group.

Castillo-Duran C, Heresi G, Fishberg M, et al. *Am J Clin Nutr* 1987;45:602.

**Editor's comment**—The authors describe marginal zinc deficiency that could not be identified by plasma zinc levels, but only by salutary clinical and immunological responses to zinc supplementation.

Zinc supplementation in these marasmic infants improved weight gain without differences in food intake and reduced the incidence of infectious morbidity. If a clinician cannot rely on standard measures to determine zinc status, ie, plasma levels, it becomes difficult to determine when supplementation is indicated and the amount required to elicit a positive response. However, mineral supplementation is recommended during recovery of malnutrition for a number of reasons. First, catch-up growth during recovery of malnutrition increases the need for zinc and other trace minerals. Additionally, diets based on cow's milk provide insufficient amounts of zinc for optimal recovery from malnutrition. Finally, marginal deficiencies of these minerals may also interfere with clinical recovery. Therefore, zinc supplementation is recommended during recovery from malnutrition since we have no other valid indication of adequate zinc levels that can permit the diagnosis of zinc deficiency in human beings. As reported in this paper, a supplement of 2 mg/kg/day is effective.

## Impact of Intensive Venous Sampling on Characterization of Pulsatile Growth Hormone Release

This study applies a computer-based pulse detection algorithm to growth hormone (GH) data collected every five minutes over a 24-hour period. Previous application of this algorithm using every-20-minute sampling has demonstrated significant differences in pulsatile GH release among different groups of individuals. The present article addresses the adequacy of traditional GH sampling rates and attempts to identify the frequency of sampling necessary to capture the majority of GH pulses.

Seven adult males were studied over a 24-hour period, with sampling for GH done every five minutes. The GH levels obtained were subjected to previously published pulse detection algorithms that excluded intrinsic measurement errors influenced by unstable baselines or non-uniform peak amplitudes. In addition, the algorithm used constrains type I statistical errors to limit the rate of false-positive peaks. In this particular study, *t* statistics were used to constrain the false-positive rate to less than 5%.

The study demonstrates that the number of GH peaks detected is maximal with five-minute sampling, and that twice as many peaks per 24 hours are detected using sampling done every five minutes as opposed to every 15 or 20 minutes. In addition, however, in not a single case is a pulse detected with sampling done every five minutes that is not contiguous to or contained within a major secretory episode, which would have been identified by sampling done every 20 minutes. Mean GH inter-pulse interval is 68 minutes with sampling every five minutes as opposed to 250 minutes with sampling every 20 minutes. With less

frequent sampling there is a progressive loss of identification of high-frequency, low-amplitude GH pulses.

Evans W, Faria A, Christiansen E, et al. *Am J Physiol* 1987; 252:E549-556.

**Editor's comment**—This group has previously utilized its computer-based pulse detection

algorithm to describe the secretion of luteinizing hormone. The results of this study suggest that sampling every 15 to 20 minutes is optimal to detect major episodes of GH secretion, but that more intensive sampling is needed to enumerate the high-frequency GH pulsations within major secretory episodes. The physiologic and pathophysiologic significance of frequent sampling applied to sub-

jects with growth retardation, acromegaly, protein-calorie malnutrition, and diabetes mellitus where GH secretion is abnormal remains to be shown. It is reassuring, however, that sampling every 20 minutes would appear to detect the major episodes of GH secretion and that more intensive sampling may not be required to identify most individuals with GH neurosecretory defects.

### Insulin-Like Growth Factors in Pygmies: The Role of Puberty in Determining Final Stature

Merimee and co-workers have previously shown that IGF-I levels were decreased in pygmies, although growth hormone (GH) and IGF-II levels were normal. They concluded in that study, in which they evaluated only adult pygmies and no control subjects, that short stature observed in pygmies is due to a deficiency of IGF-I. The present study evaluates pygmy children, adolescents, and adults and compares them with other African and American control populations.

Serum was obtained from 64 pygmies—33 adults, 14 children, and 17 adolescents—for determination of IGF-I, IGF-II, and IGF binding proteins. Careful pubertal staging was performed in each of the subjects, and no pygmy child was included in the study unless the parents could be identified. The control subjects were chosen to approximate the distribution of pygmies in the study according to age, sex, and maturational development.

Adult pygmies had mean IGF-I levels that were significantly lower than those of native African and American control adults. Over half the pygmy adults had IGF-I values of < 100 ng/ml, a level commonly accepted as diagnostic for hypo-

pituitarism. IGF-II levels, however, were similar in the pygmy adults and the African control population. Mean IGF-I and IGF-II levels in pygmy children were not significantly different from those of controls, even though there was a small (but not significantly different) reduction in height between the pygmies and the African controls. This difference in height remained constant until puberty. Pygmy adolescents showed marked reductions in serum IGF-I levels, when compared to those of American adolescents, and these reductions closely mirrored the differences in growth acceleration observed during puberty. Both pygmy boys and girls had IGF-I levels that were one-third those of American adolescents. The marked acceleration in growth known to occur during adolescence in control populations was absent in pygmy boys, while a barely detectable acceleration in growth was observed in pygmy girls. Serum testosterone levels in adolescent pygmy males were within normal limits, and IGF-II was similar within all groups. All pygmies studied had normal IGF-I carrier protein patterns.

Merimee T, Zapf J, Hewlett B, et al. *N Engl J Med* 1987;316:906-911.

**Editor's comment**—This study is the first to describe the absence of accelerated growth during adolescence in the pygmy population.

The markedly low IGF-I levels suggest that this growth factor is the principal agent of accelerated pubertal growth. Others have demonstrated a marked increase in the amplitude of GH pulsations during puberty in normal subjects. In addition, giving testosterone to sexually infantile adolescents fails to elicit a marked rise in IGF-I in those patients who are GH deficient. Therefore, the pubertal rise in IGF-I concentration appears to depend on elevated GH levels which, in turn, are influenced by sex steroid hormone concentrations. Although the secretion of GH by traditional stimulation tests is normal in the pygmy population, Merimee and co-workers have not demonstrated a failure of GH to stimulate IGF-I during adolescence and subsequent failure of growth acceleration. Many questions remain, including the overall 24-hour integrated GH level in pygmy adolescents, identification of a possible abnormality in the GH-IGF-I axis, and identification of the hypothalamic response of normally grown subjects and pygmies to sex steroids. This very important study raises many more questions than it answers, and the reader is directed to an accompanying editorial by Rechler M, Nissley S, Roth J. *Hormonal Regulation of Human Growth*. *N Engl J Med* 1987;316:941-943, for a discussion of the significance of these findings and the questions they raise.



## Fertility Onset, Spermatogenesis, and Pubertal Development in Male Rats: Effects of Graded Underfeeding

Manipulation of pubertal timing by undernutrition has proven to be a useful model for exploring the proposed link between this malnutritional event and the attainment of either a "critical body weight" or a "critical body fatness." Although a large body of data exists concerning the female, similar studies in males have been hampered by the lack of a discrete marker for puberty. Glass and co-workers have examined male rats given five different levels of food intake and have used the first successful conception in normal females as a marker of puberty. The effect of such graded undernutrition on the age and body weight at puberty in male rats was correlated with corresponding changes in androgens, gonadotropins, and spermatogenesis in order to shed light on the mechanism of puberty disruption in undernutrition and to clarify the relative importance of these factors in normal puberty.

All groups of rats were given the same diet, but at four levels of food intake. A group of rats on an ad libitum diet served as the control. Beginning at age 36 days, 10 animals from each group were placed

each night in individual mating cages, one male per cage, each of which contained two normal females. Dates of conception were back-calculated from delivery dates of the female rats.

Eight to ten animals from each group were sacrificed after 30, 60, and 100 days on the dietary regimen. Body weight and prostate, seminal vesicle, and testicular weights were recorded along with nasoanal and tail lengths. Blood was obtained for testosterone and gonadotropin levels.

Although there was a weak inverse correlation between the age at puberty and the growth rate, the mean age at puberty did not differ significantly between the fastest and slowest growing groups. By contrast, the body weight at puberty correlated strongly with the growth rate, with the underfed rats attaining puberty at lower body weights than the normally fed rats. Underfed rats went through puberty without ever having attained the percentage of body fat that normally fed rats reached at puberty.

Androgen deficiency and low gonadotropin output induced by underfeeding were apparent as early as 30 days on the diet, and progressive adaptation was seen by 100 days. Although the male accessory organs were smaller in the underfed groups, there was only minimal impairment of sperm

production. Testis histology showed mature sperm in all dietary groups at 60 and 100 days.

Glass AR, Herbert DC, Anderson J. *Pediatr Res* 1986;20:1161-1167.

**Editor's comment**—This carefully designed study showed that underfeeding of male rats by as much as 70% led to minimal delay in puberty. Based on these data (and earlier data for females), for neither male nor female rats can the "critical body weight" or "critical body fat" hypotheses of pubertal timing be substantiated.

Can these conclusions be extrapolated to human beings? It is tempting to point to the cessation of ovulatory cycles in severely undernourished females as "proving" this point, but it is more difficult to find examples among males. The sperm cycle is much longer in the human being than it is in the rat, and there is a much longer latency period between weaning and attainment of normal puberty. It would seem that undernutrition may be one of the many factors (eg, stress) that influence the human reproductive cycle and, in its severest forms, it can alter the process to the point that reproduction can be considered a "luxury" in the energy-deficient individual.

## Creutzfeldt-Jakob Disease (CJD) Dura Mater Graft Association

The Centers for Disease Control reported a case of CJD in a patient who had received a dura mater graft. This is the first association between the product Lyodur, manufactured by B. Braun Mel-sungen A.G. of West Germany, and CJD. The product was packaged in 1982 and used primarily in neurosurgical procedures although, at times, it is used in or-

thopedic ear, nose, and throat, dental, urologic, gynecologic, and cardiac procedures.

CJD agent transmission has occurred in the past through contamination of corneal transplants and intracerebral electrodes, as well as with human growth hormone (GH) derived from human cadavers.

MMWR February 6, 1987

**Editor's comment**—It is interesting that the CJD that developed in

this patient occurred relatively rapidly following the dura mater graft. This is certainly more rapid than the incubation period observed in the children who received GH as children and developed CJD as adults. It is important to recognize that the incubation period or lag time between inoculation of the CJD agent and the development of the rapidly progressive dementing illness may not be as long as previously thought, particularly when the infectious agent is placed in proximity to the central nervous system.

# Meet the Editorial Board



**William L. Clarke, M.D.**

Dr. Clarke is Associate Professor of Pediatrics in the Division of Endocrinology and Diabetes at the Children's Medical Center of the University of Virginia in Charlottesville, VA. He is also Director of Student Clerkships in Pediatrics and Chairman of the Pediatric Education Council at the University of Virginia Medical School.

After graduating from Duke University in Durham, NC, Dr. Clarke attended the Vanderbilt University School of Medicine in Nashville, TN. After graduation, he served an internship and residency at St. Louis Children's Hospital, Washington University School of Medicine, in St. Louis, MO. He also became a resident-fellow at the Division of Pediatric Metabolism and Endocrinology at St. Louis Children's Hospital, where he returned after two years as a Staff Pediatrician in Endocrine-Metabolic Disease at Wilford Hall USAF Medical Center, Lackland Air Force Base, TX.

Dr. Clarke is a member of the Board of Directors of the American Diabetes Association, Virginia Affiliate. He became President of the Virginia Affiliate in 1986 after having served as Vice-President and Secretary.

Dr. Clarke has contributed extensively to the literature on endocrinology and metabolic disorders. He has authored or co-authored 36 journal and review articles, two textbook chapters, 31

abstracts, and a textbook on the subject. He has also written three practical guides for the parents of diabetic infants, toddlers, and preschoolers for the American Diabetes Association. Dr. Clarke is a contributing editor for *Behavioral Medicine Abstracts* and a reviewer for *Diabetes, Diabetes Care, and Pediatrics*.



**James M. Tanner, M.D., D.Sc., F.R.C.P.**

Professor Tanner is Emeritus Professor of Child Health and Growth at the University of London Institute of Child Health. He is also Visiting Professor of Human Growth at the University of Texas School of Public Health in Houston.

A native of England, Professor Tanner graduated from St. Mary's Medical School in London in 1944, having meanwhile attended the University of Pennsylvania Medical School in Philadelphia, followed by further training at Johns Hopkins Hospital in Baltimore.

After returning to England in 1944, Professor Tanner served as junior medical officer at the Mill Hill Emergency Medical Service Hospital (wartime Maudsley Hospital), followed by an appointment as medical officer at Southern Hospital, Dartford, Kent.

Professor Tanner then became a demonstrator in human anatomy at Oxford University, after which he joined the medical faculty of the University of London. After serving

as a Lecturer and then Senior Lecturer in Physiology at the Sherrington School of Physiology at St. Thomas's Hospital, he was appointed Reader in Growth and Development at the Institute of Child Health, and later Professor of Child Health and Growth. During this time, he also served as Honorary Consultant Physician at the Hospital for Sick Children in London.

Professor Tanner was joint founder (1958) and later Chairman (1980-1983) of the Society for the Study of Human Biology, and served as Honorary Secretary of the Research Committee of the Mental Health Research Fund from 1951 to 1977. A member of the Health Services Human Growth Hormone Committee, he was Acting Chairman from 1973 to 1977. Professor Tanner is a corresponding member of the French, Swiss, Italian, Cuban, and Catalan Pediatric Societies and of the Society for Adolescent Medicine (United States).

The recipient of numerous awards, Professor Tanner received the John Alexander Memorial Prize from the University of Pennsylvania in 1986. He was named James Spence Medallist of the British Pediatric Association in 1980 and Rosen Von Rosenstein Medallist of the Swedish Pediatric Society in 1984. Professor Tanner is also a former Ernest Hart Memorial Scholar of the British Medical Association (1953) and a former Fulbright Research Associate (Jackson Memorial Laboratory, Bar Harbor, 1950).

Professor Tanner has been a visiting professor at Harvard University, The University of Chicago, and UCLA, and a lecturer at numerous institutions in the United States and Great Britain. He has written extensively on human growth and development and is the author or co-author of some 220 scientific papers and ten books, which have been translated into ten foreign languages.

## MEETING CALENDAR

**September 28-30** International Congress on Advances in Growth Hormones and Growth Factors Research. Milan, Italy. Contact: Drs. Daniela Cocchi and Vittorio Locatelli, Department of Pharmacology, Chemotherapy, and Toxicology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy

**October 7-10** 38th Annual Meeting of the American Society of Human Genetics. Town and Country Hotel, San Diego, California. Contact: Administrative Office, American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

**October 24-27** 39th Annual Assembly of The Endocrine Society. San Francisco, California. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**November 13-18** Postgraduate Course—Introduction to Endocrine Investigations 1987: Techniques and Concepts. Co-sponsors: The Endocrine Society and Sero Symposium, USA. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**February 21-25, 1988** 15th Annual Seminar in Pediatric Nephrology: Immunosuppression, Growth, and the Neonate. Sheraton-Bal Harbour Hotel, Miami Beach, Florida. Sponsor: Department of Pediatrics, University of Miami. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960, Miami, FL 33101 (305-549-6726)

**July 20-23, 1988** 5th International Auxology Congress. Exeter University, Exeter, England. Contact: Prof. J.M. Tanner, Room 115, Institute of Child Health, 30 Guilford Street, London, England WC1N 1EH

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# GROWTH

## Genetics & Hormones

Vol. 3 No. 4

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Please see  
reader survey inside

## Nutritional Dwarfing in Adolescents

Fima Lifshitz, M.D.

Associate Editor

*Growth, Genetics, and Hormones*

### Introduction

Inappropriate dieting because of psychological or cultural reasons is very prevalent among adolescents.<sup>1,2</sup> In this era of social and dietary fads, teenagers often diet to avoid obesity or to decrease their intake of dietary fat and "junk food" or both. Inappropriate dieting, however, may result in nutritional deficiencies that interfere with normal growth and sexual maturation.<sup>3,4</sup> Some of the causes leading to inadequate nutritional intake among adolescents were reviewed recently.<sup>5</sup> Current concepts regarding nutritional dwarfing in adolescents described and reported in that review are summarized here with the permission of the authors and publishers.

Self-imposed malnutrition among adolescents is a major problem in clinical pediatric practice.<sup>6</sup> Usually these patients present to the pediatric endocrinologist because of short stature. They have nutritional dwarfism as defined by the

Welcome Trust Classification.<sup>7</sup> For a variety of reasons, they voluntarily do not eat the appropriate quantity or quality of food to meet their needs for optimal health and normal growth and development. Therefore, pediatricians and pediatric endocrinologists must pay more attention to the growing number of eating disorders among adolescents and to the prevailing health beliefs and cultural influences that may determine nutritional intake.<sup>4,5,8</sup>

### Auxology

An evaluation of the patient's pattern of growth is the most important factor in the differential diagnosis of short stature. Much attention has been devoted to the various stages of development and growth patterns of specific patients, racial groups, and populations. However, in most instances, pediatric endocrinologists and other physicians assess the stature of the patient with little consideration of changes in body weight.

Figures 1A and 1B illustrate the importance of monitoring changes in body weight in evaluating the growth pattern of a short child. As shown in Figure 1A, nutritionally-related dwarfism could not have been diagnosed without additional weight measurements throughout life. In Figure 1B, the patient's weight is seen to have decreased when he was 12 years old, and his height increments slowed concomitantly. During this period, he failed to develop sexually. His nutritional intake provided only 54%

to 66% of his energy needs. He was an athletic boy who wanted to remain slim and trim. He did not have any primary endocrine or other organic disorder to account for his malnutrition. His self-imposed starvation was thought to be characteristic of *fear of obesity*, a syndrome we have described in detail elsewhere.<sup>3</sup>

### Nutritional Short Stature—Atypical Eating Disorders

A variety of diseases, such as chronic inflammatory bowel disease, are often associated with poor growth and short stature secondary to decreased nutritional intake and malnutrition.<sup>9</sup> Primary undernutrition is the single most significant cause of growth retardation in countries where poverty-related food deprivation results in moderate nutritional intake and poor growth.<sup>10</sup> In a pediatric endocrinology practice in a suburban middle- and upper middle-class area, surprisingly, the great majority of patients with nutritional dwarfism also have no organic cause for their short stature, and there is no poverty-related malnutrition (Table). Rather, their short stature is due to self-imposed malnutrition as a result of inappropriate dietary intake. This reduced food intake may yield no significant weight loss; it merely prevents weight gain. These patients do not appear to be suffering from anorexia nervosa, since they do not engage in self-induced vomiting, strenuous exercising, or abuse of laxatives. Most seem to

*continued on page 2*

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# Nutritional Dwarfing in Adolescents

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have exaggerated social concerns about obesity, and they strive to achieve and maintain an ideal slim, trim figure and eat a "healthy diet."<sup>4</sup>

The majority of patients with nonorganic nutritional dwarfism can only be classified as having atypical eating disorders (Table). According to the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, patients with atypical eating disorders that are not otherwise specified (NOS) belong in a category reserved for those who do not fit into any of the

**Table.** Nonorganic nutritional dwarfing in adolescents

|                                 | Number<br>of patients |  |
|---------------------------------|-----------------------|--|
| Atypical eating disorder        | 88                    |  |
| Fear of obesity                 | 21                    |  |
| Fear of<br>hypercholesterolemia | 9                     |  |
| Psychosocial                    | 2                     |  |
|                                 | 120                   |  |

*Derived from a group of 212 adolescent patients with nutritional dwarfing as the pathological cause of short stature or poor growth or both. The specific diagnostic classifications of the 120 adolescents with nonorganic nutritional dwarfing are shown. The remainder of the patients had organic causes of nutritional dwarfism, eg, chronic inflammatory bowel disease and cystic fibrosis.*

other eating disorders (anorexia nervosa, bulimia, pica, or rumination disorder). However, we have identified three specific subgroups of patients with atypical eating disorders in accordance with primary fear or a health belief that may have caused the problem: (1) fear of obesity syndrome,<sup>3,4</sup> (2) failure to thrive because of specific parental health beliefs, and (3) failure to grow because of malnutrition resulting from dietary restrictions that are based on the fear of the consequences of hypercholesterolemia.<sup>5</sup>

Patients with NOS atypical eating disorders, as well as those in whom a more specific fear or health belief was recognized, had deteriorating linear growth and delayed sexual development, preceded by at least one or two years of inadequate weight gain. These patients did not eat enough food to allow for normal growth in the absence of organic disease. However, it should be pointed out that many of the patients observed did not undergo comprehensive psychiatric or psychological testing and, therefore, we could not be sure that subtle forms of specific eating disorders or other psychological aberrations did not exist. Nonetheless, the great majority did well, and, therefore, the presence of more severe eating disorders or gross psychiatric disease was unlikely. Moreover, catch-up growth was documented in 61% of the patients once the diagnosis of nutritional dwarfing was made and nutritional counseling and nonstructured supportive therapy were initiated. The remaining 39% had no clear-cut evidence of catch-up growth but have made progress, as has been made evident by weight increments and

normal growth rates, after nutritional counseling.

Many of the patients with NOS atypical eating disorders are below the 5th percentile for height when they are referred to a pediatric endocrinologist. However, in up to one third of the patients, the stature is within the normal range, although a fall-off in height across percentiles can be documented.

## Address for Correspondence

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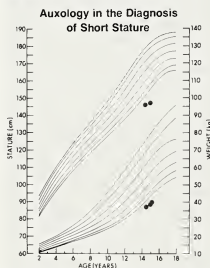
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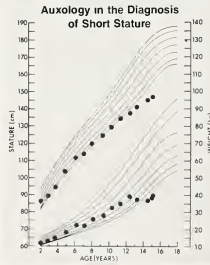


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**Figure 1A.** The patient was referred because of short stature. Initially, the heights and weights depicted in the graph were the only available data. Signs of sexual development were absent.



**Figure 1B.** The complete growth of the patient is shown. Data were obtained from school and medical records. The patient started dieting at 12 years of age to avoid obesity.



The body weight of these patients is also below the fifth percentile, but a weight deficit for height is present in only about one half. However, a cessation or a diminution of weight increments prior to examination is evident in most patients when accurate weight records are available. A decrease in arm muscle and arm fat area may be documented, but such anthropometric alterations are not found in the majority of these patients.

The dietary intake of the patients with NOS atypical eating disorders is very similar to that found in patients with the fear of obesity syndrome.<sup>3</sup> They all voluntarily ingest about two thirds of the calories required for normal growth, and they frequently skip meals. Their fat intake accounts for only about one third of the calories they do consume. They avoid whole milk, red meat, and oils and other fats; most also avoid eggs. They stay away from "junk food" and have few snacks between meals. Indeed, the dietary intake of these patients resembles the prudent diet that is currently recommended by the American Heart Association.<sup>11</sup> While this supposedly "ideal diet" is low in fat and cholesterol, it may not provide sufficient calories and micronutrients (particularly calcium, iron, and zinc) for growth and development in adolescents. Appropriate nutritional counseling should therefore be available to all teenagers who are following low-fat, low-cholesterol diets.

The incidence of atypical eating disorders leading to malnutrition and poor growth in the general population is unknown. Only those patients whose height is markedly impaired have been recognized thus far. However, growth impaired by inadequate dietary intake and resulting in a fall-off in height within the normal percentiles may not attract medical attention. In another study, we analyzed the growth patterns of more than 1,000 upper-middle-class students attending the same high school and detected 18 students who had growth alteration associated with poor weight in-

crements.<sup>4,12</sup> This finding could possibly reflect the estimated prevalence of nutritionally related growth failure in our population. However, further studies are required to ascertain the causes of poor growth in these students before we can calculate the true incidence of poor growth resulting from socially stimulated dietary restriction.

It is now known that inappropriate eating behaviors and attitudes are quite prevalent among our youth.<sup>13</sup> Dissatisfaction with body shape and appearance and unhealthy approaches toward weight reduction, such as fast dieting,<sup>14</sup> are commonly reported among adolescents. In a recent study, 13% of 10th grade students reported various types of purging behaviors, such as self-induced vomiting and the use of laxatives and diuretics.<sup>15</sup> We found that dieting to lose weight is very prevalent among high school students. A recent survey of high school students found that one third were on weight-loss diets on the day of the survey and that the remaining two thirds had attempted weight-reducing diets in the previous 4 to 6 weeks.<sup>16</sup> This occurred regardless of the body weight of the students; even those whose weight was lower than that expected for their height were concerned about dieting. The students often expressed a fear of obesity, and their distorted perception of their ideal body weight appeared to influ-

ence their actual body weight. That is, students who wanted to be thinner were indeed attaining lower weights than those who did not deliberately strive for thinness.

## Iron Deficiency

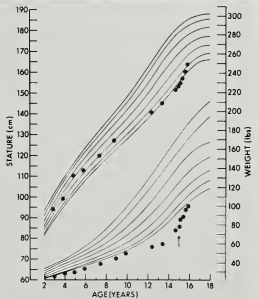
Iron deficiency is the most important nutritional deficiency in this country.<sup>17</sup> The incidence of iron deficiency in childhood peaks immediately after six months of age and again during early adolescence. Because iron deficiency is often associated with restrictive diets of any sort, it may be a confounding factor in the clinical evaluation of patients with eating disorders.

Iron deficiency is the end result of an imbalance between the sum of the patient's iron endowment, the intake and absorption of this element, and the sum of the patient's needs for growth and replacement of normal and abnormal iron losses. Thus, patients with failure to thrive or those with poor growth may have an iron deficiency, even though anemia may be very mild or absent. This deficiency may manifest itself as anorexia, which further complicates the eating disorder and leads to malnutrition and poor growth. Increased growth and improved appetite may occur after iron replacement.

The possible contribution of iron deficiency to poor growth and anorexia is shown in Figure 2. In this patient, iron deficiency was sec-

**Figure 2.** The growth pattern of a patient with an atypical eating disorder is illustrated. The main problem was anorexia. Iron deficiency without anemia was documented when the patient was first seen for nutritional rehabilitation. His hemoglobin was 13.7 g/dl, hematocrit 38.1 vol%, mean cell volume 82.7 fl, reticulocyte 2.1%. However, he had a serum iron level of 60 µg/dl, a total iron-binding capacity of 385 µg/dl with 15% saturation, and a serum ferritin level of 14.7 ng/ml despite nutritional supplements containing iron. With increased quantities of iron and appropriate supplementation, the patient gradually improved.

**Iron Deficiency in a Patient With Growth Failure Due to Atypical Eating Disorder**



*continued on page 4*

## Nutritional Dwarfing in Adolescents

continued from page 3

ondary to inadequate food intake caused by an atypical eating disorder. The clinical picture was further compounded by anorexia until the iron deficiency was corrected.

We have found that iron deficiency, usually without anemia, is common in patients with nutritional dwarfism. We saw a reduced hemoglobin level in only 12% of patients with atypical eating disorders. Only 5% had microcytosis (reduced mean cell volume level), whereas 44% had a low transferrin saturation. About one third of the patients had low serum iron or ferritin levels. In most instances, the patients achieved full nutritional rehabilitation. Thus, it is difficult to assess the exclusive role of iron deficiency in the eating behaviors of these patients. However, iron deficiency and other mineral deficiencies should be strongly suspected in patients on restrictive diets even if they do not have anemia.

A nutritional supplement containing the amount of iron ordinarily found in such formulations may not be sufficient to overcome the body's total iron deficit rapidly, particularly when the patient exhibits catch-up growth, a time when iron and other minerals are needed in higher doses.<sup>18</sup> Iron deficiency may continue to confound the clinical evaluation of the patient until its systemic effects (eg, anorexia) are eliminated.

### General Considerations

Inadequate amounts of food and adverse environmental conditions are major problems and are the main causes of malnutrition worldwide. However, inappropriate dieting for psychological or cultural reasons may be an under-recognized problem among adolescent populations in which poverty-related malnutrition is rare. Indeed, nutritional dwarfing syndromes may be more common than other classic endocrine disorders among short-stature pa-

tients who are referred to pediatric endocrinologists. On the other hand, it should be noted that for adolescents, being slightly overweight usually carries no risk and may actually be healthier than being mildly underweight.

There are no good epidemiologic studies on the prevalence of eating disorders in the United States, but the desire to be slim and trim appears to have spread like an epidemic over the past few decades. Dieting has become a multi-million dollar industry aimed at changing an individual's shape to fit an arbitrary "thin ideal." This has occurred simultaneously with an increased stigma against obesity, as well as with the pursuit of longevity through a "healthier diet." Overweight children are regarded as "responsible" for their weight, and their failure to be thin is considered to be a sign of "personal weakness" and a lack of will power,<sup>19</sup> despite medical evidence to the contrary.<sup>20</sup>

The prevalence of dieting, inappropriate eating habits, and purging behaviors by high school students to maintain a "slim and trim figure" and to follow an "ideal diet"<sup>13-16</sup> is doubly alarming because it has spawned a rise in the number of patients with serious eating disorders, such as anorexia nervosa and bulimia,<sup>21</sup> and with other atypical eating disorders and self-induced starvation syndromes.<sup>4,5,22</sup> This parallels the marked decline in the consumption of red meat and dairy products that has occurred during the last decade. Sadly, many adolescents diet to attain an "ideal" figure just when they are developing and their need for adequate nutrition is high. Consequently, they become nutritional dwarfs.

Well-intentioned dietary recommendations from reputable medical sources also appear to intensify concerns and rationalizations for self-induced dietary restrictions. Consumption of foods that are high in fat and cholesterol is widely condemned by medical authorities and the media.<sup>11</sup> The American Heart Association, the American Health Foundation, the

National Institutes of Health (NIH) Consensus Development Panel, and numerous medical authorities believe that atherosclerosis has its roots in childhood and that adherence to a prudent diet early in life will lessen the risk of this condition in later years. Thus, the medical profession may be inadvertently contributing to some of the unsound dietary practices of adolescents.

Many factors that are not necessarily tied to food or weight pervade the psychology of eating disorder patients, and these factors vary from patient to patient. Perhaps the child who is most vulnerable to social pressures to be thin is the one who adheres to a diet most strictly and, therefore, fails to grow. Perhaps he or she also has other psychological difficulties, such as poor self-esteem, feelings of incompetence, or lack of personal trust. In essence, eating disorders are a final common pathway derived from individual, familial, and cultural predisposing factors that vary in a heterogeneous patient population. In adolescents, the only visible evidence of these disorders may be poor growth.

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## In Future Issues

**Fetal Alcohol Syndrome**  
by Kenneth Jones, M.D.

**Osteogenesis Imperfecta**  
by Peter Beyer, M.D.

**Anabolic Steroid Hormones in Athletes: Efficacy or Fantasy?**  
by Alan D. Rogol, M.D., Ph.D.

**Medical Complications of Dwarfing Syndromes**  
by Judith G. Hall, M.D., and David L. Rimoin, M.D., Ph.D.

**Human Placental Lactogen and Fetal Growth**  
by Stuart Handwerger, M.D.

## Erratum

The abstract "Prenatal Diagnosis of Autosomal Dominant Polycystic Kidney Disease With a DNA Probe" (Volume 3, Number 1) incorrectly stated that the disorder (adult type) had been localized to an abnormality of the short arm of chromosome 6. The abnormality is located on the short arm of chromosome 16.

## Announcement

Readers of *Growth, Genetics, and Hormones* are urged to share the following information regarding the formation of the PEDIATRIC ENDOCRINOLOGY NURSING SOCIETY with their nursing colleagues.

As a professional nursing organization, PENS is committed to the advancement of excellence in nursing practice, research, and teaching in the field of pediatric endocrinology.

A stated objective of PENS is to promote communication and collaboration among all physicians and health professionals who work in pediatric endocrinology. To this end, PENS plans to:

- Sponsor an annual conference to provide continuing education for pediatric endocrine nurses
- Develop a directory of recommended patient education materials
- Generate written materials and audiovisual aids for use in patient education
- Organize a speaker's bureau of qualified nurses
- Publish a newsletter containing original articles, abstracts, and announcements
- Establish a clearinghouse for nurse and physician investigators active in clinical research
- Sponsor nursing research

Annual dues are \$25; newsletter subscriptions will be available for a nominal fee. Membership applications and additional information may be obtained by writing to Pediatric Endocrinology Nursing Society, 2545 Chicago Avenue South, Suite 408, Minneapolis, Minnesota 55404. Physicians and other health professionals are invited to join as associate members.

## Letter to the Editor

I read with interest Dr. Bierich's review article on constitutional delay of growth and adolescent development (CDG). He found that patients with CDG secrete less growth hormone (GH) at all stages of sexual development than do control children and suggested they have permanently diminished GH secretion.

We, on the other hand, found no significant difference between GH secretion in prepubertal children with CDG and GH secretion in controls when looking at their mean overnight GH levels, total basal GH output, total nocturnal GH pulses, mean peak nocturnal GH levels, and somatomedin-C values (Lanes et al *J Pediatr* 1986; 109:78.) Similar results were previously reported by Stubbe et al (*Ped Res* 1985;19:6). Moreover, Richter et al very recently studied a group of children with CDG and familial short stature (FSS) with the <sup>15</sup>N tracer technique and found them to have low tracer nitrogen excretion rates that were not further reduced after GH treatment; patients with GH deficiency had high tracer nitrogen excretion rates and a marked reduction of this excretion rate after GH administration. Richter et al concluded that their results yielded no evidence of partial GH deficiency in children with CDG and FSS (*J Clin Endocrinol Metab* 1987;65:74).

The variability of results so far obtained after pharmacological stimuli, as well as with measurement of spontaneous GH secretion over 24 hours or during the overnight hours, still clouds the understanding of GH secretory patterns in children with CDG, but suggests the heterogeneity of this group of patients.

Roberto Lanes, M.D.  
Department of Endocrinology  
Hospital Central Dr. Carlos Arvelo  
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# Teratogens and Growth

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Canada*

## Introduction

A teratogen may be defined as an agent that can produce a permanent abnormality of structure or function in an organism that is exposed during embryonic or fetal life. Teratogenic effects thus include not only such malformations as phocomelia or congenital heart defects but also mental retardation and behavioral abnormalities without gross malformation of the brain. Reduction of fetal growth per se is not considered to be a teratogenic effect.

Teratogenic agents can be divided into two classes with respect to the kind of effects they produce (Table). The first class includes agents that affect only a single organ or a very limited set of target organs in the developing embryo or fetus. Examples include masculinization of external genitalia in females (produced by exposure to androgenic hormones) and dental staining (produced by tetracyclines). Such agents would not be expected to affect fetal growth in general, and there is no evidence that they do.

The second and much larger class of teratogenic agents produces generalized patterns of anomalies. Fetal growth retardation often occurs in association with these syndromes.

## Teratogens That Cause Fetal Growth Retardation

Growth retardation is a cardinal feature of the embryopathies that result from intrauterine infection by rubella, cytomegalovirus, syphilis, varicella, and toxoplasmosis.<sup>1</sup> The majority of newborns with clinically apparent infections due to any of these agents are growth retarded; microcephaly is frequently present. Affected individuals often continue to grow poorly throughout child-

hood. Similarly, exposure to high doses of ionizing radiation during embryonic development regularly leads to a permanent growth deficiency, as well as to microcephaly and mental retardation.<sup>2</sup>

Typical fetal alcohol syndrome occurs among the children of mothers who drink heavily during pregnancy. Growth retardation affecting length, weight, and head circumference is characteristic of this syndrome.<sup>3</sup> Postnatal catch-up growth is usually incomplete or absent. Fetal and postnatal growth retardation are also typical features of the embryopathy associated with maternal aminopterin or methotrexate treatment during early pregnancy.<sup>4</sup>

A characteristic pattern of anomalies has been observed

among children born to women treated with the anticonvulsant trimethadione during early pregnancy.<sup>5</sup> Affected children exhibit typical facial anomalies, and congenital heart disease is frequently seen in addition to fetal growth retardation. The relationship of maternal treatment with the more commonly used anticonvulsant phenytoin to both growth retardation and the increased frequency of malformations observed among children of epileptic mothers is less clear.<sup>6</sup> Coumadin, polychlorinated biphenyls, and maternal phenylketonuria can also produce characteristic patterns of anomalies, including fetal growth retardation, in human beings.

## Teratogens That Do Not Usually Affect Fetal Growth

Although fetal growth retardation appears to be the most consistent

**Table.** Classification of Some Agents With Teratogenic Potential in Human Beings With Respect to Their Effect on Fetal Growth

| <b>Class I.</b>  | <b>Agents that affect only a single organ or a very limited set of target organs (not associated with fetal growth retardation)</b>   |
|--|---|
|  | Maternal virilizing tumors<br>Maternal lupus<br>Organic mercury<br>Androgenic hormones<br>Diethylstilbestrol<br>Tetracyclines<br>Goitrogens and antithyroid drugs<br>Radioactive iodine   |
| <b>Class II.</b>   | <b>Agents that produce generalized patterns of anomalies</b>  |
| <b>Associated with fetal growth retardation</b>                  |   |
|  | Rubella virus<br>Cytomegalovirus<br>Toxoplasmosis<br>Syphilis<br>Varicella-zoster virus<br>Maternal insulin-dependent diabetes mellitus<br>Maternal phenylketonuria<br>Polychlorinated biphenyls<br>Alcohol<br>Aminopterin and methotrexate<br>Trimethadione and paramethadione<br>Coumadin<br>Ionizing radiation |
| <b>Not consistently associated with fetal growth retardation</b> |   |
|  | Thalidomide<br>Isotretinoin<br>Valproic acid  |

single feature resulting from exposure to known teratogens in human beings,<sup>7</sup> the relationship between teratogenesis and fetal growth retardation is neither simple nor constant. Some agents with unequivocal multisystem teratogenic potential in human beings do not seem to affect fetal growth substantially. Isotretinoin is a good example. Maternal ingestion of this vitamin A analog early in pregnancy can produce serious craniofacial, cardiac, central nervous system, and thymic malformations in the offspring.<sup>8</sup> However, among 21 affected infants, only two were small for gestational age. This is exactly the number expected in the general population. Similarly, growth retardation does not appear to be a common feature of thalidomide embryopathy.<sup>2</sup>

### **Agents Without Significant Teratogenic Risk That Affect Growth**

Intrauterine exposure to certain other agents clearly reduces fetal growth but does not appear to be associated with measurable teratogenic effects. Maternal cigarette smoking during pregnancy is not considered to be teratogenic, but it clearly causes fetal growth retardation.<sup>3,9</sup> Low birth weight is twice as frequent among the infants of smokers as among the infants of nonsmokers. Birth length, and possibly head circumference, is also affected. In general, the more a pregnant woman smokes, the more likely her infant is to exhibit growth retardation. Postnatal catch-up growth in children of cigarette smokers may not be complete.

Infants born to women with such infections as hepatitis B, influenza, and listeriosis may exhibit growth retardation, as well as manifestations of the infectious disease, but malformations are usually absent.<sup>1</sup>

Fetal exposure to low doses of agents that are teratogenic at high doses may result in growth retardation only. For example, pregnant women who drink alcohol in amounts far below those associated with the occurrence of fetal

alcohol syndrome may, nevertheless, be at increased risk for having a growth-retarded infant. Significantly, reduced birth weight has been observed among the children of women who drank as little as 1 1/2 to 3 ounces of alcohol a day during pregnancy.<sup>10</sup>

### **Common Pathogenic Mechanisms**

Since agents may cause fetal growth retardation in circumstances under which they are not teratogenic, and since some human teratogens do not cause growth retardation, it seems unlikely that these are different aspects of a single phenomenon. The frequent association of fetal growth retardation with teratogenesis suggests, however, that common mechanisms might underlie both processes.

In considering what these mechanisms might be, it is important to recognize that both fetal growth retardation and teratogenesis are pathogenically heterogeneous. Even for growth retardation occurring in association with congenital infections due to the so-called "TORCH" agents, underlying mechanisms are diverse.<sup>1</sup> Cytomegalovirus causes tissue injury as a result of cell lysis, as well as vascular perfusion by damaging capillaries and small vessels. Decreased cell numbers and decreased mitotic activity occur in congenital rubella syndrome, but these may be a consequence of vascular damage. In congenital syphilis, fetal growth retardation may be caused by placental dysfunction resulting from the edema and inflammation of syphilitic placentitis.

Many processes are involved in normal embryogenesis, and interference with any of these could produce a teratogenic effect. Cell death or altered cell growth and proliferation may mediate the teratogenic effect of ionizing radiation, for example, and these factors could also impair growth of the entire fetus. Disturbed tissue interaction, defective cell or tissue migration, mechanical or vascular disruptions, and disordered pro-

grammed cell death could also lead to teratogenic lesions, but the effects of such processes on the growth of the embryo as a whole are likely to be indirect. Such indirect effects on growth could, nevertheless, be quite important. It is easy to imagine, for example, how failure of induction of critical metabolic processes or failure to form a structurally normal placenta could adversely affect cellular nutrition and thus overall growth in the embryo or fetus.

### **Clinical Recognition of Growth Retardation Due to Teratogenic Agents**

In theory, recognition of fetal growth retardation due to teratogenic agents should never present a problem, because there should always be a history of maternal exposure to the agent during pregnancy. In practice, however, this history is often not available, at least initially, for several reasons. In some instances, the mother was unaware of the exposure. This is frequently the case with cytomegalovirus or toxoplasmosis infection. However, even when an exposure is recognized, neonatal or obstetrical records may not reflect it, especially in the case of commonly-used agents, such as alcohol, in moderate amounts. Moreover, women often report exposure only from the time the pregnancy was recognized. Thus, much of the critical period in terms of teratogenic risk may have already elapsed. Therefore, in evaluating a neonate with growth retardation, it is necessary to take a thorough history directly from the mother regarding possible exposure to infectious and environmental agents, drugs, alcohol, and tobacco since the time of conception.

The second aid to clinical recognition of teratogen-induced growth retardation is that the teratogens that cause growth retardation are also those that produce characteristic patterns of anomalies. In the embryopathies associated with alcohol, aminopterin, coumadin, and the "TORCH" agents, the dysmorphic features

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## Teratogens and Growth

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are often sufficiently characteristic to suggest the diagnosis even in the absence of a history of maternal exposure. A thorough examination of major and minor dysmorphic features should be performed on every child with fetal growth retardation. Children found to have associated anomalies should be referred for evaluation by a clinical geneticist or dysmorphologist.

### Importance of Recognizing Growth Retardation Due to Environmental Agents

Epidemiologic studies suggest that as many as 40% of cases of fetal growth retardation may be associated with maternal cigarette

smoking alone or in combination with other factors.<sup>11</sup> Other environmental agents appear to be uncommon causes of fetal growth retardation, but the effect of maternal "social" drinking has not been adequately evaluated in this regard. Even if growth deficiency due to teratogenic agents is relatively uncommon, the recognition of this group of children is especially important because they are more likely to have failure of catch-up growth, developmental delay, and associated malformations. Growth retardation due to teratogens is also especially important from a public health perspective because teratogen exposures are often potentially preventable.

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# Catch-Up and Catch-Down Growth: A Review

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Associate Editor

Growth, Genetics, and Hormones

Growth in children is never consistent. Small variations occur seasonally. Frequently, significant alterations occur with illness, nutritional failure, the administration of exogenous hormones, or the increased production of endogenous hormones. Following significant alterations in growth, compensatory catch-up or catch-down growth frequently occurs. This topic has been poorly understood. The goal of this article is to provide an update on current concepts.

### Catch-Up Growth

Under normal circumstances, a child's increase in height follows a very regular path, if considered over periods of a couple of months or more. So regular is it, indeed, that the rate of such growth is one of the best indices of a child's general health. Minor illnesses cause minor irregularities: Rogers<sup>1</sup> fitted Preece-Baines curves<sup>2</sup> to the heights of individual children followed longitudinally in the Harpen-

den Growth Study and found that the deviations from the curve were greater in those children who had many episodes of minor illness than in those who had only a few. Such illnesses were not associated with lower adult heights or even with reduced growth velocities considered over a year or more. Nevertheless the children were nudged off course temporarily.

When the interruption of growth has been great, the restoring force is also great, and when the failure is repaired, the child springs forward to catch up to his previous growth curve. As he approaches his genetically predisposed channel, the restoring force diminishes, and the child's growth slows down and proceeds as smoothly as before. At least this is what happens in the most favorable circumstances, eg, juvenile hypothyroidism, where catch-up is almost always complete, provided treatment starts before the child is about 14 years of age.<sup>3</sup> In less favorable circumstances—eg, children who are admitted to the hospital with kwashiorkor and then

returned to a family environment characterized by semi-starvation—the catch-up growth may be only partial.<sup>4</sup> In children with growth hormone deficiency (GHD) who are treated with growth hormone (GH), as illustrated in Figure 1, striking initial catch-up growth occurs with treatment (14 cm/year in the first six months of treatment). However, full restoration to genetic potential, as represented by the parental target height range shown in the upper right part of Figure 1, may not occur in the long run.<sup>5</sup> Perhaps the difference between the extent of catch-up growth in children with thyroxine deficiency and those with GHD is rooted in the much greater delay in bone age that occurs in hypothyroidism, or perhaps it is simply that the child with GHD has fallen much further below the normal percentiles for age than usually occurs in hypothyroidism.

In examining the final height of 30 boys with idiopathic isolated GHD who were treated continuously with human growth hormone (hGH) until growth ceased of its own accord, Burns et al<sup>6</sup> found

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that only the standard deviation (SD) score for height at the beginning of treatment (with allowances made for parental heights) was important. The lower the SD score initially, whatever the child's age, the lower the final height, by some 2.5 cm for each SD score below the mean for the group. Of the 6 SD lost by untreated GHD patients before GHD was recognized, we were able to restore 4 SD on average, but we were not able to restore the other 2 SD. This was true even in patients who received treatment as early as four years of age.<sup>7</sup> In those first few years of rapid growth, a deficit that is only partly recoverable seems to accumulate. We do not know why this is so.

### Catch-Down Growth

Catch-down growth is the opposite of catch-up growth. If growth is artificially stimulated and then the stimulating force is withdrawn, the growth velocity drops for a while, as shown in Figure 2. We need a term for this phenomenon, and none has been forthcoming.

"Compensatory deceleration" has been suggested. The term "catch-down," however, originally suggested as a linguistic joke, seems to have caught on. Not all children treated with GH exhibit this marked deceleration in comparison with pretreatment growth rates. The reason for the differences between children remain obscure.

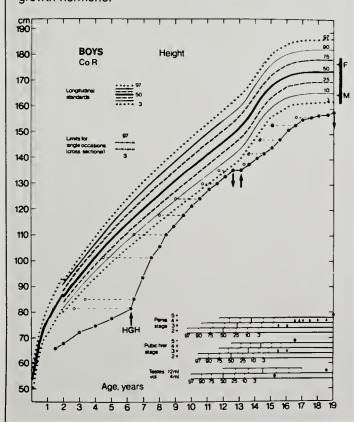
### Catch-Up and Catch-Down as Normal Occurrences

In infancy, catch-up and catch-down growth occur as normal phenomena. Soon after the longitudinal data for growth from birth to maturity became available, it was shown that the correlations between measurements taken in an individual child at various ages—birth, one month, three months, six months, and so on—and his or her measurements as an adult had a characteristic temporal pattern. The correlation between length at birth and adult height was low (approximately 0.3). By six months,

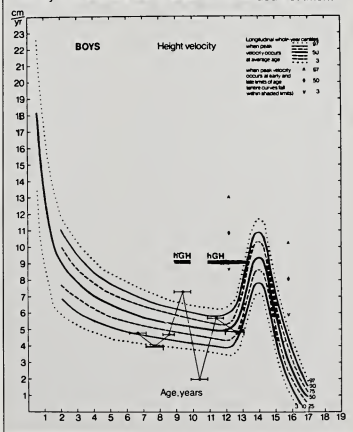
the correlation coefficient had risen to 0.5; by one year, it had risen to 0.7; by two years the stable prepubertal value of 0.8 was reached. Thus, during infancy a re-assortment of relative sizes among children comes about: Those who are larger at birth grow less; those who are small grow more. Figure 3, from an old but comprehensive study,<sup>8</sup> illustrates this. It should be noted that the velocity curves for weight of only the extreme birth weight cohorts of 5 to 6 lbs and 9 to 10 lbs are shown. In a classic paper, Smith et al<sup>9</sup> described the same thing in American middle-class, well-nourished babies.

In fact, the curves shown in Figure 3 conceal heterogeneity. Not all 9-pounders grow especially slowly, only those whose genes specify an average adult size but whose maternal uterus was highly stimulatory. Some of the 9-pounders come by it honestly: large at birth and large later. But the others slow down until they hit their proper curves. Likewise, the small

**Figure 1.** Partial catch-up growth in a boy with idiopathic isolated growth hormone deficiency treated with growth hormone.



**Figure 2.** Catch-down growth in a patient receiving growth hormone on two occasions separated by a year without treatment. The growth velocity off treatment is significantly lower than the pretreatment velocity. The growth velocity on treatment also wanes with extended treatment.



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## Catch-Up and Catch-Down Growth

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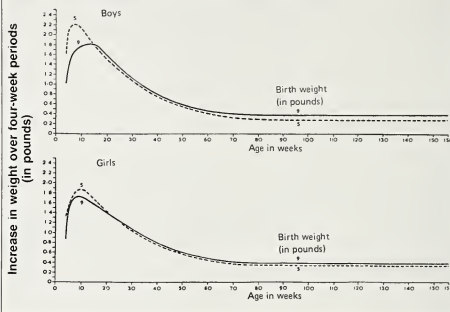
babies with genes for average final height catch up to their proper curves, a process usually completed by about 12 months. This was already well known to animal breeders: If a large Shire horse is crossed with a small Shetland pony, the size of the newborn foal closely follows the size of the mother.<sup>10</sup> But the tiny foal of the Shetland mother has half of its height-determining genes from its great Shire father, just as the large Shire-mother foal has genes from its small Shetland father. After a few months, the two foals are nearly the same size.

## The Mechanism of Growth Regulation

Catch-up and catch-down growth are simply exaggerated forms of normal growth regulation. We do not know how the extraordinary precision of growth is maintained or how it is, for example, that monozygotic twins who differ by 3 cm in length at birth grow so that there is only a 1-cm difference in adult height. We do not even know how the normal rate of growth is established or why the velocity of growth in general diminishes as a child gets older. When we fit curves to the growth of individuals, we use equations, nearly all of which assume that growth velocity at any time is a function of the remaining growth or, what is the same thing, the percentage of height completed. But how does the child "know" what percentage he has attained?

Many years ago, I suggested a "central" model to explain this,<sup>11</sup> but "peripheral" models are also possible. In the central model it is supposed that some sort of "sizostat" exists in the central nervous system; the sizostat tells the animal what size it "ought" to be at each moment during its growth. The sizostat could do this by synthesizing and/or releasing every so often, for example, a specific molecule not yet identified. The rate of synthesis would decrease

**Figure 3.** Weight velocity curves of cohorts of babies weighing 5 to 6 lbs and 9 to 10 lbs at birth. As a group, the smaller infants grow more rapidly than the larger infants.



theoretically as maturity increases. If this is true, there must be another signal that tells the animal what size it actually is, perhaps by synthesizing another series of molecules, this time in strict proportion to the amount of growth. Theoretically, these molecules would enter the central nervous system to interact with the size-stuff and bind to it so that only a certain amount is left. This remaining amount is the mismatch signal that controls the output of growth-stimulating hormone.

Recently, Mosier and his colleagues<sup>12-14</sup> designed experiments to test this model. If rats have their heads irradiated shortly after birth, their subsequent growth will be stunted. If tested later, after a 48-hour fast, their capacity for catch-up will not be impaired; they catch up to their control-irradiated curve but not to a normal-control curve. The catch-up of normal rats starved for 48 hours is associated with an increased amplitude but not frequency of GH pulses, especially during the light part of the light-dark cycle. Although the irradiated rats secrete a little less GH than do normal rats during both their control and catch-up growth, this does not necessarily mean the difference in GH secretion is causative.

Mosier thinks otherwise: The control resides in a sizostat, which the irradiation has damaged. This is the explanation I gave for the seemingly unalterable short stature of children with early intrauterine lesions (eg, Silver-Russell syndrome). Mosier has very recently localized the effect of head irradiation to the midline structures, probably the suprachiasmatic nucleus.

This model may, of course, be erroneous since the evidence is far from conclusive. Perhaps all regulation takes place in the generative and proliferative layers of cartilage, with each cell having its intrinsic rhythm of division and gathering a greater need to divide the longer it lacks the necessary local hormone to do so. However this may be, the physiology of normal growth control is clearly the key to understanding catch-up and catch-down growth, as well as the effect of the various therapeutic interventions now being used or considered for the treatment of all sorts of short stature.

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## Special Report:

### International Growth Hormone Symposium—June 15-18, 1987, Tampa, Florida

Robert M. Blizzard, M.D.

*Chairman, Editorial Board—Growth, Genetics, and Hormones*

Dr. Barry Bercu (Tampa, Florida) was the primary organizer of this excellent conference. Because a great deal of material, much of it complex, was presented, this summary highlights only the major points that were covered.

Neuroendocrine regulation of growth hormone (GH) secretion received considerable attention. Drs. Gloria Tannenbaum and Joseph Martin demonstrated very convincingly that GH-releasing hormone (GHRH) is secreted in the posterior part of the hypothalamus (tubero-infundibular region) and that growth hormone-releasing factor (GRF) is secreted in the ventromedial and arcuate nuclei. Somatostatin neurons are located primarily in the anterior hypothalamic region.

Galanin is a recently described neuropeptide of 29 amino acids; its action is additive to that of GRF. Galanin is produced in the ventromedial and arcuate nuclei, and the action is blocked by somatostatin. Dr. Eugenio Muller (Milan, Italy) emphasized that epinephrine is necessary for GH to be released by galanin. Dr. Muller also emphasized the importance of the cholinergic system and demonstrated that GH release in response to exercise, arginine, and clonidine is blocked by atropine. Clonidine, an  $\alpha_2$  adrenergic agent, was used by Muller and his co-workers to increase GH production and increase growth velocity over six- and 12-month periods in at least some children with severe short stature.

Dr. Alan Rogol presented data obtained from 20 patients who were treated with GRF in an international collaborative study. Dr. Rogol stated, "A more potent analog is needed if GRF is to be a valuable therapeutic agent." However, GRF remains a very valuable study tool for those interested in neuroendocrine interrelationships.

Dr. John Phillips discussed the presence of the two GH and three HCS genes on chromosome 17. Recently, a placental GH (derived from the hGH-V gene) has been described. This is a 22 K pituitary GH with 13 substitutions. A poster submitted by Frankenke et al demonstrated that GH was not present in amniotic fluid or in fetal serum. This produced some skepticism regarding the possible role of this hormone as a fetal GH. Dr. Phillips indicated that the first CMS gene is inactive and that apparently none of the GH or HCS genes are necessary for survival.

Drs. Gerald Baumann and James Lewis discussed the various forms of monomeric hormone in the pituitary and in serum. The human growth hormone (hGH)-N gene is responsible for production of both 22 K and 20 K GH. The latter has minimal immunologic and growth-promoting activity, but does have diabetogenic activity. Dr. Lewis concluded, "Although the number of GH variants and modifications have reached eight, there are at least an equal number of unidentified forms in pituitary extracts." A most intriguing part of Dr. Baumann's presentation was

his description of two binding proteins for GH found in plasma. The function of these binding proteins remains unknown, but there is some indication that one or both of these proteins could be receptor related and that even a partial portion of the receptor passed into the serum.

Dr. Hiroo Imura described a highly innovative sandwich enzyme immunoassay to measure GH. The assay's lower limit of sensitivity equals 50 pg/ml. Since GH is found in nearly all normal individuals at all times, Dr. Imura utilizes this assay, which he developed with his co-workers, in diagnosing GH deficiency and acromegaly. Using this technique to measure GH in urine enhances its diagnostic capability. Dr. Imura also determined that the kidney plays an important role in the degradation of GH.

Several experts discussed human insulin-like growth factor (IGF)-I and IGF-II. Dr. Matthew Rechler and others are exploring the possibility, which seems more likely to be a probability, that IGF-II plays a role in fetal growth. Drs. John Sussenback and Michael Czech discussed the role of the IGF-II gene and receptors, respectively, in the fetus and the neonate. Their data tend to substantiate an important role for IGF-II in fetal growth.

Drs. Olle Isaksson, Rudolf Froesch, and Naomi Hizuka individually presented data comparing the effects of GH and IGF-I on skeletal growth. IGF-I, as dem-

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**Special Report:**  
**International GH Symposium**  
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onstrated by Hizuka and Froesch, has a growth-promoting effect on the tibial plates of hypophysectomized rats. Isaksson infused GH locally on one of the epiphyseal plates, that of the right femur, and demonstrated unilateral growth in that femur. His thesis is that GH stimulates colony formation of epiphyseal chondrocytes adjacent to the epiphysis, while IGF-I stimulates cells isolated both in the proximal and intermediate parts of the growth plate.

Dr. David Clemmons studied the metabolic action of GH in relation to nutrition and reported that IGF-I levels do not fall in obese individ-

uals with low energy diets; they do fall in individuals who are not obese. Their preliminary data suggest that administration of GH at 0.1 IU/kg body weight every other day does not enhance weight loss in obese individuals.

Dr. Kerstin Albertsson-Wiklund presented data suggesting that integrated concentrations of GH are directly proportional to the height of children both in pubertal and prepubertal stages. Dr. Barry Bercu recapitulated his extensive studies of short children with regard to GH concentrations in serum over 24 hours.

GH neurosecretory dysfunction was discussed extensively. Most agreed that this is an appropriate term for patients who have been exposed to cerebral irradiation

and who have dysfunction of GH secretion. However, many investigators are not ready to apply the term to other entities in which there may be diminished GH secretion.

Dr. Raphael Rappaport studied the effect of cranial irradiation on GH secretion and growth in a large number of patients and reported that slowing of growth usually does not occur until at least 18 months after brain irradiation. Patients receiving spinal plus cranial irradiation have greater limitation of growth potential than do those who receive cranial irradiation alone.

Those interested in purchasing a copy of the published proceedings of this conference should contact Dr. James Posillico of Sero Symposia USA at 800-225-5185.

**Special Report:**  
**Annual Meeting of the American Pediatric Society/Society for Pediatric Research (Genetics Sessions)—April 27-30, 1987, Anaheim, California**

Judith G. Hall, M.D.

*Associate Editor—Growth, Genetics, and Hormones*

Among the highlights of this meeting was the awarding of the outstanding young investigator prize to Dr. Arthur L. Horwich for his work on the molecular structure of ornithine transcarbamylase. The gene was isolated and sequenced and found to have three sections: a leader peptide necessary for directing the molecule into the mitochondria, a propeptide section requiring removal to activate the enzyme, and the enzyme itself. Using *in vitro* mutagenesis, Dr. Horwich was able to identify the amino acid sequences that led to changes in the various parts of the protein.

Dr. D.S. Rosenblatt reported a new vitamin B<sub>12</sub>-dependent condition in which mild retardation is associated with megaloblastic anemia. The condition is responsive to vitamin B<sub>12</sub> therapy.

Studies of ovarian function in galactosemic patients conducted by Dr. Francine Kaufman and her colleagues indicate that females

who were completely deficient in galactose-1-phosphate uridylyl transferase all had ovarian failure. Only those galactosemic patients with partial presence of the enzyme were fertile.

Dr. Ian T. Thomas reported a large number of patients with chromosomal mosaicism that was not necessarily reflected in the karyotypes of the peripheral blood cells. Mental retardation, asymmetry, and striking pigmentary abnormalities, however, were present. Dr. Robert Brent reported that antiserum against yolk sac proteins could have a teratogenic effect and that this effect could not be reversed by vitamin supplementation. Dr. Ira Chasnoff confirmed previous reports that cocaine use by pregnant women is associated with genitourinary tract anomalies in the fetus.

In his paper on Rett's syndrome, Dr. John Moeschlen said that the disorder is much broader in spectrum than had been previously

recognized.

Dr. Grant Mitchell isolated a gene and at least two pseudogenes connected with the ornithine aminotransferase deficiency that may account for gyrate atrophy. Cytogenetic molecular studies of non-disjunction in trisomy 13 syndrome, which were reported by Dr. Terry Hassold, indicate that 60% to 70% of non-disjunction occurs in the first maternal meiotic division, just as it does in Down's syndrome. Molecular studies by Hassold et al demonstrated that, because of lack of crossing over, non-disjunction did not occur. Dr. Carolyn Hadley described experiments in which the gene for phenylketonuria was transferred into hepatocytes via a retrovirus. In his presentation, Dr. Aubrey Milunsky reported that the serum alpha-fetoprotein determination used to screen for chromosome defects was as efficient when used in the first trimester as it was in the second.

**Special Report:**  
**Annual Scientific Meeting of the American Diabetes Association—**  
**June 6-9, 1987, Indianapolis, Indiana**

William L. Clarke, M.D.

*Associate Editor—Growth, Genetics, and Hormones*

The plenary sessions of this annual meeting included symposia on lipid metabolism, insulin action, and the mechanisms and management of diabetic neuropathy. The Banting Memorial Lecture, given by Dr. Joseph Larner (Charlottesville, Virginia), concerned insulin signaling mechanisms.

Several papers that were presented are of particular interest to those who are studying growth. MacGorman (Rochester, Minnesota) presented a paper on the importance of growth hormone in the maintenance of basal lipolysis in normal man. Using a study design in which somatostatin, insulin, glucagon, and glucose were infused in amounts necessary to maintain euglycemia, MacGorman et al infused labeled glucose and palmitate into seven normal human volunteers. Human growth hormone (hGH) was then administered either by constant infusion or by hourly boluses. When hGH was administered in a pulsatile fashion, palmitate levels were higher than when hGH deficiency was induced. However, no differences in glucose metabolism were observed. These results indicate that hGH is important in the maintenance of basal lipolysis during the night in normal volunteers and that free fatty acid metabolism may be more sensitive than glucose metabolism to hGH.

Jacob (New Haven, Connecticut) presented a paper entitled "Effect of IGF-I to Lower Blood Glucose and Its Effect on Hepatic Glucose Production." In this study, fasted rats were infused with either saline or recombinant human IGF-I (THR59) while simultaneously being infused with tritiated glucose; these infusions were done to determine hepatic glucose production. It was demonstrated that IGF-I produced hypoglycemia in rats by selectively enhancing peripheral glucose uptake. Liver glucose

metabolism was relatively unresponsive to IGF-I in comparison with insulin, which suggests that IGF-I and insulin affect hepatic glucose production by different cellular mechanisms.

In a paper entitled "Diabetes Mellitus Influences Growth by Regulating Hepatic Insulin-Like Growth Factors I and II Gene Expression," Yang (Madison, Wisconsin) compared the effect of streptozotocin-induced diabetes on the growth rate of young rats and on the transcription and translation of IGF-I and IGF-II. Although serum IGF-I levels correlated positively with hepatic mRNA and negatively with blood glucose concentrations, neither relationship held for IGF-II. Yang et al concluded that hepatic IGF-I mRNA, serum IGF-I levels, and growth rate are decreased by poorly controlled diabetes and are normalized by insulin therapy. In contrast, IGF-II synthesis and release are only slightly altered, suggesting that this somatomedin is less important in growth regulation.

Two papers concerning GH and diabetic retinopathy were presented. The first, by Shumak et al (Toronto, Ontario), was entitled "The Effect of Growth Hormone Suppression on Established Proliferative Diabetic Retinopathy." Four Type I diabetics with preproliferative retinopathy and varying degrees of macular edema received eight weeks of therapy with a long-acting somatomedin analog (SMS 201-995). Glycosylated hemoglobin bA<sub>1c</sub> concentrations did not change during the study, while 24-hour integrated GH concentrations declined by about 42%. Visual acuity improved in all eight eyes but was not associated with detectable morphologic changes on stereofundus photography or fluorescein angiography. Within two months of discontinuing ana-

log treatment, visual acuity returned to pretreatment levels. The mechanism by which visual acuity improved is unclear, although subtle changes in the degree of macular edema may have occurred.

The second paper, presented by Sundkvist (Sweden), was entitled "Absent Elevations in Growth Hormone and Endothelial Factors During Exercise Predict a Resistance Against Retinopathy." Plasma levels of GH, endothelial factors, and GH factor VIII-related antigen, and plasminogen activator activity were recorded during exercise in 22 patients with insulin-dependent diabetes. The patients were reevaluated five to seven years later for the absence or presence of retinopathy in relation to previous exercise test results. Patients with retinopathy at follow-up showed significant elevations in GH factor VIII-related antigen and plasminogen activator activity during exercise. In contrast, patients without retinopathy at follow-up did not show significant elevations of these values during exercise. Sundkvist et al concluded that the absence of elevations in GH and endothelial factors during exercise may predict resistance against retinopathy in patients with insulin-dependent diabetes mellitus.

Further information regarding these and other papers concerned with growth can be found in *Diabetes* Vol. 36, Supplement I, 1987.

**Address  
for Correspondence**

Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

## Growth in Turner's Syndrome: Long-Term Treatment With Low-Dose Ethinyl Estradiol

The use of low-dose estradiol (100 ng/kg/day) was previously reported by Ross et al (*N Engl J Med* 1983;309:1104) to increase the growth rate of patients with Turner's syndrome after six months of treatment. In this study, Martinez et al attempted to reproduce and extend the studies of Ross et al.

Nine patients with Turner's syndrome between the ages of 8.6 and 13.3 years were treated with ethinyl estradiol, 100 ng/kg/day, for 18 months. The growth rates increased in all nine patients during the first six months of treatment (3.09 cm/yr  $\pm$  1.05 v 7.09 cm/yr  $\pm$  1.47), but fell progressively during

the 6th to 12th months (6.08 cm  $\pm$  1.78) and the 12th to 18th months (4.03 cm  $\pm$  1.65). The bone ages increased by more than two years in six of the nine patients during the 18 months of treatment (from 9.23  $\pm$  1.60 to 11.63  $\pm$  1.27), and the predicted heights did not change.

The integrated concentrations and the number of peaks of GH observed during a seven-hour period (07:30 to 14:30) did not change, as was the case for IGF-I levels as determined by both bioassay and radioimmunoassay. The predicted heights also did not change.

Sexual development was noted during the first six months of treatment in all patients, but regression occurred in seven patients over the next 12 months.

The authors conclude that growth velocity is increased with this regimen but ultimate height is

probably not affected.

Martinez A et al. *J Clin Endocrinol Metab* 1987;65:253.

**Editor's comment**—The patients in this study have been treated with estrogen longer than those in any other study reported to date. The effect of oxandrolone alone, growth hormone (GH) alone, and both together also has resulted in increased growth over a 12-month period (*J Pediatr* 1986;109:936-943). My personal observations using oxandrolone over the years are that growth rates return to their low levels by the third year of treatment. Will oxandrolone or GH or both increase ultimate height when used over an extended period? The answer to this question will not be known for several years.

RMB

## Testosterone Treatment of Constitutional Delay of Growth and Development: Effect of Dose on Predicted Versus Definitive Height

Boys with constitutional delay of growth and adolescent development (CDGAD) are treated with different hormones or anabolic steroids or testosterone or a combination of the three. In adolescents, testosterone is favored, since it stimulates both growth and sexual development. While there is no doubt about the short-term success of such treatment, opinion regarding the long-term effects is divided, particularly with respect to ultimate height.

Martin et al carried out a large longitudinal study on 44 adolescents with CDGAD who received different doses of testosterone enanthate. A group of 14 untreated boys with CDGAD served as controls. The inclusion criteria for the study were pretreatment growth

velocity less than 4.0 cm/year, height less than the 5th percentile, retarded puberty ( $P_1$  or  $P_2$ ), and a delay in bone age corresponding to that in height. Treatment was started when the boys were 14 or 15 years of age. Group I received 200 mg testosterone enanthate monthly, group II received 100 mg, and group III, 50 mg. During a year of continuous treatment, the growth velocity advanced considerably in all treated groups (10.9 cm  $\pm$  0.5 cm in group I, 11.0 cm  $\pm$  0.6 cm in group II, 10.7 cm  $\pm$  0.4 cm in group III). The  $\Delta BA/\Delta HA$  ratio was 2.0:1.8 in group I, 1.8:1.7 in group II, and 1.5:1.5 in group III. In the year after the discontinuation of therapy, the growth rates dropped—most markedly in group I (4.6 cm/year), less in group II (5.0 cm/year), and least in group III (7.0 cm/year). This led to a statistically significant loss of ultimate height in group I. Measured adult height in these patients was 167.8 cm, or 3.3 cm less than the predicted ultimate heights that were calculated at the start of treatment ac-

cording to RWT. No significant differences were seen between predicted and measured adult height in groups II and III.

Martin MM, Martin ALA, Mossman KL. *Acta Endocrinol* 1986; 279(suppl):147-153.

**Editor's comment**—This study of a fairly large number of patients brings about important, statistically ascertained results. With testosterone treatment, the desired and expected growth spurt occurs in all three treatment groups. However, in the year after treatment, a rebound phenomenon occurs, which depresses the gain in height age to 0.7 years/year and leads to a significant loss of ultimate height. In light of these results, long-term therapy with 200 mg testosterone per month is certainly contraindicated. On the other hand, treatment with 50 mg testosterone per month provides satisfactory results and is free of undesired side effects as well.

JRB

## Short-Term Metabolic Effects of Recombinant Human Insulin-Like Growth Factor (IGF-I) in Healthy Adults

Recombinant human IGF-I was administered intravenously (IV) at a dose of 100  $\mu\text{g/kg}$  to eight healthy adult volunteers after a ten-hour fast. In addition, on a separate day, 0.15 U/kg of IV insulin were administered to the same subjects. Serum was obtained after the fast and after each injection for determinations of total and free circulating IGF-I, glucose, growth hormone (GH), cortisol, lactate, epinephrine, norepinephrine, glucagon, and free fatty acids.

The mean fasting blood glucose level was  $4.65 \pm 0.30$  mmol/l. Similar reductions in blood glucose were observed with either IGF-I or insulin ( $1.98 \pm 0.44$  and  $1.78 \pm 0.29$  mmol/l, respectively, 30 minutes after injection). Blood glucose levels returned to  $3.50 \pm 0.56$  and  $3.48 \pm 0.25$  mmol/l within 120 minutes of IGF-I and insulin administration, respectively. The glycemic curves for those studies were not significantly different. Serum IGF-I rose from  $144 \pm 38$  ng/ml to  $424 \pm 56$  ng/ml 15 minutes after administration and fell to  $261 \pm 56$

ng/ml after 60 minutes and remained at that level for seven hours. Serum levels of free IGF-I rose within 15 minutes after injection from  $26 \pm 8$  ng/ml to  $343 \pm 87$  ng/ml and then decreased to its initial value within seven hours. Blood glucose values returned to normal after two hours, although total IGF-I was still elevated. Peak GH values were reached at 45 minutes ( $19.3 \pm 9.4$  ng/ml) after IGF-I injection and at 90 minutes ( $29.8 \pm 14.3$  ng/ml) after insulin injection. No statistically significant differences between the two GH curves were observed. Serum insulin fell below the limit of sensitivity of the radioimmunoassay ( $< 8.0$  ng/ml) after injection of IGF-I. Glucagon, epinephrine, norepinephrine, cortisol, and lactate levels were similar after both injections. In addition, free fatty acids were suppressed by both hormones, and they reached a nadir 30 minutes after injection. Free fatty acid levels at 60 and 90 minutes, however, were significantly lower ( $P < 0.01$ ) after injection with insulin than with IGF-I.

The authors conclude that IGF-I, when administered in a supra-physiologic dose, is a hypoglycemic agent in human beings. The potency of IGF-I in producing hypoglycemia was 7.5% of that of

insulin on a molar basis. The disappearance curve of free IGF-I between 15 and 60 minutes after injection was similar to that of insulin, but the apparent half-life of free IGF-I was twice as long as that of insulin (20 minutes v 10 minutes). The authors state that the kinetic features of disappearance of IGF-I are similar to those of insulin in subjects with substantial levels of anti-insulin antibodies. In addition, the effect of free IGF-I on free fatty acids is in keeping with in vitro data obtained in rat adipose tissue, demonstrating that insulin is about 100 times more potent than IGF-I in inhibiting lipolysis. Finally, these data do not support the hypothesis of a negative feedback loop of IGF-I on GH secretion.

Guler H-P, Zapf J, Froesch ER. *N Engl J Med* 1987;317:137-140.

**Editor's comment**—This carefully performed study provides much information concerning both the metabolic and the pharmacokinetic effects of recombinant human IGF-I. Further studies will be required to determine the long-term effects of this hormone in individuals with GH deficiency and, possibly, insulin-dependent diabetes.

WLC

## Effect of Growth Hormone Releasing Hormone (GHRH) on Growth Hormone (GH) Secretion in Type II (Non-Insulin-Dependent) Diabetes Mellitus

Pietschmann and Scherthaner studied GH response to GHRH in 21 non-obese and 26 obese patients with Type II diabetes mellitus. Subjects received an intravenous bolus injection of 1  $\mu\text{g/kg}$  GHRH (1-44) following an overnight fast. In addition, nine Type II diabetic patients received hpGHRH during hyperglycemia and after reductions in blood sugar with insulin. The increase in

GH levels following GHRH administration was less marked in obese Type II diabetic patients compared to non-obese Type II diabetic patients ( $P < 0.02$ ). However, the GH response to GHRH in non-obese Type II diabetic patients was not significantly different from that in control subjects. In addition, reduction in mean fasting plasma glucose values from 247 mg/dl to 131 mg/dl did not influence GH response to GHRH.

Pietschmann P, Scherthaner G. *Diabetologia* 1987;30:13-15.

**Editor's comment**—GH response to GHRH in normal subjects has been shown to be influenced by age and obesity. This

paper suggests that obesity might be the factor that is predominantly responsible for the GH responses to GHRH in obese Type II diabetics. The authors suggest that GH responses to GHRH may be due to enhanced secretion of hypothalamic somatostatin, since rats with genetic obesity have a greater release of somatostatin from the hypothalamus than do non-obese rats. In addition, the finding that improvement in glucose control does not alter GH response to GHRH is consistent with similar findings in patients with Type I diabetes. Those individuals failed to exhibit glucose-mediated suppression of GHRH-induced GH levels.

WLC



## MEETING CALENDAR

**February 16-19, 1988** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, California. Contact: David K. Stevenson, MD, Department of Pediatrics, Room S222, Stanford University School of Medicine, Stanford, CA 94305 (415-723-5711)

**February 18-20, 1988** Canadian College of Medical Genetics. Mont Gabriel, Quebec. Contact: Canadian College of Medical Genetics, Alberta Children's Hospital, 1820 Richmond Road SW, Calgary, Alberta T25C7

**February 21-25, 1988** 15th Annual Seminar in Pediatric Nephrology: Immunosuppression, Growth, and the Neonate. Sheraton-Bal Harbour Hotel, Miami Beach, Florida. Sponsor: Department of Pediatrics, University of Miami. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960, Miami, FL 33101 (305-549-6726)

**May 2-6, 1988** Annual Meeting of the American Pediatric Society/Society for Pediatric Research. Sheraton Hotel, Washington, DC.

Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2350 Alamo SE, Suite 106, Albuquerque, NM 87106 (505-764-9099)

**May 6, 1988** Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Sheraton Hotel, Washington, DC. Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2350 Alamo SE, Suite 106, Albuquerque, NM 87106 (505-764-9099)

**May 9-13, 1988** Clinical Disorders of Bone and Mineral Metabolism. Detroit, Michigan. Sponsor: Henry Ford Bone and Joint Specialty Center. Contact: Henry Ford Hospital, Continuing Medical Education, 2799 West Grand Boulevard, Detroit, MI 48202 (800-662-8242, within Michigan; 800-521-7946, other states and international)

**June 8-10, 1988** 70th Annual Meeting of The Endocrine Society. New Orleans, Louisiana. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**July 10-13, 1988** 20th Anniversary of the Clinical Genetics Conference. Baltimore, Maryland.

**July 17-23, 1988** 8th International Congress of Endocrinology. Kyoto, Japan. Contact: Travel Planners—Kyoto Congress, Suite 150, GPM Building, San Antonio, TX 78216-5674 (512-341-8131)

**July 20-23, 1988** 15th International Auxology Congress. Exeter University, Exeter, England. Contact: Prof. J.M. Tanner, Room 115, Institute of Child Health, 30 Guilford Street, London, England WC1N 1EH

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# GROWTH

## Genetics & Hormones

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### The Fetal Alcohol Syndrome

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The fetal alcohol syndrome is a pattern of altered growth, structure, and function in the offspring of chronically alcoholic women who continue to drink heavily throughout pregnancy. Since the initial delineation of this condition in 1973, it has become clear that prenatal alcohol exposure can lead to a variable spectrum of defects. At the most severe end is an increased frequency of fetal wastage; less severely affected children manifest prenatal onset of growth deficiency, mental retardation, and facies characteristic of fetal alcohol syndrome, while the most mild end of the spectrum includes otherwise normal children with learning disorders and mild growth deficiency.

#### Incidences

Since children at the most mild end of the spectrum are frequently not identified as being prenatally affected by alcohol, the true incidence of this syndrome is difficult to determine. In a study conducted in Goteborg, Sweden, Olegard et al estimated the incidence of the full-blown fetal alcohol syndrome to be one in 600 liveborn infants, while one in 300 had partial or milder manifestations of the syndrome. In the United States, the fetal alcohol syndrome is thought to be the third most common recognizable cause of mental retardation, with an incidence of one to two per 1000 live births. However,

in areas where maternal alcohol abuse is high, the incidence of the fetal alcohol syndrome is far higher.

#### Pattern of Malformation

Features most frequently seen in children with the fetal alcohol syndrome are listed in the table and are further described below.

#### Growth

Prenatal and postnatal growth deficiency occur in the vast majority of affected individuals. In the first eight patients reported to have this disorder, mean gestational age was 38 weeks, while birth length and weight were in the 50th percentile for gestational ages of 33 and 34 weeks, respectively. Regarding postnatal growth, a follow-up study of the same eight children, plus three additional children also reported to have the fetal alcohol syndrome, indicates continued growth deficiency with respect to both length and weight over a ten-year period. Although some degree of catch-up linear growth during the first 1½ years of life was evident in most patients, weight decreased during the

same time period for the majority. Thereafter, length remained relatively constant while catch-up growth occurred with respect to weight. Initially, all the children were strikingly underweight for their length, with a weight-for-height age in the preschool years averaging between the fifth and tenth percentile. However, with the onset of puberty, two of the three females had become overweight for height; the weight of the third pubertal girl was appropriate for her height age.

With respect to brain growth, head circumference, which at birth was below the third percentile for gestational age in the vast majority of patients, decreased relative to height age during the first one and 1½ years. Thereafter, head circumference remained two to four standard deviations below the mean for chronologic age in the majority of patients.

The etiology of the growth deficiency seen in this disorder is unknown. Tze et al evaluated five patients and found a normal or slight hyper-response of growth hormone (GH) and normal somatomedin activity in those samples with high GH levels. Normal function of the hypothalamic-pituitary axis was seen in four additional affected children studied by Root et al. These findings suggest that the growth deficiency is not due to hormonal factors.

#### Performance and Central Nervous System Defects

Of greatest significance is the effect that prenatal alcohol exposure can have on brain development. Although the average IQ of children with fetal alcohol syndrome is

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## The Fetal Alcohol Syndrome

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63, a wide range of developmental outcomes has now been documented. At one end are children with profound mental deficiency and cerebral palsy; at the other are those with normal intelligence who manifest learning disorders and other behavioral aberrations.

Characteristic neuropathologic findings noted in the brains of some affected children include multiple heterotopias throughout the leptomeninges and cerebral mantle resulting from aberrations of neuronal migration. Meningo-myelocoele and hydrocephalus have also been seen, but in extremely small numbers.

### Facies

Many of the characteristic facial features of the fetal alcohol syndrome are believed to be a function of the effect of alcohol on early brain development. For example, since the optic vesicles that develop as evaginations from the neural ectoderm are responsible for induction of normal palpebral fissures, it is suggested that alcohol-related defects of brain development—and thus optic vesicle development—lead secondarily to an alteration in palpebral fissure size. In addition, it has been suggested by Sulik et al, who utilized a prenatally exposed mouse model, that the long, smooth phil-

trum and thin vermillion of the upper lip are secondary to a defect in forebrain development resulting in closely set olfactory placodes and underdeveloped medial nasal processes. Since the medial nasal processes form the columella, the philtrum, the portion of the dental-alveolar ridge containing the upper incisors, and the anterior portion of the hard palate, Sulik suggested that deficiency of the medial nasal processes leads to fusion of the maxillary processes at the midline to form the characteristic long philtrum seen in children with the fetal alcohol syndrome.

### Skeletal Defects

Many of the skeletal defects seen in this condition are also due to the effect of alcohol on brain development. For example, since joint development depends on fetal activity, the joint contractures seen in affected children are secondary to decreased fetal activity and decreased joint mobility. Similarly, since development of palmar crease patterns depends on movement of the hands, the altered palmar crease patterns are secondary to the effect of alcohol on early development of the brain.

### Cardiac Defects

Of the 76 children with the syndrome who were diagnosed by Smith et al, 31 had a cardiac defect, and an additional 12 had

functional murmurs. Although only three of the 15 children with isolated ventricular septal defects required surgery, ten of the 16 children with complex cardiac defects required or had undergone surgery at the time the report was published (1981).

### Other Defects

Other defects that become significant as the child grows older include dental malalignment and malocclusions, eustachian tube dysfunction, and myopia. The incidence of renal anomalies is unclear. Intravenous pyelograms in 19 affected children revealed two with ureteropelvic obstruction, one with a neurogenic bladder secondary to meningomyelocoele, and one with malrotation of one kidney.

### Etiology and Pathogenesis

Despite numerous studies in humans and laboratory animals, no clear-cut explanation of the pathogenetic mechanisms and risk factors associated with the fetal alcohol syndrome has yet been demonstrated. Although it is becoming increasingly clear that the occurrence of the fetal alcohol syndrome cannot be predicted by alcohol consumption alone, a crude dose-response effect has begun to emerge. Based on a number of retrospective and prospective studies, the incidence of serious problems in the offspring of alcoholic women who continue to drink heavily throughout pregnancy ranges from 30% to 50%. Moderate alcohol consumption—defined as one to two ounces of absolute alcohol per day (two to four ounces of whiskey or two to four glasses of wine)—has been associated with an 11% incidence of babies who show evidence of the adverse prenatal effect of alcohol. The extent to which lesser amounts of alcohol at various times during pregnancy can cause problems in fetal development is unknown. However, the full-blown fetal alcohol syndrome has not been seen in babies born to women who drink less than one ounce of absolute alcohol per day.

Variables other than the amount

**Table.** Pattern of Malformation

**Growth:** Prenatal and postnatal growth deficiency

**Performance:** Developmental delay; fine motor dysfunction manifested by weak grasp and poor eye-hand coordination; irritability, hyperactivity, and poor attention span; speech problems

**Craniofacial:** Microcephaly; short palpebral fissures; ptosis; maxillary hypoplasia; long, smooth philtrum; thin vermillion of upper lip

**Skeletal:** Joint alterations, including camptodactyly, flexion contractures at elbows, congenital hip dislocations, and foot positional defects; radioulnar synostosis; tapering terminal phalanges, with hypoplastic fingernails and toenails; cervical spine abnormalities; altered palmar crease pattern

**Cardiac:** Ventricular septal defect; atrial septal defect

**Other:** Cleft lip, palate or both; myopia; strabismus; epicanthal folds; dental malocclusion; hearing loss; protuberant ears; abnormal thoracic cage; renal anomalies; strawberry hemangiomas; hypoplastic labia majora

of alcohol consumed may be important in the expression of the fetal alcohol syndrome. These include parity, socioeconomic status, smoking, marital status, use of other drugs, and altered placental function.

Recently, an animal model has been utilized to evaluate the genetic background of the mother and the developing fetus. Using three inbred strains, Chernoff demonstrated that the incidence of malformations in the offspring of alcoholic mice was dependent on the genetically determined rates of maternal alcohol metabolism, as well as on the amount of alcohol consumed. This study implies that the incidence of the fetal alcohol syndrome is dependent upon the maternal genotype as well as on the maternal consumption of alcohol.

That the fetal genotype may also play an important role in humans is suggested by the documentation of discordance for the fetal alcohol syndrome in dizygotic twins. Evidence in humans of a genetic contribution to alcohol metabolism has been documented. Moreover, Vegheli and co-workers demonstrated elevated blood acetaldehyde levels in a chronic alcoholic woman who had previously given birth to a child with the fetal alcohol syndrome. The elevated levels, compared to levels from three normal, nonalcoholic controls, gave further credence to the concept that differences in maternal alcohol metabolism are important determinants of the fetal alcohol syndrome.

To test further the hypothesis that susceptibility to the fetal alcohol syndrome is dependent on genetic differences that result in differences in maternal alcohol and acetaldehyde metabolism or both, Cooper et al monitored breath alcohol and acetaldehyde levels for 240 minutes following administration of 0.75 cc of 95% ethanol/kg body weight in five nonpregnant alcoholic women who had given birth to affected babies, five nonpregnant alcoholic women who had given birth to normal babies, and five nonalcoholic controls. Al-

though no statistically significant differences among the three groups of women could be documented, a trend towards elevated alcohol and acetaldehyde levels in the group of alcoholic women who gave birth to affected babies was demonstrated. This trend was strongest during the first 120 minutes after ingestion.

Although the data do not provide any concrete information regarding pathogenesis of the fetal alcohol syndrome, a recent study has documented two factors that may be helpful in predicting the ultimate prognosis for affected children. Of greatest importance is the extent and severity of the pattern of malformation, including the growth deficiency. Those children noted during the newborn period as being severely affected were noted at follow-up to have the most severe degree of microcephaly, the shortest stature, and the most impaired intellectual function. Next in importance is the severity of the maternal alcoholism. Three of the four mothers with the most seriously handicapped children who were followed for ten years by Streissguth et al died of alcohol-related causes within six years after giving birth to their affected children.

### Conclusion

Despite the fact that alcohol has now clearly been identified as a significant human teratogen, a number of practical questions remain unanswered. Foremost among them is the extent to which lesser amounts of alcohol (one ounce or less per day) can affect fetal development. Pregnancy outcome associated with lesser amounts of alcohol is difficult to study since many of the more subtle effects of prenatal alcohol exposure may involve learning disorders that often do not become obvious until a child reaches the first grade in school. At present, it is important to recognize that no study has established a "safe" amount of alcohol for all pregnant women. Depending on unknown factors, which may well be genetically determined, what may be a

"safe" amount of alcohol for some women may have devastating effects on the unborn babies of others. Therefore, it is the author's belief that total abstinence from alcohol is the best policy throughout pregnancy.

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# Human Placental Lactogen and Fetal Growth

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Placental lactogen, or chorionic somatomammotropin, is a protein hormone of the placenta that has striking chemical and biologic homologies to growth and prolactin. Early studies of the physiology of placental lactogen focused primarily on the biologic actions of the hormone in the mother, while more recent studies have focused on the actions of the hormone in the fetus. This presentation will deal predominantly with investigations of the action of placental lactogen action in the fetus that strongly suggest a direct role for the hormone in the regulation of fetal growth and metabolism. In addition, some recent studies of secretion of placental lactogen will be presented; these studies implicate the involvement of several novel factors in the regulation of release of this placental hormone.

## Physiologic Studies of Placental Lactogen

In the pregnant woman, human placental lactogen (hPL), like growth hormone (GH), has been shown to induce peripheral insulin resistance, elevate blood glucose and amino acid concentrations, and stimulate insulin secretion. Some studies have also shown that hPL stimulates lipolysis. Since these metabolic actions of hPL are qualitatively similar to those of GH, and since plasma hPL concentrations increase markedly during gestation (while GH concentrations do not change), many investigators have suggested a major role for hPL as a maternal "growth hormone" of the second half of pregnancy. Although the effects of the increase in maternal hPL concentrations on the fetus are unknown, the resultant changes in maternal substrate concentrations may promote the transport of nutrients to the fetus and thereby stimulate fetal growth.

Because concentrations of placental lactogen in maternal serum greatly exceed the concentration of placental lactogen in umbilical cord blood, the effects of placental lactogen on fetal metabolism were felt to be mediated only indirectly by changes in maternal metabolism and not by direct effects of the hormone on fetus tissues. However, with the recent demonstration that placental lactogen has direct growth-promoting actions in the fetus, it appears that placental lactogen may affect fetal growth *by promoting substrate transport to the fetus and by acting directly on fetal tissues.*

Initial studies of placental lactogen action in fetal tissues focused on the biologic actions of ovine placental lactogen (oPL) in fetal rat and sheep tissues. oPL was shown to stimulate dose-dependent increases in amino acid transport into fetal rat skeletal muscle and glycogen accumulation in fetal sheep and rat hepatocytes. The increase in hepatic glycogen content resulted from both stimulation of glycogen synthesis and inhibition of glycogenolysis. Although GH stimulated amino acid transport in postnatal skeletal muscle, GH had no effect on amino acid transport in the fetus, even at concentrations considerably greater than the half-maximal effective concentration of oPL. In addition, the potency of GH in stimulating glycogen synthesis in fetal hepatocytes was only about one tenth that of oPL. These studies indicate that placental lactogen has metabolic actions in the fetus and that GH has little or no metabolic activity in fetal tissues. Since the biologic actions of placental lactogen in the fetus are qualitatively similar to those of GH in postnatal animals, these studies further support a role for placental lactogen as a fetal "growth hormone."

The lack of somatotropic and metabolic activity of GH in the fetus is in accordance with other clinical and experimental observations suggesting that GH does not

play a central role in the regulation of fetal growth. For instance, a deficiency or absence of GH in the mammalian fetus does not limit fetal weight gain or linear growth *in utero* and does not reduce fetal plasma somatomedin concentrations. Furthermore, an excess of fetal GH, as noted in transgenic mice bearing metallothionein-hGH fusion genes, does not accelerate fetal growth.

In additional studies, oPL has also been observed to stimulate the activity of ornithine decarboxylase (ODC) in the fetal liver directly. Since ODC is the rate-limiting enzyme in the synthesis of the polyamines, a group of compounds that play a critical role in the regulation of protein and in nucleic acid metabolism, this finding further supports a direct role for placental lactogen in the regulation of fetal growth. In contrast, GH, which stimulates ODC activity in the postnatal liver with a potency identical to that of oPL, has no effect on fetal hepatic ODC activity.

OPL at physiologic concentrations has also been shown to stimulate an increase in dose-dependent, insulin-like growth factor-II (IGF-II) synthesis in fetal rat embryo fibroblasts, while GH and a variety of other hormones were found to have no effect. However, when tested in fibroblasts from postnatal rats, both oPL and GH stimulated the synthesis of IGF-I but not IGF-II. Since the plasma concentration of IGF-II in the fetal rat and lamb greatly exceeds that of IGF-I, these studies suggest that fetal growth may be regulated, in part, by oPL through its actions on the synthesis of fetal IGF-II. Additional support for a role of placental lactogen in fetal growth comes from recent studies indicating that hPL stimulates somatomedin production, DNA synthesis, and amino acid transport in human fibroblasts and myoblasts. The table summarizes the evidence supporting a role for placental lactogen.

**Table.** Evidence suggesting a direct role for placental lactogen in fetal growth and development

- Placental lactogen detected in human, ovine, and bovine fetal sera
- Distinct placental lactogen receptors demonstrable in fetal tissues
- Placental lactogen has anabolic effects on fetal amino acid and carbohydrate metabolism and stimulates somatomedin production in fetal tissues
- Low levels of hPL in cord blood may be associated with intrauterine growth retardation

Placental lactogen competes with GH and prolactin for binding to GH and prolactin receptors in mammalian postnatal tissues. Consequently, several investigators have suggested that the metabolic effects of placental lactogen may be mediated through binding to GH or prolactin receptors or both. However, the studies demonstrating marked differences in the potencies of oPL and ovine GH (oGH) in fetal tissues strongly suggest that there are distinct placental lactogen receptors in the fetus.

The presence of distinct receptors for placental lactogen in the fetus is further substantiated by more recent studies. Ontogenetic studies of the binding of placental lactogen and GH to hepatic membranes of fetal sheep indicate the presence of specific oPL binding sites as early as mid-gestation, with a marked increase in the number of oPL binding sites during the latter half of gestation. Specific binding for GH, on the other hand, does not appear until shortly after birth. Biochemical studies of the hepatic membrane receptors for oPL and oGH also indicate striking differences in the binding sites of the two hormones. oPL binds to a single binding site in fetal hepatic membranes; the binding site has an apparent molecular weight by SDS-PAGE of 38-43 kD. In contrast, neither GH nor prolactin binds to fetal liver membranes. In hepatic membranes from postnatal sheep, the GH receptor appears to be a complex of disulfide-linked subunits with apparent molecular weights of 53 and 118 kD. Thus, the GH receptor in the postnatal sheep liver is structurally distinct from the

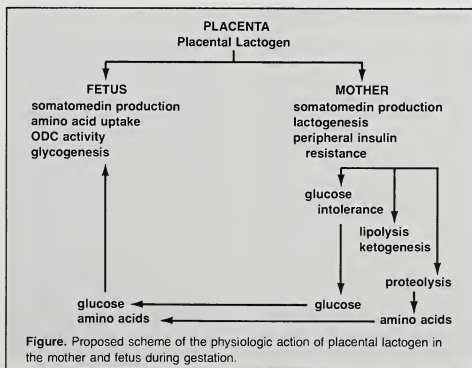
placental lactogen receptor in the fetal liver. These observations strongly suggest that a new and structurally distinct GH receptor appears soon after birth in the sheep.

In summary, placental lactogen appears to have numerous metabolic effects in the mother and fetus and these effects promote fetal growth (Figure). In the mother, placental lactogen induces insulin resistance and stimulates lipolysis and proteolysis, biologic effects that promote the transfer of glucose, amino acids, and to a lesser extent, fatty acids to the fetus. In the fetus, placental lactogen acts directly to stimulate ODC activity, somatomedin production, glycogen accumulation, and amino acid transport into cells. Since fetal tissues contain specific receptors for placental lactogen, and since the biologic actions of placental lac-

togen in fetal tissues occur at physiologic concentrations, the effects of placental lactogen on fetal growth appear to result from a concerted action of the hormone on both maternal and fetal tissues.

The observation that women with very low hPL concentrations resulting from a deletion of two of the three genes coding for hPL have normal pregnancies and give birth to normal-sized babies indicates that placental lactogen is not the only hormonal factor involved in the regulation of fetal growth. Since fetal growth, like postnatal growth, is undoubtedly controlled by many factors, the low hPL concentrations in the fetus may have resulted in compensatory changes in GH and other factors involved in somatomedin production. One possible explanation for normal fetal growth in pregnant women with gene deletions for hPL comes from the recent studies of Frankenne et al. These studies demonstrate that the placentas from these pregnancies may produce hPL-like or hGH-like molecules through expression of placental genes that are not expressed under normal conditions. It is possible that these hPL-like or hGH-like gene products may assume hPL-like roles in the mother or the fetus or both, sus-

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**Figure.** Proposed scheme of the physiologic action of placental lactogen in the mother and fetus during gestation.

## Human Placental Lactogen and Fetal Growth

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taining normal fetal growth and development during those pregnancies complicated by a deficiency or an absence of normal hPL production. It is also possible that low hPL concentrations during pregnancy may cause an up-regulation of hPL receptors or an increase in the affinity of the hPL receptor or both.

### Regulation of hPL Release

Because the chemical and biologic properties of placental lactogen are very similar to those of GH, initial investigations to delineate the factors regulating the release of hPL focused on factors known to regulate the release of GH. In these studies, hyperglycemia and insulin-induced hypoglycemia produced no consistent effects on plasma hPL concentrations, and growth hormone-releasing factor and somatostatin had no effect on hPL release. These results, therefore, strongly suggest that the regulation of placental lactogen re-

lease is different from that of GH.

Recently, an hPL-releasing factor that selectively stimulates the release of hPL has been partially purified from the serum of pregnant women. Chemical investigations to date indicate that the releasing factor is a protein with an apparent molecular weight of approximately 28,000. However, the amino acid composition and sequence of the protein and its site of origin are unknown. HPL release has also been shown to be stimulated by high-density lipoproteins (HDL). However, unlike the actions of HDL in other tissues, the stimulatory effect of HDL on placental lactogen release is not due to the lipid constituents of HDL but is due to apolipoproteins AI, AII, and CI. Since human placental tissue contains specific receptors for HDL, and plasma HDL concentrations increase progressively during pregnancy with a pattern similar to that of hPL, these studies strongly suggest a physiologic role for HDL in the regulation of hPL release.

IGF-I has also been recently demonstrated to stimulate hPL re-

lease from human placental explants. Since the human placenta synthesizes IGF-I and placental plasma membranes contain specific IGF-I receptors, it is possible that somatomedin may also be involved in the regulation of hPL release. These findings indicate a complex interaction of hormones, growth factors, and nutrients in the regulation of maternal metabolism, placental function, and fetal growth.

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# Creutzfeldt-Jakob Disease: Current Reports and Comments

Robert M. Blizard, M.D.

Chairman, Editorial Board

Growth, Genetics, and Hormones

There has been much concern since the association between Creutzfeldt-Jakob disease (CJD) and pituitary growth hormone (GH) was first reported. Two recent reports—"Human Growth Hormone Therapy and Creutzfeldt-Jakob Disease: A Drama in Three Acts" (Brown, P. *Pediatrics* 1988; 80) and "Iatrogenic Creutzfeldt-Jakob Disease" (Rappaport, E. *Neurology* 1987;37:1520)—deserve special comment.

Dr. Brown, author of the first report, is a virologist and epidemiologist who has worked at the NIH for many years with Dr. Carlton Gajusek and other pioneers who study kuru, CJD, and other brain diseases attributed to long-acting

viruses. Dr. Rappaport, a pediatric endocrinologist, wrote the second report. She became involved in CJD research while serving as a medical officer for the United States Food and Drug Administration.

Dr. Brown's article is derived from the Lawson Wilkins Lecture, which he delivered in the spring of 1987 at the Lawson Wilkins Pediatric Endocrine Society meeting. Dr. Brown records the events that prompted the termination of distribution of native growth hormone (GH), reviews epidemiologic studies that are currently under way as a follow-up to the initial report of three patients who received GH many years before their deaths from CJD in 1985, and reports four

additional cases of CJD—two abroad and two in the United States. He also reports two other patients who received native GH and who have a cerebellar syndrome, but whose histories and clinical courses suggest that their disease may be different from CJD, although the possibility of CJD cannot be ruled out completely.

There are, therefore, seven proven cases of CJD worldwide among patients who received GH many years ago (five in the United States, one in New Zealand, and one in England). Six have had clinical disease with pathological confirmation and the seventh had one brain lesion consistent with CJD

identified at autopsy, although the death was due to other causes. All except the British patient received pituitary GH prepared in the United States between 1966 and 1969. A review of laboratory records indicates that one batch of pituitary glands harvested in 1966 had found its way into at least one lot of the GH preparation given to the five American patients. However, this batch could not be incriminated as the cause of CJD in the patients from England and New Zealand. Priority has been given to a search for all recipients of the early lots of pituitary GH.

Studies in animals given injections of GH prepared from the early batches have not revealed deaths attributable to CJD but, unfortunately, negative results do not necessarily demonstrate freedom from contamination.

Dr. Brown believes that the possibility of a major epidemic of iatrogenic CJD seems less and less likely, and a reasonable guess is that the final tally will be perhaps 15 to 20 cases, with an occasional case reported over the next two decades. Epidemiologic studies are under way. The endocrinologic, social, psychologic, and medical data that will be obtained from them will go beyond data that are used for the prospective and retrospective study of the incidence of CJD.

In reply to an inquiry from me in late October, 1987, Dr. Brown said that there were no additional known cases of CJD and that the two patients with cerebellar syndrome were alive. He also stated that the epidemiologic studies are progressing and that the animal studies are continuing.

Dr. Rappaport focused on the transmission of CJD in her article. In addition to transmission via injections of GH, CJD has been inadvertently transmitted during surgical procedures, particularly those necessitating grafts of tissue, placement of electrodes in the brain, or both. In animals, CJD has been transmitted via the blood and urine of infected patients. Apparently, the infecting agent can be present in the blood and urine

of asymptomatic patients. If so, the risk of the iatrogenic spread of CJD will be proportional to the prevalence of potentially infectious people in the population. Dr. Rappaport calculates the possible prevalence in different ways, and depending on the assumptions used, the prevalence of the carrier state may be considerable.

On the basis of this possibility, Dr. Rappaport makes several recommendations:

(1) The medical community needs to be made aware of the potential infectivity of tissue and body fluids obtained from patients with CJD and of the potentially significant increase in the number of people who may be harboring the CJD pathogen.

(2) Hospitals should routinely review their sterilization procedures for surgical instruments and operating suites and their procedures for decontamination of the CJD pathogen. These should comply with current recommendations for inactivation of the CJD agent.

(3) Epidemiologic studies in patients exposed to native GH should include a tabulation of the surgical procedures performed before and after GH therapy.

(4) Consideration should be given to prohibiting recipients of pituitary-derived GH from donating blood, tissue, or organs.

(5) Regulations should be instituted to minimize the chance of manufacturing biologic products from the blood, tissue, or organs of individuals who may harbor the CJD pathogen.

Summaries of these articles are presented to update our readers about CJD. I asked Dr. Judith Fradkin to comment further. As Chief of the Division of Endocrinology and Metabolic Diseases, Program Branch, at the National Institute of Diabetes and Digestive and Kidney Diseases, she is responsible for coordinating many of the ongoing CJD studies that are sponsored by the NIH.

#### **Dr. Fradkin's comments:**

Clearly, recipients of pituitary GH are at increased risk for CJD. The extent of the risk will be better un-

derstood after the epidemiology study is complete. However, a comparison of the number of cases of CJD identified in GH recipients in the United States (five over several years) with the total number of cases of CJD in the United States (142 deaths in 1980) suggests that cases associated with GH administration will constitute a small fraction of the total number of cases of CJD in America. Thus, while there is the *potential* for a significant increase in the number of people harboring the CJD pathogen, current experience indicates that GH administration is unlikely to have increased the prevalence significantly. Because recipients of pituitary GH are at increased risk for CJD, and because there is no test to detect infection, and because CJD may be transmitted through blood or tissue, these individuals should not serve as blood or tissue donors. Patients and physicians have been notified of this recommendation, and blood banks will not collect blood from pituitary GH recipients. The routine precautions currently recommended to protect medical personnel from exposure to body fluids and tissues of patients with certain infectious disease should be sufficient to protect against the CJD pathogen and similar infectious agents. There are no additional special precautions for health care workers who treat recipients of pituitary GH. It is also reassuring that household contacts, including spouses of persons who have CJD, have not been at increased risk for infection. Thus, we do not recommend that a person who has received pituitary GH make any changes in social or family interaction.

The epidemiology study is well under way. Identification of the cohort that received pituitary GH through the National Hormone and Pituitary Program is nearly complete. The questionnaire to be administered has been developed under the guidance of an advisory panel. The study is designed to determine the incidence of CJD in the cohort and the association of CJD with specific lots of hormone;

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## Creutzfeldt-Jakob Disease:

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to identify other possible adverse outcomes associated with GH deficiency or therapy; and to determine the long-term outcome of this cohort in terms of pituitary function, psychosocial development, and final height. The telephone survey will begin early in 1988 and

will be completed by the end of the year. Medical records and available neuropathologic specimens are being obtained and reviewed for all deceased patients who were identified through the National Death Index and subsequently through the survey. No cases of CJD have been identified through the epidemiology study other than the five already described.

**Editor's note:** A fact sheet on human growth hormone and CJD and a reference list of the scientific literature in this area may be requested from:

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National Institute of Diabetes and Digestive and Kidney Diseases  
Building 31, Room 9A04  
Bethesda, Maryland 20892

## Special Report: International Turner Syndrome Symposium— November 9-11, 1987, San Francisco, California

Robert M. Blizzard, M.D.

*Chairman, Editorial Board*

and Judith G. Hall, M.D.

*Associate Editor*

*Growth, Genetics, and Hormones*

Several aspects of Turner syndrome—including its natural history, therapy of short stature, and intellectual and psychosocial development—were discussed at this five-session symposium.

During one session on the genetics, organogenesis, and incidence of Turner syndrome, much attention was directed to the genes found on the X and Y chromosomes. Portions of that session are highlighted in this report.

Dr. L. Shapiro (University of California, Los Angeles) discussed an area of complete homology on the tips of the p (or short) arm of the X and Y chromosomes. This region, called the "pseudoautosomal region," escapes inactivation in the inactivated X chromosome. Currently, two functional genes are known to exist in this region: a steroid sulfatase gene in mice and a MIC 2 (surface antigen) in human beings. The pseudoautosomal region has 5-7 megabases. There is room for several hundred as yet undefined genes in this region.

Dr. C. Epstein (University of California, San Francisco) emphasized that aneuploidy is a disorder of gene dose and that gene dosage effects may perturb structure, function, or both. The Y chromosome carries a gene or genes for sex determination and prevents Turner syndrome. The "inactive" X chromosome does not have inac-

tivation of the pseudoautosomal region. Regions of the X chromosome appear to have an oocyte-maintaining function, as there are families whose members have premature menopause associated with minor deletions of the X chromosome. Dr. Epstein suggested that the Y chromosome be inspected for genes that affect somatic development because the Y chromosome is much easier to study than the X in many respects. He described two phenotypic females (karyotype 46, XY) with lymphedema who had minor deletions of the Yp.

The intrauterine mortality of 45, X fetuses is 95% to 99%, but death does not necessarily result from fetal defects; however, death could occur because of an abnormal placental karyotype. Interestingly, the paternal X is lost more frequently than the maternal X in Turner syndrome, and the paternally derived X is more likely to be the inactivated X in 46, XX individuals.

Dr. D. Page (Massachusetts Institute of Technology) hypothesized that a single gene on the Y chromosome, known as the gene for testicular differentiating factor (TDF), is sex determining. Its locus is on the p arm just proximal to the pseudoautosomal region. The question was raised as to whether a similar region is also present on the X chromosome. If the TDF gene is present on both the X and Y chromosomes, testicular differentiation alternatively could be an effect of gene dosage and not necessarily due to one gene on the

Y chromosome. Of 26 XX males studied, 24 had Y material on the X chromosome. These 24 may have had XY interchange at paternal meiosis of the sperm.

Among the 155 46,XY individuals with Y deletions who were studied, there were nine subgroups based on mapping of the missing loci on the Yp. Of the 24 XX males with Y material, all had Y region 1A2 present. This region, which appears to be critical for male differentiation, was "lost" in 46, XY females. The TDF gene is not the gene for the HY antigen since the two map to different areas of the Y chromosome; HY antigen maps to the 4B region of Yp. The TDF does not code for a hormone but rather for a DNA-binding protein. Dr. Page postulated that the two XX males without identifiable Y material, of the 26 XX males studied, may have a mutation of another gene, possibly an autosome.

An extension of this type of study was reported by Dr. C. Disteche (University of Washington), who used cytogenetic assays and DNA probes to study XY females who were not short. A deletion of the TDF loci area was identified. The area on Yp, which is believed to account for the short stature seen in Turner syndrome, is therefore not the TDF loci.

Dr. Disteche also discussed the seven regions on the Y chromosome. Region 7, a long segment at the distal end of the long arm, is brightly fluorescent. Its deletion does not produce significant problems, although two patients

with a small Y had small teeth and petite bones. Stature was unaffected. The development of gonadoblastoma in those patients with Turner syndrome who have a partial Y chromosome(s) could result from a normal Y gene that continues to function in the absence of normal testicular tissue. It could also be caused by a defective gene on the Y chromosome, or it might be secondary to tissue disorganization.

Dr. S. Ohno (Loma Linda, California) led a discussion about homologies between the testis and the ovary. Spermatogonia are homologous with oogonia, Sertoli cells with granulosa cells, and Leydig cells with thecal cells. He then described a species of fish whose gonads can change from ovary to testis during adulthood. There is always one male in the school of fish. If the male dies or disappears, the dominant female becomes the male for the school by differentiation of the ovary into a testis.

The HY antigen (HY Ag) was discussed by several symposium participants. Three assays for HY Ag exist: (1) transplant rejection, (2) cell mediated cytotoxicity, and (3) serological. The latter assay employs a male-specific polypeptide antigen, 18-20 D in size, that is present in body fluids and expressed in Sertoli and Leydig cells. Dr. Page tried to clarify the issues: HY Ag was initially defined as a transplantation-rejection antigen. T-cell assays recognize transplant Ag. There is no evidence that the serological Ag is

the same HY Ag that is identified by the other two assays. The conclusion was that the nomenclature regarding HY antigens is totally inadequate. The result is that we are often talking about different things when we discuss Hy Ag.

Dr. C. Lau (University of California, San Francisco) extended the discussion, stating that the serological HY Ag in humans is a glycoprotein of 185 amino acids; it is found in both males and females. The gene is not localized on the Y chromosome but on chromosome 6. The Hy Ag gene identified by the other two assays is located on the Y chromosome.

Dr. J.L. Simpson (Memphis, Tennessee) postulated that (1) the X and Y chromosomes are not solely responsible for gonadal differentiation; (2) X and Y determining factors are solely regulatory; (3) the structural loci for gonads are on autosomes; and (4) X and Y gonadal determining genes are identical and the different effects result because of a dosage effect produced by X inactivation in the female. The latter postulate would be theoretically possible and consistent, in part, with the concepts presented by Dr. Page. For example, the female would have half the gene dosage for TDF as the male since one X is inactivated.

Dr. Simpson reported that more than 12 45,XO patients have become pregnant. He emphasized that oocytes can develop in testicular tissue, and gonadal stroma may therefore play a role in gonadal differentiation, at least in cer-

tain instances. The existence of 46,XY individuals with female phenotype or ambiguity as part of the gonado-palato-cardiac syndrome supports this concept. The gonads in persons with this syndrome have been variously reported to be streaks, ovaries, and testes.

Primary amenorrhea resulting from deletions of the short arm and from small deletions of Xq 11.2-11.4 was also reported. Deletions at Xq 13 may be associated with secondary amenorrhea. A gene or genes in that region would appear to be associated with ovarian maintenance and thus prevent premature menopause. Individuals with deletions between or including Xq 21-25 have minimal abnormalities (although they are not necessarily normal) and frequently have secondary amenorrhea.

Dr. Judith Hall (University of British Columbia) said that many of the phenotypic defects observed in Turner syndrome may be secondary to intrauterine lymphedema and pressure phenomena resulting from this edema. Even coarctation of the aorta could be secondary to this phenomenon. She states that the dysmorphology in Noonan's syndrome might be related to intrauterine lymphedema. This concept, if true, would make it unproductive to look for specific genes responsible for each phenotypic feature.

The data presented during this session add greatly to our understanding of Turner syndrome.

## Special Report: The March of Dimes Clinical Genetics Conference— July 19-22, 1987, Minneapolis, Minnesota

Judith G. Hall, M.D.  
*Associate Editor*  
*Growth, Genetics, and Hormones*

The theme of this conference was "disorders of the neural crest and craniofacial skeleton." Thus, neural crest development and the possible effects of teratogens upon it were reviewed. Clearly, neural crest cells in the cranial area are more diversified in their

actions than neural crest cells in the trunk.

A number of new experimental techniques permit identification of neural crest cells and observation of their migratory patterns in experimental animals. In the craniofacial area, neural crest cells or ectomesenchymal tissue separate from the neural tube laterally and migrate within the mesoderm to form somitomeres, which are

segmental areas within the cranial region. The neural crest cells provide muscle and bone, as well as endothelial cells for angiogenesis, in the cranial area. In the trunk, neural crest cells play a role primarily in pigmentation and in development of the autonomic nervous system.

Utilization of a Nile blue stain to indicate areas of necrosis in an embryo permits the suggestion

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**The March of Dimes  
Clinical Genetics Conference**  
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that there are large areas of cell death that are programmed as part of normal embryological development. These areas that are programmed for cell death seem to be accentuated by teratogens,

appearing earlier and resolving later than they normally would.

It was clear from the presentations that simple neurocristopathies, which are associated with single defects, should be distinguished from complex neurocristopathies, which are characterized by structural anomalies and/or ongoing problems, such as dysplasia and overgrowth.

A number of new craniofacial syndromes were described, but the classification of neural crest disorders is still very difficult and complex. New imaging techniques, such as magnetic resonance imaging and stereoradiophotogrammetry, are improving our abilities to diagnose and describe the natural history of already described conditions.

**Special Report: 26th Annual Meeting of the European Society for Pediatric Endocrinology (ESPE)—September 6-8, Toulouse, France**

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The presentations at the annual meeting of the ESPE reflect the broad spectrum of interests of ESPE members, who come from many countries, each with different administrative structures and facilities for clinical and basic research in pediatric endocrinology. The main topics of ESPE meetings are therefore aimed at reviewing current developments for a broader audience and at informing selected audiences about recent advances in endocrine research. This year's plenary lectures were devoted to pediatric aspects of endocrine autoimmunity, regulation of growth hormone (GH) secretion by growth-hormone-releasing hormone (GHRH) and somatotropin-release-inhibition hormone (SRIH), and the cDNA of the human insulin receptor and its potential pathologic expression.

Dr. H. A. Drexhage reviewed the more conventional disorders of endocrine autoimmunity. The primary purpose was to point out the contrasting effects of autoimmune reactions, which may stimulate endocrine tissues but may also lead to their destruction. The basic principles of these reactions and the methodologies used to evaluate them have been derived from investigations of disorders of the thyroid gland. Recently, however, a variety of other disorders that mimic classical endocrine disorders but also have an auto-

immunologic pathogenesis have been discovered. Examples are pigmented adrenocortical micronodular dysplasia (PAMD) and precocious puberty. Dr. Drexhage's reference to the high incidence of transplacental passage of thyroid-growth-blocking antibodies as a cause of thyroid agenesis (or ectopia)—a mechanism suggested many years ago by Dr. R. Blizzard—was also supported by a report of Grüters et al (Berlin, West Germany). These investigators found cytotoxic thyroid autoantibodies in 32% (12 of 37) of newborns with hypothyroidism. Dr. Drexhage left his audience with the impression that more advanced techniques to quantitate autoimmune processes would make this field one of the most important areas to investigate in endocrinology.

Dr. W. Wehrenberg summarized what is currently known about GH regulation by GHRH and SRIH. Numerous clinical and experimental investigations are under way in this area, and new data emerge almost daily. Although SRIH appears to play a dynamic role in controlling GH secretion, the pulsatility of GH secretion does not appear to be determined solely by SRIH. Because a multitude of factors influence GH secretion, the two prominent hypothalamic hormones obviously cannot explain all the phenomena of GH secretion that are observed in various clinical states. There are also indications that GHRH exerts a negative effect on GH secretion by reducing GHRH receptors at the

pituitary level. Dr. Wehrenberg also reported a remarkable finding from his work on prenatal growth control: Administration of GHRH antibody to pregnant rats results in smaller offspring.

Two sessions dealt with Cushing syndrome and neonatal hyperinsulinism. Since both disorders are relatively rare in childhood, clinical experience with children with these disorders is still limited. A multicenter approach to standardize modes of diagnosis and treatment was advocated for neonatal hyperinsulinism, a condition whose prognosis is essentially determined by effective prevention of hypoglycemic states. Dr. A. Aynsley-Green pointed out that glucose, diazoxide therapy, and surgery are still the most important modalities in the management of neonatal hyperinsulinism. Based on his experience in cases of disseminated  $\beta$ -cell hyperplasia, Dr. Aynsley-Green advocated a subtotal pancreatectomy (about 95%) to prevent recurrences, which are observed frequently after less radical surgery.

A major segment of the meeting focused on problems related to the potential of GH to improve growth in growth disorders other than "classical" GH deficiency (GHD). Rather than provide solutions to these problems, investigators presenting papers attempted to put the confusion regarding GH measurement into perspective. Several presentations were devoted to GH testing and measurement of spontaneous GH secretion. With respect to spontaneous

GH measurements, for example, there is a diversity of blood-sampling methods and wide variability in approaches to evaluation of the data obtained by these methods. The critical observer was left with the impression that standardization of GH measurements and other parameters

among investigators is badly needed to ensure consistency in reporting data. Similarly, trials evaluating treatment of different growth disorders with GH and GHRH need to be conducted with great care and more patients to validate results. Similar standardization problems appear to be

prevalent in various studies evaluating the treatment of precocious puberty with luteinizing-hormone-releasing-hormone analogues.

The next ESPE meeting will be held in Copenhagen in June, 1988. It will focus on the testis and endocrine problems associated with malignancies.

## **Special Report: 8th Annual Workshop on Malformation and Morphogenesis—August 15-19, 1987, Greenville, South Carolina**

Judith G. Hall, M.D.  
*Associate Editor*  
*Growth, Genetics, and Hormones*

Several reports on a variety of congenital defects and inherited syndromes in animals and humans were presented at this workshop. Dr. W. Webster (Sydney, Australia) reported that handling the uterus of the pregnant rat during surgery, or for nonsurgical reasons, can lead to limb defects and central nervous system damage compatible with vascular compromise. This may have clinical implications, although there is no evidence that the same sort of anomalies are seen with manipulation of the human uterus during pregnancy.

Dr. C. Stevens (University of Utah) described a carefully done study of the development of embryonic and fetal palmar and digital creases. Finger creases are well-defined by nine weeks and palmar creases by 12 weeks. These observations are important for timing various effects on limb development.

Dr. S. Clarren (University of Washington) described a carefully controlled experiment assessing "binge drinking" in monkeys. Large doses of alcohol (2.5-4.1 g/kg) given during the first week of pregnancy (and on a weekly basis thereafter) can have a significant impact on the fetal development of the monkey in terms of behavior and cognitive developmental delay measured after birth.

Dr. J. Cordero (Atlanta, Georgia) discussed research that suggested the use of multivitamins before conception may reduce the

risk for neural tube defects and possibly for other congenital anomalies.

Excellent studies by Dr. S. Cassidy (University of Connecticut) on Prader-Willi syndrome and Dr. C. Morris (Phoenix, Arizona) on Williams syndrome provided data that allow much better definition of the natural history of these conditions. The studies also suggest that many features thought to be part of the syndromes—such as obesity in Prader-Willi syndrome and behavior in Williams syndrome—can be modified. Long-term follow-up of patients with Weaver syndrome and Robinow syndrome was discussed by their namesakes.

Several reports suggested the possibility that many disorders with patchy areas of dysplasia (such as the McCune-Albright and Proteus syndromes) may represent somatic mosaicism due to single gene changes and chromosome changes.

Dr. K. Jones (University of California, San Diego) described research in which it was demon-

strated that the supraorbital ridge has a role in inducing the contour of the eyebrow. Individuals with aberrant supraorbital ridges will have aberrant placement of the eyebrows.

Dr. P. Duncan (Downstate Medical Center, Brooklyn, New York) presented an analysis of a family with three generations of Russell-Silver syndrome, a condition that is usually nonfamilial.

Reporting on Joubert syndrome, Dr. D. Flannery (Medical College of Georgia) showed a videotape that visually demonstrated the functional changes that occur in affected patients. These changes are sometimes hard to describe, but recognizing them is essential for an accurate diagnosis. The pattern of respiration in Joubert syndrome is quite striking, with episodic hyperpnea and abnormal eye movements. CAT scans of patients with these breathing and movement patterns may demonstrate the aplasia of the cerebellar vermis associated with Joubert syndrome.

### **In Future Issues**

**Osteogenesis Imperfecta: An Update**  
by Peter Byers, M.D.

**Lipodystrophy**  
by William L. Clarke, M.D.

**Medical Complications of the Skeletal Dysplasias**  
by Judith G. Hall, M.D. and  
David L. Rimoin, M.D., Ph.D.

Please send all correspondence to Robert M. Blizzard, M.D.,  
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Charlottesville, VA 22908.



## Statural Development Parallels IGF-I Levels in Subjects of Constitutionally Variant Stature

Serum levels of insulin-like growth factor I (IGF-I) were estimated in 92 children with stature greater than 2 SD above normal for age (tall) and in 109 with stature less than 2 SD below normal for age (short). A protein-binding assay was used on acid gel-filtrate serum. In this assay, IGF-II has half the displacing capacity of IGF-I.

The level of IGF-I increases as age increases. However, for a given bone age (Greulich-Pyle), IGF-I levels are significantly higher in tall than in short children (over the bone age range 2 to 16 years). Also, for a given pubertal stage (Tanner), tall children have significantly higher IGF-I levels in P2, P3, and P4-5 (definition of P not given). At completion of growth, tall children had significantly higher IGF than short children.

The authors conclude that differences in the secretion rate of IGF-I may be associated with differences in stature.

Binoux M, Gourmelen M. *Acta Endocrinol* 1987;144:254.

**Editor's comment**—The question of whether tall people have greater

growth hormone secretion or IGF-I production than short people, or both, is unresolved. Although this article speaks in favor of the association between high IGF-I levels and tall stature, the degree of association is quite small. The most that can be said is that the difference in secretion rate (already a jump from serum level) is only a relatively minor factor among the perhaps multiple causes of tallness or shortness. At each stage of puberty, for example, there is overlap between the distributions, although there is also separation of the means. One of the authors' graphs shows that within neither the tall nor the short group is there a significant relationship between growth rate for age and IGF-I for age; only when these two extreme groups are pooled does a correlation emerge, which is therefore an overestimate of the true population value. It may be, of course, that larger people produce a little more IGF on average than smaller ones, but they do not clear it from the blood relatively faster. It is unclear how cartilage levels of IGF-I relate to blood levels. Even if we take this association between serum IGF and height at its face value, it is clear that the main cause of human stature differences resides elsewhere.

James M. Tanner, M.D., D.Sc.

## The Effect of Exercise on Plasma Somatomedin-C/Insulin-like Growth Factor-I Concentrations

Nutritional status is well known to be an important determinant of plasma somatomedin-C/insulin-like growth factor (Sm-C/IGF) levels, and reductions are observed in malnutrition and during prolonged fasting. Previous studies have suggested that both dietary protein and energy intake modulate Sm-C concentrations. The present study was undertaken to determine whether an energy deficit induced by vigorous exercise is associated with a reduction in Sm-C.

Six healthy, exercise-conditioned males were fed a constant diet and were exercised, and they expended 14.1-16.3 kcal/kg/day. Their plasma Sm-C concentrations declined significantly during the last two days of the seven-day exercise period.

After three days of re-acclimation, the subjects had their calorie intakes reduced by the same number of calories that had previously been expended in the form of exercise. Once again, a fall in Sm-C concentrations was

## A Longitudinal Study of the Relationship of Plasma Somatomedin-C Concentration in the Pubertal Growth Spurt

Beginning at puberty, 12 boys and eight girls underwent yearly measurements of plasma somatomedin-C (Sm-C) levels. For each subject, age at peak height velocity (PHV) was determined and the Sm-C levels plotted in relation to years before and years after PHV. Not all subjects were followed for the entire study period: There were 19 subjects at PHV and PHV + 1

year, 15 at PHV-1, 16 at PHV + 2, 11 at PHV - 2, and ten at PHV + 3. Sm-C levels were determined by radioimmunoassay (control adult value, 1.0 U/mL).

Sm-C levels rose sharply in parallel with the increase in height velocity, but the peak Sm-C level occurred one year after PHV, averaging 3.5 U/mL in both boys and girls. The minimum peak value was 1.5 U/mL. After the peak level occurred, the decline was very slow, and quite unlike the decline in height velocity. By PHV + 3 the Sm-C level still averaged about 2.8 U/mL. Therefore, plasma Sm-C

levels correlate with height velocity only in early puberty.

Cara JF, Rosenfield RL, Furlanetto RW. *Am J Dis Child* 1987;141:562.

**Editor's comment**—This paper is a good example of how the correct use of auxological methods enables the extraction of important conclusions from a small study. Although the use of years before and after PHV as a scale of developmental age was initiated in the 1930s, it is still far from universal.

The authors' result is striking: The Sm-C curve looks more like

noted, and the magnitude of this fall was not different from that observed during exercise. Nitrogen balance, measured daily throughout the studies, averaged  $-1.6 \pm 1.7$  g/day during the last three days of exercise but  $-3.5 \pm 1.73$  g/day during dietary restriction.

According to the authors, these results demonstrate that strenuous exercise produces a negative calorie balance and results in a fall in Sm-C concentrations equivalent to those seen in caloric restriction without exercise. However, exercise appears to have a nitrogen-sparing effect. This observed nitrogen-sparing effect with exercise could not be explained by the study design.

Smith AT, Clemons DR, Underwood LE, et al. *Metabolism* 1987; 36:533-537.

**Editor's comment**—This study suggests that children who exercise strenuously and limit their calorie intake may have reduced plasma Sm-C concentrations, which could lead to attenuation of linear growth. All care should be taken to make certain that children who are involved in strenuous athletic training have an adequate intake of nutrients.

William L. Clarke, M.D.

the testosterone curve than that of any growth variable. When does the Sm-C level eventually drop to the adult value? And why? The authors suggest that the high levels seen several years after PHV may reflect bone and muscle growth, which continues somewhat after PHV. By PHV + 3, however, there is little additional lean body mass to come. Clearly, more extended longitudinal studies are needed before we can grasp the mysterious relationship between plasma Sm-C levels and growth rate.

James M. Tanner, M.D., D.Sc.

## Impaired Response of GHRH Measured in Plasma After L-Dopa Stimulation in Patients With Idiopathic Delayed Puberty

The advent of an assay to measure growth-hormone-releasing hormone (GHRH) permitted the authors of this report to compare GHRH and growth hormone (GH) levels in two groups of patients: 16 with idiopathic delayed puberty (IDP) or constitutional delay of growth, and 12 with what the authors call constitutional short stature, which is synonymous with genetic short stature. All patients were in stage 1 or early stage 2 of sexual development. The mean GH values after stimulation with L-Dopa were  $8.6 \pm 1.4$  ng/mL (SEM) in those with IDP and  $12.0 \pm 0.8$  ng/mL in those with genetic short stature. The difference was not statistically significant. The GHRH values were  $41.0 \pm 10$  pg/mL in the IDP subjects and  $96 \pm 25$  pg/mL in those with genetic short stature ( $P < 0.02$ ). Five patients with IDP and the most severe delay in growth velocity (more than 2 SD below the bone age growth

velocity) had a mean value of 17.3 pg/mL. Values were  $75.0 \pm 14.5$  pg/mL for those with normal growth velocity.

The authors state that these data suggest a hypothalamic dysfunction in patients with IDP.

Argente JD, Evain Brian D, Donnadieu M et al. *Acta Paediatr Scand* 1987;76:260.

**Editor's comment**—The conclusions of the authors are logical. Although the difference in GH values in the two groups was not statistically significant, a closer look at the range of GH values (presented in a graph) indicates that seven of the 16 patients with IDP had values lower than any of those obtained from the 12 patients with genetic short stature. These data would suggest that there is a decreased peak of GH secondary to L-Dopa stimulation in patients with IDP. Unfortunately, the authors do not correlate the GHRH and peak GH values for each patient, nor do they give or refer to data pertaining to total GH output following L-Dopa administration. Nevertheless, the data do support the concept presented.

Robert M. Blizzard, M.D.

## Knemometry in Assessment of Linear Growth

Four health care professionals, one an auxologist, the others doctors or nurses, measured 18 children aged 5.5 to 10.5 years every month between 5 PM and 8 PM for six months. Each child was measured sequentially and independently by two observers at each measuring session, during which single measurements of stature and sitting height and six measurements of lower leg length (LLL) were taken. Children were asked to walk a few steps between each of the LLL measurements. In a second series, four LLL measurements were made weekly for six weeks on six children.

The coefficients of variation of the six sets of measurements in the first series averaged 0.10%, with the highest being 0.25%. The coefficients of variation of the four sets of measurements in the second series had a highest value of 0.18%. Thus, four measurements, with the most aberrant deleted in determining the average, are as good as six, and are recommended in assessing linear growth.

Although there were significant intra-observer differences in LLL (due to differences in initial positioning of the child), there were no such differences in LLL increments. However, the stature measurements made by the auxologist were considerably more reliable than those made by the doctors

continued on page 14

### **Knemometry In Assessment of Linear Growth continued from page 13**

and nurses. When lines were fitted to the monthly stature values for each child, the SD of the points around the line was only half as large for the auxologist's measurements as for the measurements made by the others.

The velocities over the six months (obtained by linear fit for LLL and stature) correlated poorly, ranging from 0.32 in nine children measured by a doctor or nurse to 0.76 in 11 children measured by the auxologist. The agreement on these fitting velocities between observers was no better for LLL than for stature.

The values of growth velocity seen at six months were very badly predicted by values seen at one month, with the average deviation about the prediction being no less than 50%. Deviations in predictions from values seen at two and three months were about 25% and 17%, respectively. The deviations were nearly the same for LLL and stature.

In the second series, in which measurements were taken weekly, significant week-to-week variations in LLL velocity were seen. These variations were sometimes, but not always, associated with transient illness. Six children measured for three weeks before and three weeks after tonsillectomy had decreased LLL velocity for two weeks, followed by catch-up growth.

Wales JKH and Milner RDG. *Arch Dis Child* 1987;62:166.

**Editor's comment**—*This is a very important paper on knemometry because the design is comprehensive and the results clear-cut. The authors summarize their conclusions by saying that velocities seen at six months cannot be usefully predicted by velocities measured at one, two, and even three months. LLL can be measured ac-*

*curately, as claimed by its originator, but LLL growth rates vary from month to month and week to week. Thus, the authors suggest that similar discrepancies in stature are due to inherent inaccuracy of the technique rather than to inherent variation. However, this does not necessarily follow from their results. If they are correct, then stature would definitely be a more accurate index for short-term prediction of longer-term measurements, for it has to be said that the physicians and nurses taking the measurements in these studies were not very accurate. Their maximum intra-observer difference of 1.6 cm can turn up occasionally, but their standard deviation of intra-observer differences averaged 0.5 cm, which means that*

*values approaching 1 cm were not that infrequent. We need to evaluate stature measurements as long-term predictors, but the measurements must be highly accurate and the design similar to that for LLL. My clinical impression (supported by an analysis of my data) is that the inherent variability of short-term velocities for stature, though perhaps less than LLL variability, precludes using one-, two-, and three-month rates as useful predictors for six-month or 12-month ones. Dr. Michael Hermanussen, who has had much experience with the knemometer, makes the same point in a letter [Arch Dis Child 1987;62:763] supporting the findings of Wales and Milner discussed here.*

James M. Tanner, M.D., D.Sc.

### **Fragile X Syndrome**

Fragile X syndrome is a common cause of mental retardation. Since it is almost always familial, recognition of the syndrome is important for the whole family. Affected boys tend to be large, with large heads and ears, and they are occasionally confused with patients who have Soto's syndrome. Typically, they have long faces and macroorchidism, although these features may not be present until after puberty. About 20% of patients have hyperextensible joints, mitral valve prolapse, and pectus excavatum.

A particularly distressing feature of the Fragile X syndrome is that a large number of males exhibit autistic behavior and many have repetitive hand movements. Hyperactivity may be present prior to puberty and can be very distracting. Cluttered speech, including a rapid rate of disfluency, stuttering, and perseveration, is typical.

About one third of girls who are carriers of the gene for Fragile X syndrome also manifest the syndrome's clinical features. Recent work suggests that about one in 1,500 males and one in 750 females carry the abnormal gene. About 4% of cases of severe men-

tal retardation among males can be attributed to Fragile X syndrome, although males may transmit the abnormal gene without being affected themselves.

Investigators working at the molecular genetic level have demonstrated a number of linked markers on the X chromosome. Therefore, prenatal diagnosis of the Fragile X syndrome may be possible with the aid of cytogenetic and linkage studies.

Chudley AE, Hagerman RJ: *J Pediatr* 1987;110:821.

**Editor's comment**—*Because of its genetic implications, clinicians must be aware of this very important syndrome. A major effort is being made to isolate the gene, which would improve the accuracy of linkage studies and provide better understanding of the pathogenesis.*

Judith G. Hall, M.D.

Please send all correspondence to Robert M. Blizzard, M.D., Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908.

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Lawson Wilkins—Pioneer in Pediatric Endocrinology and Growth Disorders"

Robert M. Blizzard, M.D.

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The David Smith Workshop on Malformations and Morphogenesis—August 1986, Burlington, Vermont

25th Annual Meeting of the European Society for Pediatric Endocrinology—August 31-September 3, 1986, Zurich, Switzerland

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The Origin of 45,XO Males

Treatment of Duchenne's Muscular Dystrophy With Growth Hormone Inhibitors

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Constitutional Delay of Growth and Adolescent Development

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Formation of Pediatric Endocrinology Nursing Society (PENS)



## MEETING CALENDAR

**May 2-6** Annual Meeting of the American Pediatric Society/Society for Pediatric Research. Sheraton Hotel, Washington, DC. Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2350 Alamo SE, Suite 106, Albuquerque, NM 87106 (505-764-9099)

**May 6** Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Sheraton Hotel, Washington, DC. Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2350 Alamo SE, Suite 106, Albuquerque, NM 87106 (505-764-9099)

**May 9-13** Clinical Disorders of Bone and Mineral Metabolism. Detroit, Michigan. Sponsor: Henry Ford Bone and Joint Specialty Center. Contact: Henry Ford Hospital, Continuing Medical Education, 2799 West Grand Boulevard, Detroit, MI 48202 (800-662-8242, within Michigan; 800-521-7946, other states and international)

**June 8-10** 70th Annual Meeting of The Endocrine Society. New Orleans, Louisiana. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**July 8-10** International Symposium on the Marfan Syndrome. Baltimore, Maryland. Contact: Diane Heydinger, Johns Hopkins University School of Medicine, 720 Rutland Road, Baltimore, MD 21205 (301-955-2959)

**July 10-13** 20th Anniversary of the Clinical Genetics Conference. Baltimore, Maryland. Contact: Carlita Kearney, Johns Hopkins University School of Medicine, 720 Rutland Road, Baltimore, MD 21205 (301-955-2959)

**July 20-23** 15th International Auxology Congress. Exeter University, Exeter, England. Contact: Prof. J.M. Tanner, Room 115, Institute of Child Health, 30 Guilford Street, London, England WC1N 1EH

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# GROWTH

## Genetics & Hormones

Vol. 4 No. 2

June 1988

### Osteogenesis Imperfecta: An Update

Peter H. Byers, M.D.  
*Professor of Pathology  
Departments of Pathology and  
Medicine (Medical Genetics)  
Center for Inherited Disease  
University of Washington  
Seattle, Washington*

For most families, the birth of a child with osteogenesis imperfecta (OI) raises three questions: How will my child do? Will it happen again? Why my family?

The clinical, biochemical, and genetic approaches taken during the past several years to understanding OI are beginning to provide answers to these questions. We now know something about the natural history of each of the phenotypes observed with OI, and we know a little about the molecular basis and heterogeneity of the disease(s). Thus, we can predict (and prevent) recurrence, even though we understand relatively little about why it occurs.

Attempts to develop a classification of OI were made as soon as heterogeneity was recognized. The efforts had two goals: to determine the natural history of OI and to identify families at risk for recurrence. No classification has yet achieved these goals. Because of the dynamic nature of the disease, the occasionally marked intrafamilial variability, and evidence of recurrence due to germline mosaicism for dominant mutations, no classification system will succeed completely.

The classification that most successfully meets the goals, and is generally used by geneticists, was developed by Silience and his colleagues. Four types of OI were distinguished on the basis of clinical presentation, radiologic criteria, and mode of inheritance:

(1) OI type I, a dominantly inherited variety with blue sclerae, bone fragility, and normal or near normal stature;

(2) OI type II, a form that is lethal in the perinatal period and was thought to be inherited in an autosomal recessive fashion but is now known to result from new dominant mutations;

(3) OI type III, a severe, progressively deforming form first thought to be inherited in an autosomal recessive fashion but now recognized to be genetically heterozygous;

(4) OI type IV, a dominantly inherited form with normal sclerae, mild to moderate (but variable) short stature, and mild to moderate deformity. Additional clinical, biochemical, and molecular genetic studies have expanded and clarified the initial classification (Table).

To put the current biochemical and genetic approaches in appropriate perspective, one must understand the structure and synthesis of type I procollagen.

#### Biosynthesis and Structure of Type I Procollagen

Type I procollagen, the precursor of type I collagen found in tissues, is a heterotrimer that contains two identical  $\alpha 1(I)$  chains and a single  $\alpha 2(I)$  chain. The genes that encode the two proteins are members of a multigene family (the collagens) and are located on chromosome 7 (where COL1A2 encodes the  $\alpha 2(I)$  chain) and chromosome 17 (where COL1A1 encodes the  $\alpha 1(I)$  chain). The genes are complex, contain more than 50 exons each, and are transcribed, spliced, and processed in the nucleus. The mature messenger RNA (mRNA) is transported to the rough endoplasmic reticulum and translated on membrane-bound ribosomes. Although each gene is present in a single copy, they are differentially transcribed so that the steady-state mRNA levels reflect the 2:1 chain ratio found in normal molecules.

The  $\alpha$  chains have several major domains (Figure), an amino terminal globular domain, a long triple-helical segment characterized by the repeating amino acid triplet sequence of (Gly-X-Y)<sub>338</sub>, and a globular carboxyl-terminal

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## Osteogenesis Imperfecta: An Update

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extension. During chain elongation, most prolyl residues in the Y position (about 100 per chain) of the triple helix are hydroxylated, some lysyl residues are hydroxylated, the rare hydroxyllysyl residue is glycosylated, and heterosaccharide is added to a single residue in the carboxyl-terminal propeptide extension.

Assembly of chains into molecules begins once chain synthesis is terminated through domains located in the carboxyl-terminal propeptide extension and triple helix propagates from the carboxyl-terminal end toward the amino terminus. Glycine in every third position is necessary for triple helix propagation and the structure is stabilized by interchain hydrogen bonds. Hydroxyproline is also important for thermal stability.

Post-translational modification of the triple-helical domain is terminated with the formation of a stable triple helix. The intact molecules are transported to the Golgi apparatus, packed into secretory vesicles that fuse with the cell membrane, and discharged into the extracellular space. Outside the cell, the amino-terminal and carboxyl-terminal propeptides are removed and collagen molecules self-assemble into fibrils that are stabilized by lysine-derived covalent cross links.

### Osteogenesis Imperfecta Type I

OI type I is characterized by blue sclerae, normal or near normal stature, and autosomal dominant inheritance. Early studies indicated that individuals with OI type I had less type I collagen (compared with type III collagen) in skin than did normals. Decreased production of type I procollagen by

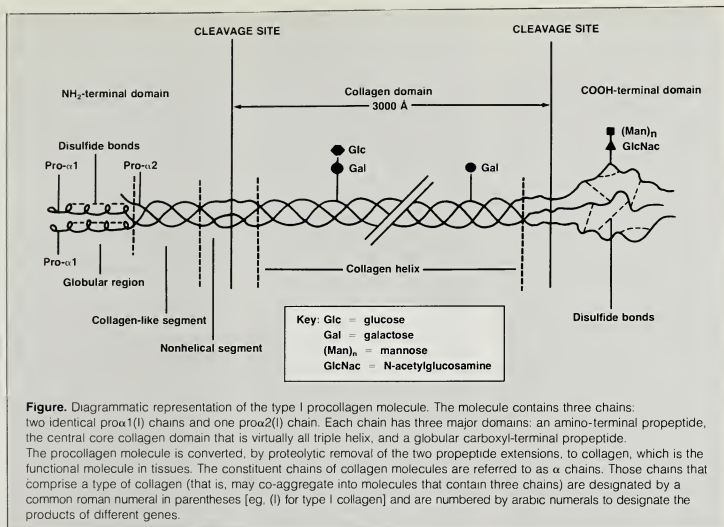
cells cultured from such patients results from synthesis of only half the normal amount of  $\text{pro}\alpha 1(\text{I})$ , which reflects the half normal levels of steady-state  $\text{pro}\alpha 1(\text{I})$  mRNA levels. Most individuals with the OI type I phenotype have mutations in one allele of the COL1A1 gene.

Our analysis of the COL1A1 genes from more than ten individuals with OI type I, and of the linkage data from more than twice that number of families, indicates that loss of the entire gene must be a rare event (no case has been identified yet). Instead, it appears that either the expression of the gene itself or its nuclear processing is disturbed. The decrease in production (and secretion) of type I procollagen occurs because stable molecules cannot be formed without two  $\text{pro}\alpha 1(\text{I})$  chains. Thus, when the number of  $\text{pro}\alpha 1(\text{I})$  chains is decreased to half normal, the amount of type I procollagen formed is also half normal and the

**Table.** Osteogenesis imperfecta: Clinical heterogeneity and biochemical defects

| OI type | Clinical features   | Inheritance pattern* | Biochemical defects   |
|---------|---|----------------------|---|
| I       | Normal stature, little or no deformity, blue sclerae, hearing loss in about 50% of individuals; dentinogenesis imperfecta is rare and may distinguish a subset                                  | AD                   | Decreased production of type I procollagen  |
| II      | Lethal in the perinatal period, minimal calvarial mineralization, beaded ribs, compressed femurs, marked long bone deformity, platyspondyly   | AD (new)             | Rearrangements in the COL1A1 and COL1A2 genes<br>Substitutions for glycyl residues in the triple-helical domain of the $\alpha 1(\text{I})$ chain                   |
|         |   | AR (rare)            | Small deletion in $\alpha 2(\text{I})$ on the background of a null allele   |
| III     | Progressive deformation of bones, usually with moderate deformity at birth; sclerae variable in hue, but often lighten with age; dentinogenesis common, hearing loss common; very short stature | AR                   | Frame-shift mutation that prevents incorporation of $\text{pro}\alpha 2(\text{I})$ into molecules<br>Noncollagen defects  |
|         |   | AD                   | Point mutations in the $\alpha 1(\text{I})$ or $\alpha 2(\text{I})$ chain   |
| IV      | Normal sclerae, mild to moderate bone deformity, variable short stature; dentinogenesis is common and hearing loss occurs in some   | AD                   | Point mutations in the $\alpha 2(\text{I})$ chain<br>Rarely, point mutations in the $\alpha 1(\text{I})$ chain<br>Small deletions in the $\alpha 2(\text{I})$ chain |

\*AD = autosomal dominant; AR = autosomal recessive



remaining pro $\alpha$ 2(I) chains are degraded. Linkage studies suggest that in some families the OI type I phenotype results from mutations in the COL1A2 gene, but better clinical descriptions are required to be sure that members of those families do not have OI type IV.

### Osteogenesis Imperfecta Type II

OI type II is generally lethal in the perinatal period. Radiographs of affected infants demonstrate a virtual absence of calvarial mineralization, beaded ribs, platyspondyly, and marked deformity of long bones with accordionlike compression of the femurs. Although OI type II was originally thought to be an autosomal recessive condition, recent clinical studies and the preponderance of biochemical and molecular genetic studies indicate that this phenotype usually results from heterozygosity for new dominant mutations in the genes that en-

code the chains of type I collagen.

OI type II is a biochemically heterogeneous disorder in which four classes of mutations have been recognized: (1) multiexon rearrangements (deletions or insertions) in the COL1A1 and COL1A2 genes, (2) point mutations in the COL1A1 gene, (3) short deletions from the COL1A1 gene, and (4) compound heterozygosity for mutations in the COL1A2 gene.

Three rearrangements have been characterized: (1) deletions of the triple-helical residues 327-411 in the  $\alpha$ 1(I) chain, (2) deletion of triple-helical residues 586-765 in the  $\alpha$ 2(I) chain, and (3) duplication of about 60 residues between triple-helical residues 123 and 200 in the  $\alpha$ 1(I) chain. In each case, molecules that contain the mutant chains are poorly secreted and less stable than normal molecules.

Point mutations in five separate individuals have been characterized and each resulted in a substi-

tution for a glycyl residue within the triple-helical domain of the products of one COL1A1 allele (cysteine for glycine at 988, 904, or 748; aspartic acid for glycine at 883; and arginine for glycine at 391). The preponderance of cysteine-for-glycine substitutions reflects the ease of identification and characterization, *not* the relative frequency of the substitution. Only six of about 150 infants characterized have this mutation, slightly less than the predicted frequency. Cells from these infants produce two populations of type I procollagen molecules, some that are normal and others that contain the abnormal chains. The latter have undergone excessive post-translational modification of all chains (largely lysyl hydroxylation and hydroxylysyl glycosylation) from the site of the altered residue to the amino terminus of the triple helix.

The increased modification is  
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thought to reflect an alteration in the rate of triple helix propagation beyond the substitution or an alteration in triple helix integrity in that domain. Either alteration would allow continued modification. The "overmodified" molecules are delayed in their secretion, and the rate of intracellular degradation of abnormal molecules is increased. Assuming random association, a mutation in  $\text{pro}\alpha 1(\text{I})$  chains would affect 75% of all molecules produced so that 25% of the normal amount of collagen would be secreted. Depending on the stability of the abnormal molecules, variable amounts of the abnormal molecules would appear in the matrix. The abnormal molecules synthesized by cells that carry small deletions in the  $\text{pro}\alpha 1(\text{I})$  chains and by cells with compound heterozygosity for mutations in the COL1A2 gene are unstable, poorly secreted, and degraded within the cells.

*How do these disparate mutations result in the same clinical and radiologic phenotype?* One unifying concept is that two factors are required to produce OI type II: a marked decrease in production of normal molecules, and the secretion of some type I collagen molecules in which the structure of the triple helix is disrupted. In this model, substitutions for a triple-helical glycine in the  $\alpha 1(\text{I})$  chain would disrupt winding and result in an unstable molecule that was overmodified from the site of substitution to the amino terminus and was poorly secreted. Overmodification probably affects the incorporation of abnormal molecules into fibrils and alters the interactions of these molecules with other matrix macromolecules. The rearrangements and small deletions similarly derange structure and appear to interfere with normal modification, stability, and secretion.

Although in most instances OI type II results from new dominant

mutations, there is an empiric recurrence rate of about 6%. Recurrence usually results from germline mosaicism for a mutation in one of the genes that encodes a chain of type I procollagen. In rare families, OI type II may be inherited in an autosomal recessive fashion. Prenatal diagnosis by ultrasound examination at about 15 weeks' gestation is reliable and analysis of collagen synthesized by cells from chorionic villi biopsied at ten weeks' gestation can provide diagnostic information.

## Osteogenesis Imperfecta Type III

OI type III is characterized by markedly short stature and progressive bone deformity during childhood and adolescence. The sclerae may be blue in infancy but are usually only pale blue or normal in adulthood. Dentinogenesis imperfecta is seen in some and hearing loss occurs frequently. Individuals with OI type III sustain multiple fractures. They often develop severe scoliosis that is difficult to treat, ultimately leading to cardiorespiratory compromise. OI type III was originally designated as an autosomal recessive condition, but it is now apparent that the phenotype is genetically heterogeneous, and both autosomal recessive and autosomal dominant varieties are known. The recessively inherited OI type III is probably rare except in certain populations, such as South African blacks, among whom it may be the most common form of OI.

With a small number of important exceptions, OI type III has been difficult to characterize biochemically. Among infants with recessively inherited forms, one child whose cells secreted type I procollagen that contained only  $\text{pro}\alpha 1(\text{I})$  chains has been identified. The child, a product of a consanguineous mating, was homozygous for a four base-pair deletion near the end of the coding region in the COL1A2 gene; the frameshift resulted in a short extension of the carboxyl-terminal propeptide. The change in sequence prevented the assembly of those  $\text{pro}\alpha 2(\text{I})$

chains into type I procollagen molecules. Both asymptomatic parents were heterozygous for the mutant allele.

In other families in which a single child is affected, and in some families in which a parent and a child are affected because of autosomal dominant inheritance, point mutations in COL1A1 or COL1A2 that affect triple helix stability have been identified. For example, a new mutation that results in a substitution of cysteine for glycine at position 526 of the triple helix in about half the  $\text{pro}\alpha 1(\text{I})$  chains synthesized has been identified in one infant. The de novo appearance of cysteine in  $\text{pro}\alpha 2(\text{I})$  between residues 6 and 327 (from which it is normally excluded) was identified in another infant. Thus, the dominant OI type III phenotype may overlap at the biochemical level with OI type II (point mutations in the COL1A1 gene) and with OI type IV (see below). The phenotypic effect of a specific point mutation probably depends on the nature of the substituting residue and its location in the chain.

## Osteogenesis Imperfecta Type IV

OI type IV is a dominantly inherited disorder characterized by normal or greyish sclerae and normal to moderately short stature with significant deformity. Affected children are often born with femoral bowing that straightens with time and ambulation. Dentinogenesis imperfecta is common. Linkage studies have implicated mutations in the COL1A2 gene as the cause of the OI type IV phenotype in several families. In one such family, substitution for the last glycine in the triple helix of  $\alpha 2(\text{I})$  (residue 1012 of the triple helix) results in OI type IV. Molecules that incorporate the abnormal  $\text{pro}\alpha 2(\text{I})$  chain are overmodified along the entire length of the triple helix and are secreted less efficiently than those that incorporate the normal chains.

Other mutations that result in small deletions or point mutations in about half the  $\text{pro}\alpha 2(\text{I})$  chains are responsible for the phenotype in most individuals with OI type IV.

These mutations all result in secretion of some molecules that have undergone excessive post-translational modification, and it is often difficult to distinguish between OI type II and OI type IV at the biochemical level. In OI type IV the mutations are usually in the COL1A2 gene and, as a result, only half the type I procollagen molecules are abnormal (compared with three quarters when mutations affect COL1A1 in OI type II). This quantitative difference may account for the milder disease phenotype in OI type IV.

### Other Forms of Osteogenesis Imperfecta

Not all individuals with OI readily fit the classic types. For example, one family has a phenotypic mixture of OI type IV and Ehlers-Danlos type VII features, probably explained by a short deletion from the pro $\alpha$ 2(I) chain near the amino-terminal end of the triple helix that interferes with the proteolytic conversion of procollagen and alters the bone matrix to produce bone fragility. As more families are studied, it is likely that similar "overlap" syndromes will emerge.

### A Unifying Picture

How do different mutations produce similar phenotypes, and why do similar mutations often result in markedly different phenotypes? While we do not have a clear answer to either of these questions, some integrating concepts are emerging. First, multiexon rearrangements are lethal if they are expressed in proteins. Second, the effect of point mutations depends on the chain in which they occur, the residue substituted (glycine versus an X- or Y- position residue), the position in the chain, and the nature of the substitution. Third, there is a polar effect in the  $\alpha$ 1(I) chain such that point mutations in the carboxyl-terminal end of the triple helix (substitutions for glycine) are lethal while those toward the amino-terminal end may not be. Fourth, point mutations or small deletions in the  $\alpha$ 2(I) chain are generally milder in their phenotypic effects than those in the  $\alpha$ 1(I)

chain. Fifth, the phenotypic effect is a consequence of the presence of normal and abnormal collagens in the matrix and interaction among these molecules and the other components of the bone matrix. Finally, interfamilial heterogeneity is generally explained by different mutations while intrafamilial variation is likely to result from genetic differences in the expression or structure of other matrix components.

### Implications for Management and Treatment

The majority of individuals with OI have dominantly inherited disorders, either as a result of new dominant mutations or inheritance of the condition from a parent. Autosomal recessive phenotypes are rare. The distinction between the two can often be made from analysis of collagens synthesized by fibroblastic cells cultured from affected individuals. Biochemical analysis helps to distinguish among the types of OI. This, in turn, often allows for more appropriate counseling about natural history and facilitates prenatal diagnosis either by linkage analysis in families or analysis of collagens synthesized by chorionic villus cells. It should be stressed that intrafamilial variability can be marked and that our understanding of the correlation between the nature and location of the mutations and their phenotypic consequences is not yet complete.

Fractures can be treated by standard orthopedic measures. Disabling bone deformity can be corrected orthopedically to restore anatomic limb position and function, but early intervention is recommended. Surgery to correct major spinal deformities produces limited therapeutic results in the most severely affected individuals because of the compliant nature of the defective bone. Many medical treatments have been advocated through the years but their effectiveness is, at best, controversial. The variable responses to treatment that have been reported to date may reflect the biochemical heterogeneity of the condition. For

example, treatments designed to increase collagen production may be effective if the only effect of the primary mutation is to decrease production of normal collagen (eg, OI type I). These same treatments may be of little benefit if the OI phenotype results from incorporation of abnormal collagen into the matrix.

The immediate future holds immense promise for understanding the nature of mutations that result in the OI phenotypes. Only slightly beyond is the prospect of rational medical therapies that may emerge from understanding the molecular defects that produce the several forms of OI.

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# Medical Complications of Dwarfing Syndromes

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Major progress in delineating the many specific skeletal dysplasias that present with disproportionate short stature has been made in the last 15 years. Each dysplasia has its own natural history, genetic basis, and specific pathological findings. However, some general comments can be made about the most common medical complications seen in children with these dwarfing conditions since they are of importance to the practitioner caring for these children.<sup>1-4</sup> These have been grouped into intrauterine, respiratory, central nervous system (CNS), skeletal, muscular, otolaryngologic, ophthalmologic, dental, and nutritional complications (Table).

## Intrauterine Complications

The most common problems seen during gestation are polyhydramnios and edema. In general, these occur in fetuses with the lethal chondrodystrophies, such as achondrogenesis and thanatophoric dysplasia. Occasionally, however, polyhydramnios is a late gestational complication in an otherwise nonlethal condition, such as achondroplasia.

Prenatal diagnosis of the chondrodysplasias by midgestational ultrasound is possible in many such conditions since normal measurements of limbs and trunk are available for comparison. When ultrasound is done because polyhydramnios is present, disproportionate fetal development may be noted. Observing the rate of growth over several weeks is often helpful in establishing a diagnosis. Prenatal diagnosis by ul-

trasound depends on whether the particular chondrodysplasia manifests itself before birth. Diagnosis of a specific chondrodysplasia during the second trimester permits two options: termination of the pregnancy or preparation of the family for the birth of a dwarfed child.

## Respiratory Complications

Respiratory distress is seen in a number of dwarfing conditions,<sup>5</sup> particularly those associated with small chests, such as asphyxiating thoracic dysplasia. Respiratory distress occurs when there is a small chest and lungs, a small or collapsing trachea, or a small upper airway; distress is worsened by respiratory tract infections. Norms for evaluating the size of the respiratory tree in children with chondrodysplasias are not readily

available. Respiratory rates and retractions are often the best indication that there is a problem. Monitoring oxygen and carbon dioxide levels is important in order to determine whether true respiratory distress exists in dwarfed infants.<sup>6</sup>

As the child grows, there is often a disproportionate increase in the size of the chest. During the first six months of life, the trachea in children with chondrodysplasias increases in size. During the second year of life, upper airway obstruction becomes a more significant problem than lower airway obstruction, particularly if the tonsils and adenoids are enlarged. Snoring and sweating during sleep are frequently seen in dwarfed infants. If persistent, these symptoms indicate that the child is having hypoxic episodes during sleep and requires further evaluation.

**Table.** Medical Complications of Dwarfing Syndromes

| Intrauterine           | Polyhydramnios; edema   |
|------------------------|---|
| Respiratory            | Respiratory distress secondary to small chest and lungs, small or collapsing trachea, or small upper airway (often complicated by upper respiratory infections); asphyxiating thoracic dysplasia; snoring; upper airway obstruction; hypoxic episodes |
| Central nervous system | Hydrocephaly; spinal cord compression; nerve damage secondary to instability of cervical vertebrae  |
| Skeletal               | Kyphosis; instability of cervical vertebrae; various vertebral abnormalities; hip dysplasia; tight and loose joints; osteoarthritis; bowed legs; fractures  |
| Muscular               | Truncal hypotonia; disease of muscles; muscle contractures  |
| Otolaryngologic        | Frequent otitis media; hearing loss (conductive and neurosensory)   |
| Dental                 | Malocclusions; dental crowding; structural abnormalities of teeth   |
| Ophthalmologic         | Severe myopia; retinal detachment   |
| Nutritional            | Obesity   |

## Central Nervous System Complications

### Hydrocephaly

True hydrocephaly occurs in several dwarfing conditions, notably the achondroplasias, the metatopic dysplasias, and conditions that affect the base of the skull, and results in a decrease in the size of the foramen magnum and jugular foramen. Hydrocephaly can occur prior to delivery and, if the head is sufficiently enlarged, may lead to cephalopelvic disproportion and a traumatic birth. An infant with true hydrocephaly must be distinguished from one who has apparent hydrocephaly: ie, one with a normal-sized head and a small body.

In general, hydrocephaly, if present at birth or developing shortly afterwards, progresses during the first year of life. If disproportionate head growth occurs, a neurologic evaluation is essential. During the first year of life, serial ultrasounds through the fontanelle every three months give an indication of relative ventricular size. In the past, shunting operations were not performed unless the increase in head size was judged to be abnormal when compared with the growth charts of head circumference. However, there is increasing evidence that shunting should be considered in certain children with neurologic symptoms even if the head size remains stable, since increased intracranial pressure may be present and lead to CNS damage.<sup>7</sup>

### Spinal Cord and Nerve Damage

Compression of the spinal cord is seen at a number of vertebral levels in many of the chondrodysplasias. If the foramen magnum is small, compression may occur during the birth process. Instability of the cervical vertebrae can occur at this time when there is odontoid and vertebral or ligamentary laxity. The latter is seen in mucopolysaccharidosis conditions or when vertebral abnormalities are present, such as those associated with the spondyloepiphyseal dys-

plasias and spondylodysplasias. Laxity of the ligaments is also seen in diastrophic dysplasia, metatropic dysplasia, Larsen syndrome, and camptomelic dysplasia.<sup>8</sup>

Instability of the cervical vertebrae can best be evaluated by lateral views of the neck during flexion and extension. If stability and subluxation are present, further evaluation by imaging and electrophysiologic techniques is indicated. The results may indicate the need for cervical fusion to prevent possible compression and quadriplegia. Sudden infant death, which has been reported with increased frequency in older infants with achondroplasia,<sup>9</sup> may be a function of high cervical cord compression. Occasionally, procedures to enlarge the foramen magnum must be considered.<sup>6</sup>

In conditions characterized by either vertebral abnormalities or short pedicles, or in disorders in which spinal abnormalities ultimately develop, spinal cord compression can occur at any time during life. In achondroplasia, symptoms of lower spinal cord and root compression usually do not occur until patients reach their early 20s. Symptoms of claudication and numbness must be taken seriously in patients with achondroplasia and should be treated medically by traction or bracing. If no relief occurs, surgical treatment is necessary in order to avoid permanent damage to the spinal cord and/or nerves.

### Skeletal and Muscular Complications Hypotonia and Kyphosis

Patients with truncal hypotonia, which occurs in infants with achondroplasia or mucopolysaccharidoses, may develop kyphosis at the first or second lumbar vertebra. Kyphosis may be a function of weight distribution leading to compression of the anterior part of the vertebra. It may be possible to avoid this by positioning the baby properly so that increased weight is not placed on the anterior part of the first lumbar vertebra. Therefore, during the first year of

life or until good trunk strength develops, forward slumping of the body (eg, curling into the fetal position) should be corrected by repositioning the baby.

### Hip Joint Abnormalities

Hip dysplasia is common in a variety of chondrodysplasias because the pelvis acetabulum is slow to form, the femoral head may be dysplastic (poorly ossified), or the femoral neck is short. In addition, the full range of motion of the hip joint is frequently limited by bony abnormalities. It is important that attention be given to proper acetabular formation during the first year of life; frog-leg positioning may be necessary to encourage good hip joint development. Severe osteoarthritis frequently occurs at the hip joint in children with epiphyseal and spondyloepiphyseal dysplasias, and early total hip replacement is required.

### Bowed Legs

Bowing of the legs is frequent in many dwarfing conditions because the long bones of the leg grow disproportionately and the ligaments of the knee and ankle are relatively loose. In achondroplasia, the fibula overgrows and pushes the ankle inward. It is important to try to maintain normal distribution of weight on the joints of the leg to avoid asymmetric growth and wear and tear of the joint surfaces, since this produces osteoarthritis at a later time.

### Fractures

Individuals with chondrodysplasias may have a higher incidence of bone fractures because of falls or trauma. Except in osteogenesis imperfecta, chondrodysplastic bones are generally of normal strength. In all dwarfing conditions, bone-healing rates seem to be normal.

### Joint Stability

In certain dwarfing conditions, joint instability occurs because some joints are loose while others are tight. Loose joints lead to dislocation and excessive wear and

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## Medical Complications of Dwarfing Syndromes

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tear on the outer joint edges. Tight joints may be caused by bony limitation, such as the bony fusions that are seen in diastrophic dysplasia, or to disuse and contractures of the muscles in other dwarfing conditions. It is important to determine the cause of the tight joint in order to initiate appropriate therapy. In diastrophic dysplasia, where there is bony fusion causing tight joints, physical therapy would be inappropriate. However, in Kniest syndrome, where muscular atrophy frequently results from disuse of muscles, physical therapy is very helpful in maintaining range of motion and strength.

### Arthritis

Osteoarthritis (degenerative wear-and-tear arthritis) is a frequent complication of the chondrodysplasias. In many of the epiphyseal dysplasias, delayed ossification of a normal-sized epiphysis may lead to excessive wear and tear of the articular cartilage. Premature arthritis frequently develops in the weight-bearing joints of children with various chondrodysplasias. The activities of childhood and daily living should therefore be approached in a manner that minimizes excessive trauma and overuse of joints. Contact sports, which cause repeated wear and tear on the joints, should be avoided in children with conditions that correlate strongly with the development of arthritis.

### Hearing and Speech

Otitis media is a frequent complication of several skeletal dysplasias (eg, achondroplasia, spondyloepiphyseal dysplasia congenita, and Kniest dysplasia) in childhood. Aggressive antibiotic treatment is needed to avoid scarring of the eardrum and middle ear structures and to ensure that the child's hearing is normal so that he or she can develop normal social skills. There is some evidence that neurosensory loss develops if

chronic conductive loss occurs during childhood. Some conditions, such as osteogenesis imperfecta and diastrophic dysplasia, are associated with anomalies of the ossicles that lead to deafness. Since there is an increased incidence of hearing problems and otitis in most of the chondrodysplasias, hearing should be tested on a regular basis, particularly after upper respiratory infections.

### Dental Crowding

Many chondrodysplasias are associated with dental malocclusions or crowding because of overgrowth or undergrowth of the mandible or maxilla and because of structural abnormalities of the teeth. Regular dental checkups are necessary, as is appropriate planning for orthodontic correction.

### Ophthalmologic Complications

Severe myopia and retinal detachments are seen in several spondyloepiphyseal dysplasias and in Kniest dysplasia. In children with these conditions, careful monitoring of vision is essential. Prophylactic laser treatment of the retina may be required to avoid retinal detachment.

### Anesthesia

Anesthesia can be a problem for individuals with some chondrodysplasias. Since frequent orthopedic surgical procedures are required, special care should be taken before and during the administration of anesthesia. The dosage of the anesthetic should be adjusted for the patient's weight. The possibility that the patient has unstable cervical vertebrae should also be kept in mind when the head is manipulated for intubation. Prior to the induction of anesthesia, flexion and extension of the cervical spine should be evaluated by lateral radiographs. Magnetic resonance imaging may also be necessary to evaluate flexion. Because the patient's trachea may be small, tracheal cuffs in several sizes should be available

in the operating room. Tracheal swelling can occur after extubation in diastrophic dysplasia. Some chondrodysplasias, such as osteogenesis imperfecta, seem to be associated with an increased incidence of malignant hyperthermia.

### Obesity

Obesity is often a problem in individuals with disproportionate short stature, particularly those with achondroplasia. The origin of obesity in achondroplasia is not clear, but it does not appear to be entirely due to psychosocial maladjustment. Because of the social ramifications of obesity and because of its significant contribution to increased wear and tear on joints, it is important for dwarfed individuals to try to maintain a normal weight. It is often hard to establish what the "normal" weight should be since there are no standard charts for weight in relation to disproportionate short stature. The best indicators of extra weight are general body appearance and the "pinch" test, in which the skin of the underarm or abdomen is pinched and the skinfold measured. If it measures more than 1 cm, the individual is probably overweight.

### Obstetric and Gynecologic Care

A number of obstetric and gynecologic problems are common in women with disproportionate short stature. Pregnant women will require a cesarean section for delivery because of a contracted pelvis. In women with achondroplasia, general anesthesia should be used for the cesarean section because of bony abnormalities of the spine. In addition, women with some types of disproportionate short stature reportedly have an increased incidence of fibroid tumors.

Sexual intercourse may be difficult for some women with dwarfing conditions because of the abnormal tilt of the pelvis. Because many of these women have short arms, personal hygiene can also be a problem. Both of these complications need to be discussed openly

with the woman and her partner, and practical solutions that are appropriate for them should be suggested.<sup>10</sup>

## Summary

The medical complications of chondrodysplasias are significant and require appropriate management. Therefore, physicians must be aware of these and other potential complications. Although visceral disorders such as congenital heart disease and renal anomalies are sometimes seen in patients with certain chondrodysplasias, they are generally not problematic.

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## In Future Issues

Lipodystrophy  
by William L. Clarke, M.D.

The Genetics of Various  
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Anabolic Steroids in Athletes:  
Efficacy or Fantasy?  
by Alan D. Rogol, M.D., Ph.D.

## Abstracts From the Literature

### Serum Concentrations of IGF-I, IGF-II, and Unsaturated Somatomedin Carrier Proteins in Children With Chronic Renal Failure

Growth failure in children with renal disease has multiple causes, including renal rickets, metabolic acidosis, chronic infection, and malnutrition. However, in some instances, chronic growth failure cannot be attributed to any of these causes. Because insulin-like growth factor (IGF)-I levels are often low in children with growth failure and renal disease, Powell et al examined the possible role of IGF-I, IGF-II, and their binding proteins.

Serum samples from 16 patients with glomerular filtration rates <50% of normal, who were on chronic peritoneal dialysis but did not have acidosis, rickets, or chronic infection, were compared with serum samples from normals. Acid chromatography permitted measurement of the actual amounts of IGF-I, IGF-II, and somatomedin carrier proteins (SmCP). The results revealed no diminution of the IGF levels, and an increase in SmCP.

On the basis of these data, di-

minished absolute values of IGF-I or IGF-II in serum cannot be the cause of the observed growth retardation. The authors suggest that the possible role of increased SmCP needs investigation.

Powell DR, Rosenfeld RG, Sperry JB, et al. *Am J Kidney Dis* 1987; 10:287-292.

**Editor's comment**—These findings both clarify and confuse the issue of the etiologies of growth retardation in chronic renal dis-

ease. Low serum IGF-I and IGF-II values in patients with chronic uremia, as tested by bioassay, radioimmunoassay, and radio-receptor assay, were repeated. Powell et al have demonstrated that there are inhibitors of the assay systems that can be removed by acid chromatography. The confusion arises because the cause of growth retardation remains obscure in many children with chronic renal disease who are not acidotic.

Robert M. Blizzard, M.D.

### Editor's Note

In the special report on the International Growth Hormone Symposium (Volume 3, Number 4), the second paragraph read as follows: "Drs. Gloria Tannenbaum and Joseph Martin demonstrated very convincingly that GH-releasing hormone (GHRH) is secreted in the posterior part of the hypothalamus (tubero-infundibular region) and that growth hormone-releasing factor (GRF) is secreted in the ventromedial and arcuate nuclei." While the statement is technically correct, the use of both GHRH and GRF in the same sentence may be misleading, since the terms are synonymous. The editor extends his apologies for any confusion this may have caused.

## The Measurement of Stature: Letter to the Editor

Recently Genentech, Inc. and Ross Laboratories distributed to many pediatricians a plastic instrument for the measurement of "standing" stature. This instrument consists of a vertical track (graduated in 1.0-mm and 0.16-inch increments), a movable horizontal headboard to be placed on the individual's head for measurement, and a track for the headboard, which is fixed to a wall to provide a flat vertical surface against which the heels, buttocks, scapulae, and head can be aligned. Installation is easy, and a bubble level is provided to ensure that the headboard is horizontal.

Dr. Alex Roche and colleagues conducted a small study of 30 children in which they compared the reliability of the apparatus with that of the Holtain stadiometer and the Healthometer instrument, which is a scale for weight as well as a measuring device. In a Letter to the Editor, they reported that the Accustat stadiometer, distributed by Ross Laboratories and Genentech, Inc., is more reliable than the Healthometer and can be recommended as an alternative to the more expensive Holtain stadiometer.

Roche AF, Guo S, Baumgartner RN, Falls RA. *Am J Clin Nutr* 1988; 47:922.

**Editor's comment**—The companies are to be commended for providing this device at no charge. Several members of the Editorial Board of Growth, Genetics, and Hormones have found that the data derived from its use are reproducible, as did Roche et al. Readers who would like to acquire the device for their use in the office or clinic are encouraged to contact their local Ross Laboratories representatives.

Robert M. Blizzard, M.D.

## Altered $G_s$ and Adenylate Cyclase Activity in hGH-Secreting Pituitary Adenomas

Three investigators from Milan report two groups of human growth hormone (hGH)-secreting adenomas. The groups are differentiated by the adenylate cyclase activity in the cells grown in culture and the amount of hGH released in the basal, stimulated, and inhibited states.

The results in Group 1 are similar to those observed in normal rat pituitary cells. The results in Group 2 were completely different. The stimulatory effect of magnesium in Group 1 was significant but was even greater in Group 2. This hyperresponse, which occurred only with magnesium stimulation, could account for the high cyclic adenosine monophosphate (cAMP) levels observed in cultured cells because it was already appreciated at physiologic magnesium concentrations.

The authors noted that the altered regulation of adenylate

cyclase in tumors in Group 2 concerned only the guanine-stimulatory ( $G_s$ ) mechanism and not the guanine-inhibitory ( $G_i$ ) mechanism on cAMP activity. The authors postulate that tumors in Group 2 probably have a disturbance of stimulatory transmembrane signalling, which is located in the  $G_s$  protein, while adenylate cyclase activity and its resulting function (hGH release) resides in the  $G_s$  but not the  $G_i$  protein.

Since both secretion and growth of pituitary somatotropes are known to be under the control of cAMP, the authors suggest that a direct causal relationship between the alteration of guanine and the high secretory rate of these cells and their growth is possible.

Vallar L, Spada A, Giannattasio G. *Nature* 1987;330:566-568.

**Editor's comment**—Professor Henry R. Bourne, of the University of California at San Francisco, addressed this subject in the same issue of *Nature*. In his commentary

## Risk of Hypoglycemia With Alternate-Day Growth Hormone Injections

This paper describes three children with growth hormone deficiency (GHD) who presented with fasting hypoglycemia 36-60 hours after an injection of growth hormone (GH). Each child was receiving thrice-weekly injections of synthetic GH; hypoglycemia no longer occurred once injections were begun on a daily basis.

The first patient was a 5-year-old boy with isolated GHD. He was placed on therapy with non-methionyl GH (Eli Lilly, Indianapolis), 60  $\mu$ g/kg IM, three days a week. This child began to experience nightmares 36-60 hours after GH injection, and his plasma glu-

cose values fell below 2.2 mmol/L. Simultaneous insulin-like growth factor-I (IGF-I) levels (measured by Nichols Institute) were 350-500 U/L, compared with 230 U/L prior to the initiation of therapy. Once daily injections of 30  $\mu$ g/kg IM were instituted, overnight plasma glucose levels rose and remained above 4.7 mmol/L.

The second child, a male with panhypopituitarism diagnosed at birth, was initially treated with thyroxine and cortisol. This resulted in complete resolution of hypoglycemia. When he was 20 months old, he was placed on therapy with methionyl GH (Genentech Inc, San Francisco) for growth failure and received 50 mg/kg subcutaneously three times a week. His blood glucose concentrations

|  | Group 1           | Group 2            |
|--|-------------------|--------------------|
| hGH secretion/30 min/2 × 10 <sup>5</sup> cells | 53.1 ± 12.6 ng    | 246.7 ± 78.3 ng    |
| With GHRH                                      | 132 ± 0.0 ng      | no increase        |
| cAMP levels                                    | 2.2 ± 0.1 pmoles  | 49.5 ± 18.7 pmoles |
| With GHRH                                      | 17.6 ± 0.0 pmoles | no increase        |
| Adenylate cyclase activity                     | 12.64 ± 1.51*     | 102.49 ± 23.2*     |
| With GHRH                                      | 40.60 ± 3.59*     | 125.38 ± 29.64*    |
| With GTP                                       | 26.30 ± 5.25*     | 92.14 ± 14.71*     |
| With Gpp(NH)p                                  | 21.45 ± 3.58*     | 67.17 ± 11.08*     |
| With NAF                                       | 127.09 ± 16.94*   | 94.90 ± 15.74*     |
| With Forskolin                                 | 55.61 ± 10.68*    | 263.53 ± 30.71*    |
| With SRIF                                      | 10.53 ± 2.22*     | 68.96 ± 7.52*      |

\*pmol cAMP mg<sup>-1</sup> protein min<sup>-1</sup>

("G Proteins and cAMP: Discovery of a new oncogene in pituitary tumors?"), Bourne presented two possible explanations for the autonomous function of the tumors in Group 2. These are either a covalent modification or an activating mutation of G<sub>s</sub>. Bourne favors the

latter. He postulates that a somatic mutation in Group 2 tumors activates G<sub>s</sub> directly and cites precedent for a mutational replacement of residues in the nucleotide-binding pocket of normal cellular ras proteins. These mutations produce proteins with

reduced intrinsic capacity for hydrolyzing guanosine triphosphate (GTP) and, consequently, markedly diminished sensitivity to a GTP-ase-activating regulatory protein. Expression of these activated ras proteins causes malignant transformation of cells in vitro and contributes to oncogenesis in animals. Bourne also stated that cAMP can stimulate, inhibit, or have no effect on proliferation. It is already known that cAMP stimulates hGH secretion and proliferation of somatotropes. Other tropic hormones that use cAMP as a second messenger include the thyroid, adrenal, and sex glands. It is easy to imagine that tumors of these glands might result from the activation of mutations in G<sub>s</sub> protein or in other elements of the cAMP-signalling pathway.

Further speculation might be in order. Could the McCune-Albright syndrome, characterized by sexual precocity, café-au-lait spots, and polyostotic fibrous dysplasia, result from abnormal or mutated G<sub>s</sub>? We may know the answer in a few years.

Robert M. Blizzard, M.D.

were as low as 1.9 mmol/L 38 hours after GH injections; a simultaneous plasma IGF-I level was 360 U/L, compared with 130 U/L prior to the start of therapy. Fasting plasma glucose levels remained low when the child was given thrice-weekly injections of non-methionyl GH, but he was not hypoglycemic.

The third child, also a male with panhypopituitarism, exhibited hypoglycemia on the first day of life and was treated with thyroxine and hydrocortisone. Thrice-weekly injections of methionyl GH were begun when he was one year old. Like the other two children, he also experienced hypoglycemia, with blood glucose levels as low as 2.3 mmol/L 36 hours after each injection. Treatment with daily in-

jections resulted in complete resolution of hypoglycemic symptoms.

The authors suggest that the high levels of somatomedin-C (IGF-I), which often fail to peak until 19 hours after GH injection, contribute to the total insulin-like activity in the serum since they are not accompanied by GH, which would usually antagonize the glucose-lowering effects of insulin.

Press M, Notarfrancesco A, Genel M. *Lancet* 1987;1:1002-1004.

**Editor's comment**—These interesting case reports suggest that all patients requiring GH therapy, even those who do not initially present with hypoglycemia, should be carefully observed for the presence of low blood glucose

levels when receiving thrice-weekly GH injections.

Haymond et al (JCEM 1976;42:846) previously demonstrated that the hypoglycemia observed in untreated patients with panhypopituitarism is substrate-mediated and characterized by low circulating concentrations of plasma alanine and glutamine. However, these patients, when receiving cortisone and daily GH injections, did not become hypoglycemic after 30 hours of fasting. No subject receiving GH every third day was studied. From the data presented by Press et al, it would seem reasonable to repeat Haymond's fasting study with more traditional GH therapy and measurements of IGF-I.

William L. Clarke, M.D.



## Short-Term Testosterone Treatment at Bone Age 12-13 Years Does Not Reduce Adult Height in Boys with Constitutional Delay of Growth and Adolescence

Zachmann, Studer, and Prader retrospectively compared the adult heights of two groups of males with constitutional delay of growth and adolescence (CDGA). The first group (22 patients) had received no therapy. The second group (19 patients) had been treated with long-acting testosterone, at a dose of 100-250 mg/month for periods of 2-45 months. Target height calculations (mid-parental heights) and predicted heights (calculated by three methods) were used.

The mean adult height was not compromised in the treated group, but was comparable with or exceeded the predicted heights for both groups. The authors conclude that there was no correlation of the total testosterone dose (absolute and corrected for surface area) with adult height and with the differences between the three height predictions and adult height. The authors also state that the fear that testosterone treatment might later impair gonad function and fertility is not warranted.

Zachmann M, Studer S, and Prader A. *Helv Paediatr Acta* 1987;42:21.

**Editor's comment**—These analyses are for groups and not individuals, which limits their value somewhat. One must be cautious in using mean data for groups and applying the interpretation of those data to treatment of individuals. For example, a minority of patients may grow markedly and a majority grow moderately less than the mean for the group. This can be interpreted to mean that treatment may be contraindicated for most

## Does Growth Hormone Cause Relapse of Brain Tumors?

This report compares tumor relapse rates in two groups of patients: 31 growth hormone (GH)-treated patients with brain tumors distant from the hypothalamic-pituitary axis and all patients with similar tumors in the North-West Tumor Registry between 1972 and 1982. Those in the latter group did not receive GH.

Patients treated with GH for growth failure secondary to cranial irradiation included 14 with medulloblastoma, eight with glioma, two with ependymoma, six with leukemia, and one with T-cell lymphoma. Five relapses occurred: one optic nerve glioma, two medulloblastomas, and two ependymomas. Three relapses occurred during GH therapy, and two occurred after GH therapy was completed. The relapse and survival rates, which were presented according to tumor type, indicated that GH therapy did not increase the risk of tumor relapse. Patients treated with GH did not have more relapses, either during or after discontinuation of therapy, than those who did not receive GH. Patients who relapsed tended to be older at

diagnosis and have slightly later onset of puberty.

Clayton PE, Gattamaneni HR, Shalet SM, et al. *Lancet* 1987;1: 711-713.

**Editor's comment**—This paper presents important information for physicians caring for children who have received cranial irradiation and have subsequently developed growth failure. Although a significant number of patients with central nervous system (CNS) tumors will experience relapse, it is reassuring that those treated with GH do not appear to be at increased risk. However, as the prognosis for patients with CNS tumors begins to improve, it is important to identify the long-term sequelae associated with either the tumor or GH treatment of growth failure. For those receiving craniospinal irradiation, hypothalamic pituitary dysfunction is common. There is often a reluctance to begin GH therapy in these patients, since it has been considered by some to contribute to tumor regrowth or relapse. The findings of the present study suggest that GH treatment does not increase this risk.

William L. Clarke, M.D.

patients with the condition under consideration. Therefore, caution is urged in using mean data of groups to determine therapeutic approaches. I invite the authors to write to Growth, Genetics, and Hormones and supply data on individuals and/or comment more fully on their study findings.

The authors' statement regarding the absence of long-term effects of testosterone therapy is related not to patients in this study but to data published elsewhere by Zachmann et al (*J Pediatr* 1976;88:116).

The authors' findings may be in accord with those of Martin et al [published in *Illig R*, ed: *Pediatr Endocrinol*, and Visser HKA, ed: *Acta Endocrinol* (1986;Suppl:279)].

Martin et al examined individual data and group data. They concluded that a monthly dose of 50 or 100 mg of testosterone cypionate for approximately 9-12 months did not diminish predicted height, although a dose of 200 mg/month was associated with a trend toward stature that was lower than predicted.

My approach to therapy for patients with CDGA is to use a monthly dose of only 50-100 mg of testosterone enanthate for 6-12 months and only in boys 14 years of age and older. Younger boys are better treated with oxandrolone ( $\leq 0.1$  mg/kg body weight/day).

Robert M. Blizzard, M.D.

## Tubular Bone Alterations in Familial Short Stature: Two Reports

Familial short stature (FSS) accounts for about 50% of the children who are seen by pediatric endocrinologists for assessment of growth. These children usually are in good health, have no obvious dysmorphism, and grow at a consistently normal rate. Growth usually proceeds parallel to the 5th percentile, and the predicted adult height is within range for the child's family. Essentially, these patients are short because their parents and families are short. Since such children are considered "normal," their skeletal anthropometric characteristics are presumed to be similar to those in the rest of the "normal" population.

In these two reports, however, a high prevalence of skeletal alterations in individuals with FSS was noted. The occurrence of these characteristics has not been reported previously in this patient group.

A detailed anthropometric study was performed in 40 white children with FSS. Measurements were compared with those from 40 children of normal stature who were matched for age, race, and sex, and from 958 adolescent boys and girls with normal stature. Anthropometric measurements were also obtained from 30 short parents of FSS children and compared with those from 26 normal-statured parents with FSS children and from 33 unrelated normal-statured adults.

Shortening of the fifth metacarpal bone was more prevalent in the 40 FSS children (78%) than in the children with normal stature (28%) and the healthy adolescents (39%,  $P < 0.001$ ). Rhizomelia was also more prevalent in all FSS children (42%) than in the children with normal stature (15%,  $P < 0.01$ ) and the healthy adolescents (17%,  $P < 0.001$ ). Likewise, shortening of the fifth metacarpal bone was more prevalent in the short parents

of the FSS children (73%) than in the unrelated adults with normal stature (27%,  $P < 0.001$ ). Also, the prevalence of rhizomelia was higher in the short parents of the FSS children (33%) than in the unrelated adults with normal stature (12%,  $P < 0.05$ ).

Disproportionate shortening of the lower limbs was more prevalent in the FSS children (32%) than in the healthy adolescents (11%,  $P < 0.001$ ). Disproportionate shortening of the arms was more prevalent in the FSS children (35%) than in children with normal stature (10%,  $P < 0.01$ ) and healthy adolescents (8%,  $P < 0.001$ ).

The presence of more than one form of tubular bone alteration occurred more frequently in the children and adults with FSS than in the groups with normal height. Most children and adults with FSS had one to four types of tubular bone alteration, while the majority of individuals with normal stature had either no tubular bone defect or only one type of this defect.

In view of the high prevalence of brachymetacarpia V in FSS patients, the authors also performed detailed radiologic anthropometry of the hand in 28 FSS children. Lengths of each of the hand bones were measured and compared with the normal standards developed by Garn and Poznanski. Moreover, the fifth metacarpal was compared with the other metacarpal bones by obtaining ratios and comparing these ratios with the normal standards set by Garn.

The results of this latter study revealed that most patients with clinical brachymetacarpia V had radiologic evidence of fifth metacarpal bone shortening. The metacarpal pattern profiles of the patients with and without brachymetacarpia V differed. The shortest metacarpal bones in children with clinical brachymetacarpia V were the first and fifth, while in the children without clinical brachymetacarpia V, the shortest metacarpal bone was the fourth, followed by the third and fifth. The

phalangeal pattern profiles of these two groups were similar.

The ratios between the fifth metacarpal and the other metacarpal bones in the children with brachymetacarpia V showed that the fifth metacarpal bone was short in relation to the third and fourth metacarpal bones, while the opposite was true in the group without clinical brachymetacarpia V. Moreover, it was also observed that there is a significant positive correlation between height reduction and metacarpal and proximal phalangeal bone shortening in the group with clinical brachymetacarpia V. There was no correlation between height reduction and the length of the distal and middle phalanges.

Cervantes C, Lifshitz F. *Human Biology* 1988;60:151-165.  
Cervantes C, Lifshitz F, Levenbrown J. *Pediatr Radiol* 1988;18:248-253.

**Editor's comment**—The results of these two studies indicate a very high prevalence of tubular bone alterations, mainly disproportionate shortening of the limbs, rhizomelia, brachymetacarpia V, and possible brachymetacarpia I, in children and adults with FSS. Since these characteristics are frequently seen in various syndromes characterized by skeletal dysplasia, it seems reasonable to speculate that children who fall in the lower end of the normal growth standards are short statured because of an inherited abnormality in endochondral growth, the major process responsible for increase in stature. The possibility of mild hypochondroplasia in FSS patients cannot be entirely ruled out, since this condition can present with no stigmata other than short limbed FSS and brachydactyly. There are isolated reports of families with dominantly inherited brachymetacarpia and short stature but no other associated abnormalities. The presence of brachymetacarpia V in the parents of

*continued on page 14*

continued from page 13

affected FSS children suggests an autosomal dominant mode of inheritance of this characteristic. This trait is also fairly common even in the normal population. Therefore its association with FSS may be coincidental. However, since these tubular bone alterations were significantly more prevalent in the FSS children and their parents and siblings than in the normal population, an inherited trait is most likely responsible for these pleomorphic manifestations. Segregation analysis may help determine the mode of inheritance of these skeletal alterations.

The findings in these two studies illustrate the major role that endochondral ossification plays in de-

termining stature by expressing itself not only in overall height but also in disproportionate shortening of tubular bones in those with FSS.

The presence of tubular bone alterations in an otherwise healthy patient with FSS should be carefully evaluated before instituting therapy with growth hormone (GH). This is especially important since GH is now available in unlimited supply and pressure to treat the short child with the drug is again high. It may be reasonable to expect that children with FSS who also have skeletal abnormalities would respond less favorably to GH or require a higher dose of GH than would those with FSS and no bone abnormalities.

Fima Lifshitz, M.D.

## Parental Health Beliefs as a Cause of Nonorganic Failure to Thrive

Parental health beliefs and misconceptions about the constituents of a normal diet for infants are reported as a cause for failure to thrive in seven children (four male, three female), aged 7 to 22 months, who were evaluated for poor weight gain and deteriorating linear growth.

After a medical and nutritional evaluation, it was found that the caloric intake for these children had been restricted by their parents to such a degree that they were receiving much less than the recommended caloric allowance for their age and sex.

The parents instituted diets that were consistent with health beliefs that are currently in vogue and recommended by the medical community for adults who are obese or at risk for cardiovascular disease or both. These parents were concerned that their children would become obese, develop atherosclerosis, become dependent on junk food, or develop eating habits that the parents felt were unhealthy. However, these diets resulted in inadequate

weight gain and a decreased linear growth rate in the infants.

Nutritional counseling was provided, all unnecessary food restrictions were lifted, and the caloric intake was increased to the recommended allowance for age. The weight gain rate increased soon thereafter, and the linear growth rate increased within three months of improved nutritional therapy.

Exaggerated parental concerns over excessive food intake in childhood have resulted in additional cases of failure to thrive during infancy.

Pugliese M, Wyman-Daum M, Moses N, Lifshitz F. *Pediatrics* 1987;80:179.

**Editor's comment**—In the past few years, we have heard from many self-appointed experts in child care and nutrition who advocate numerous and often unsubstantiated "health beliefs." This report demonstrates what can happen if this "advice" is followed without appropriate medical supervision. Fear of obesity is quite prevalent in our population, and parents are well aware of the commonly held belief that if obe-

## Recovery From Post-Traumatic Anterior Pituitary Insufficiency

Usually, it is assumed that traumatic damage to the hypophysis persists and is barely reversible, except when the damage is due to an acute condition, such as diabetes insipidus. Eiholzer et al describe a patient in whom the sequelae of a severe trauma disappeared after many years.

At 7 years of age, the patient was in a car accident and was comatose for four months. His condition improved after insertion of a ventriculo-atrial shunt. A discrete spastic tetraparesis persisted, as did insufficiency of the anterior hypophysis, which led to

stity occurs in infancy it may persist throughout life. This may not be true, but it can certainly lead to unnecessary dietary restrictions in infancy nonetheless. Similarly, following a "healthy diet" to prevent atherosclerosis and eliminating so-called "junk food" from the diet are also very prevalent and are endorsed by the medical community. Even though the American Academy of Pediatrics and the American Medical Association have never endorsed low-fat, low-calorie diets for infants younger than 2 years of age, parents with misguided health beliefs have enforced these dietary recommendations for their infants, who then fail to thrive.

Junk food is an abused term. Indeed, there is no junk food, but there may be junk diets. High-calorie snacks are necessary for children, since they contribute up to one third of a child's usual dietary intake. A cookie or a chocolate sundae may be necessary and appropriate if the remainder of a child's diet is well-balanced. Eliminating these high-calorie snacks could result in an inability to ingest the calories that are necessary for growth in childhood.

Fima Lifshitz, M.D.

progressive growth retardation. The provocative tests showed an unsatisfactory response to growth hormone (GH). Plasma testosterone was low, and a secondary hypothesis was established. The child received replacement therapy for his endocrine deficiencies, and his growth rate was promptly normalized. A spontaneous increase in testicular volume was observed when the patient was 17 years old, and treatment with testosterone and human growth hormone (hGH) was discontinued. Two years later, treatment with thyroxine was stopped.

Eiholzer U, Zachmann M, Gnehm HE, Prader A. *Eur J Pediatr* 1986; 145:128-130.

**Editor's comment**—Post-traumatic deficiencies of the adeno-hypophysis arise in the following different ways: through hypothalamic lesions, following denervation due to damage of the stalk, by interruption of the long portal vessels, and by lesions directly affecting the anterior lobe. In the present case, the hypothalamic origin of the hormone deficiency could be excluded since the administration of thyrotropin-releasing hormone (TRH) did not produce an increase of thyroid-stimulating hormone (TSH). Apparently the pituitary was touched directly. The presupposition for the gradual recovery that was observed is the re-innervation of the stalk, the recanalization of the portal vessels, the regeneration of the necrotized pituitary tissue, or a combination of these (as shown in animal experiments conducted by Daniel and Prichard in 1975). The observation is especially important to the clinician, since it demonstrates that the pituitary may not be permanently damaged after a severe acquired lesion and that the function of the gland should be checked repeatedly after such a trauma.

Jürgen R. Bierich, M.D.

## Gross and Fine Motor Development in 47,XXY and 47,XYY Males

Males with sex-chromosome anomalies come to the clinician's attention because of their tall stature, as seen in those with Klinefelter syndrome, gynecomastia, and hypogonadism. In an ongoing study to define the natural history of children with sex-chromosome abnormalities, 14 boys with XXY and four with XYY were compared with matched controls.

Neuromuscular deficits, such as motor awkwardness and slow movement, were described in early childhood and continue to be present in the school-age boys. XXY boys have significantly lower mean scores for limb coordination, speed, dexterity, and gross motor activity than the matched controls. School intervention for reading deficiency had occurred in 15 of the 18 boys with aneuploidy in contrast with none of the 14 controls. In addition, auditory processing deficits and dyslexia were believed to play a greater role in decreased school performance than would have been expected. Hypermobility of the finger joints

and poor grasp seemed to hinder writing skills.

Findings from this study suggest that mild to moderate dysfunction in sensory motor integration occurs frequently in boys with sex-chromosome aneuploidy and is likely to be an additional factor that influences classroom performance.

Salbenblatt JA, Meyers DC, Bender BG, et al. *Pediatrics* 1987; 80:240.

**Editor's comment**—As the authors point out, these mild changes found in males with sex-chromosome aneuploidy can have a significant influence on both classroom performance and social integration of self-concept and adequate peer interaction. Clinicians must be aware of these problems and institute early intervention when they are recognized. Perhaps males with neuromuscular deficits such as those described should be screened by buccal smear determinations for the presence of Barr bodies and double quinacrine bodies. This could make an appropriate study.

Judith G. Hall, M.D.

## Life Expectancy in Down Syndrome

Life expectancy among patients with Down syndrome may be much higher than suspected, based on data from 1,341 Down syndrome patients in the British Columbia Health Surveillance Registry from 1952 to 1981. The important factor seems to be the presence or absence of congenital heart disease.

Among Down syndrome patients with congenital heart disease, 23% died during the first year of life and only 53% survived to age 20. In contrast, 90% of Down syndrome patients without congenital heart disease survived

to age 1 year and almost 80% survived to age 30. Clearly, patients with Down syndrome and congenital heart disease have a disproportionately higher mortality than those without congenital heart disease, particularly during the first year of life.

Baird PA, Sadovnick AD. *J Pediatr* 1988;110:849.

**Editor's comment**—These data are very important for pediatricians caring for children with Down syndrome, since families need assistance in planning appropriately for the child's lifetime and life expectancy.

Judith G. Hall, M.D.



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## MEETING CALENDAR

**July 8-10** International Symposium on the Marfan Syndrome. Baltimore, Maryland. Contact: Diane Heydinger, Johns Hopkins University School of Medicine, 720 Rutland Road, Baltimore, MD 21205 (301-955-2959)

**July 10-13** 20th Anniversary of the Clinical Genetics Conference. Baltimore, Maryland. Contact: Carlita Kearney, Johns Hopkins University School of Medicine, 720 Rutland Road, Baltimore, MD 21205 (301-955-2959)

**July 17-23** 8th International Congress of Endocrinology. Kyoto, Japan. Contact: Travel Planners—Kyoto Congress, Suite 150, GPM Building, San Antonio, TX 78216-5674 (512-341-8131)

**July 20-23** 15th International Auxology Congress. Exeter University, Exeter, England. Contact: Prof. J.M. Tanner, Room 115, Institute of Child Health, 30 Guilford Street, London, England WC1N 1EH

**October 15-20** 57th Annual Meeting of the American Academy of Pediatrics. San Francisco, California. Contact: American Academy of Pediatrics, 141 Northwest Point Boulevard, PO Box 927, Elk Grove Village, IL 60009 (800-433-9016 outside Illinois, 800-421-0589 in Illinois)

**October 27-31** 40th Postgraduate Assembly of The Endocrine Society. Franklin Plaza Hotel, Philadelphia, Pennsylvania. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

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# GROWTH

## Genetics & Hormones

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### Renal Disease and Growth Retardation

David R. Powell, M.D.

*Assistant Professor of Pediatrics  
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Medicine  
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Many individuals who develop chronic renal failure (CRF) as children fail to achieve an adult height consistent with their genetic potential.<sup>1</sup> Moreover, they often do not achieve a final height that is within the normal range despite delays in bone age and in the onset of pubertal development, events that should allow a prolonged period of growth to occur.<sup>1,2</sup>

While there is wide variability in the level of residual renal function capable of maintaining normal growth, the risk for growth failure probably increases when residual function is less than 30% of normal for age.<sup>3</sup> The lowest growth rates and the greatest losses of growth potential occur in infants with CRF.<sup>2,3</sup> Growth rates are also subnormal for older children with end-stage renal disease who are treated with hemodialysis<sup>4</sup> and probably for those treated with peritoneal dialysis as well.<sup>4,5</sup> In contrast, older children with milder degrees of CRF who do not require dialysis grow at rates that are closer to normal.<sup>2</sup> Interestingly, growth rates of children who have received renal transplants are usually not above the norm for age. However, the mean rate has steadily improved in recent years, especially with the advent of cyclospo-

rine therapy.<sup>5,6</sup> Of perhaps the greatest significance, catch-up growth is rarely observed either in children with CRF or in those with a renal transplant.<sup>2</sup>

Many factors contribute to growth failure in children with CRF. These factors are listed in the Table on page 2 and discussed in some detail below.

#### Hormonal Imbalance

The hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axes are two major endocrine systems that interact with growth-plate cartilage to regulate growth. Each axis is perturbed in individuals with CRF, and the uremic milieu may also directly impair the function of growth-plate cartilage itself. While mild hypothyroidism in adults with CRF results from abnormalities at multiple levels of the hypothalamic-pituitary-thyroid axis,<sup>7</sup> children with CRF nevertheless appear to be clinically euthyroid.<sup>8</sup> In contrast, the hypothalamic-pituitary-gonadal axis does appear to be compromised in children with CRF. At first, hypogonadotropic hypogonadism persists and puberty is delayed; later, hypergonadotropism is found.<sup>7</sup> These abnormalities do not necessarily lead to a significant shortfall in final height, however, since children with CRF often exhibit normal pubertal growth spurts.<sup>2</sup>

The growth hormone (GH)-insulin-like growth factor (IGF) axis is probably the major hormonal sys-

tem regulating bone growth. Apparently, these two hormones act in concert to produce growth: GH stimulates both differentiation of chondrocyte precursors in growth-plate cartilage and IGF-I production by many tissues including cartilage; IGF-I then stimulates clonal expansion of these differentiating chondrocytes.<sup>9</sup> GH levels are elevated in adults and children with CRF, probably because of GH overproduction.<sup>7,10</sup> Although past data suggested that IGF levels were low in CRF patients, recent work indicates that these earlier IGF measurements were in error,<sup>11</sup> and concludes that IGF-I and IGF-II levels are normal or increased in adults and growth-retarded children with CRF.<sup>11-14</sup> These data suggest that children with CRF may be resistant to the growth-promoting action of these two hormones. Alternatively, GH or IGF may be carbamylated or otherwise modified by the uremic milieu so that their biologic potency is decreased.

#### Energy Malnutrition

Inadequate energy intake clearly contributes to growth failure in

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## Renal Disease and Growth Retardation

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children with CRF, especially during infancy, when higher energy requirements for each kilogram of body weight are frequently not met.<sup>2,3,15,16</sup> Children and infants with CRF and severe associated malnutrition often respond to dietary supplements with improved growth, although gastrostomy or nasogastric feedings are often required in infants to ensure adequate energy intake.<sup>16</sup>

**Table.** Factors contributing to growth failure in children with chronic renal failure

|                                     |
|-------------------------------------|
| Hormonal Imbalance                  |
| GH-IGF axis                         |
| Hypothalamic-pituitary-thyroid axis |
| Hypothalamic-pituitary-gonadal axis |
| Energy Malnutrition                 |
| Uremic Toxins and Inhibitors of     |
| Hormone Action                      |
| Renal Osteodystrophy                |
| Sodium Bicarbonate Wasting          |
| Sodium Chloride Wasting             |
| Hyposthenuria                       |
| Prednisone Therapy                  |
| Psychosocial Dysfunction            |
| Hypertension                        |
| Anemia                              |

Rat models of CRF also demonstrate the association of malnutrition and growth failure: CRF rats eat much less food and gain much less weight and length than sham rats fed ad lib. However, malnutrition is not the sole cause of growth failure, since CRF rats gain significantly less weight and length than their pair-fed counterparts despite comparable energy intake.<sup>17,18</sup>

Indeed, malnutrition does not seem to be the major cause of growth delay in many children with CRF, since they often fail to grow better even when given dietary supplements.<sup>16</sup> In one study, only those children who initially ingested less than 75% of the recommended calories for age responded to caloric supplements

with improved growth, and growth velocities above the mean for age were rare.<sup>15</sup>

The way low energy intake causes growth failure is not understood, although a direct inhibitory effect on growth-plate chondrocyte metabolism seems likely. Energy malnutrition is also associated with high serum GH and low serum IGF-I levels; since growth improves and these levels return to normal with nutritional rehabilitation, it is possible that the effects of malnutrition on growth are modulated by IGF-I. However, energy-deficient and growth-retarded individuals with CRF have normal IGF-I levels, suggesting that malnutrition does not cause growth failure in CRF by affecting the GH-IGF axis.<sup>12,17,18</sup>

### Uremic Toxins and Hormone Inhibitors

CRF is associated with the accumulation or increased production of many molecules, some of which may directly impair growth. Phillips et al<sup>14</sup> found increased levels of a 14 kDa (kD) molecule in uremic adult serum. This poorly characterized substance is a generalized inhibitor of cartilage metabolism that blocks the growth stimulating effects of IGFs and other growth factors.

Also, IGF-binding protein activity and radioimmunoassay (RIA) levels of IGF-binding proteins are increased in the serum of CRF patients.<sup>12,19</sup> Recent evidence suggests that a purified 25 kD IGF-binding protein<sup>20</sup> markedly inhibits the growth of fetal cartilage in organ culture (Powell et al, unpublished observations). Further work will establish whether these uremic toxins and inhibitors contribute to growth failure in vivo and whether inhibitor levels are affected by malnutrition or by high protein intake.

### Renal Osteodystrophy

The inability of damaged kidneys to excrete excess phosphate and to synthesize 1,25 dihydroxyvitamin D leads to renal osteodystrophy (ROD) and associated secondary hyperparathyroidism.

Poor growth may be the result of disturbed cartilage metabolism and/or frank bony deformities. Radiologic and biochemical evidence of ROD is seen more often in short children and in those with renal function that is less than 20% of normal. However, evidence of ROD is often found in children with CRF who have normal height, while short children with CRF frequently have no signs of ROD.<sup>21</sup>

Treating older CRF children with phosphate restriction and vitamin D derivatives can result in accelerated growth, often by healing frank skeletal deformities, but true catch-up growth is rare.<sup>16,22</sup> However, preliminary studies suggest that children under 2 years of age who are treated with calcitriol grow at more normal rates. This therapy, then, may prevent some of the lost growth potential that usually occurs in infants with CRF.<sup>22</sup>

### Bicarbonate, Salt, and Water Losses

Children with obstructive or dysplastic renal disease and CRF often have renal tubular dysfunction and excessive urinary loss of sodium bicarbonate, sodium chlo-

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ride, and water. Children who are otherwise normal but have renal wasting of bicarbonate and chronic hyperchloremic metabolic acidosis grow poorly. The physiology responsible for this poor growth is unknown, but complete catch-up growth often occurs with oral bicarbonate therapy.

Growth failure can also occur when salt wasting leads to dehydration or when hyposthenuria leads to hypertonicity. In these cases, replacement therapy with salt or water should improve growth. Although these fluid and electrolyte disturbances can be effectively treated, salt and water wasting are sometimes difficult to diagnose, and all of these disorders can be difficult to manage in infants with CRF.<sup>2,16,23</sup> Undoubtedly, poor management of these disorders during infancy can lead to permanent loss of growth potential.

### Prednisone and Other Factors

Prednisone therapy, which is most often used in children with kidney disease for post-transplant immunosuppression, causes growth failure in a dose-dependent fashion. The mechanism for this growth failure is not clear, but it is thought that prednisone may directly damage chondrocytes or stimulate production of generalized inhibitors of cartilage metabolism.<sup>24</sup> Unfortunately, higher doses are used in those patients who have lost some degree of renal function due to episodes of graft rejection, and persistent growth failure is common in these individuals. Recently, it has been recognized that immunosuppression with cyclosporine permits the prednisone dosage to be lowered or discontinued, resulting in improved or catch-up growth in some transplanted children.<sup>6</sup>

Psychosocial problems may contribute to poor growth in some children with CRF.<sup>2</sup> Severe hypertension and anemia have been implicated as well. Further study is needed to evaluate the extent of the contributory roles of these factors in growth delay in these children.

### Treatment and Catch-Up Growth

In the past few years, pediatric nephrologists have aggressively attempted to maximize growth in infants with CRF. Most nephrologists now feel that infants and even older children with reduced renal function will remain short for chronologic age despite aggressive therapy to correct energy malnutrition, renal osteodystrophy, acidosis, salt wasting, and hyposthenuria.

Research continues into the factors responsible for this persistent growth failure. However, the almost uniform lack of catch-up growth in children with CRF is striking, suggesting that the mechanism for catch-up growth may be directly affected in these children. Unfortunately, the physiologic basis for catch-up growth is poorly understood.<sup>25</sup> Although the GH-IGF axis does not appear to play a major role in catch-up growth, and despite the fact that GH levels are already elevated in individuals with CRF, preliminary studies in children with CRF<sup>26</sup> and rats<sup>18,27</sup> show a short-term improvement in growth for those individuals receiving GH therapy.

These data suggest the need for long-term studies to examine whether GH therapy produces sustained catch-up growth in CRF children, and whether this treatment is associated with complications such as insulin resistance, worsening ROD, or accelerated decline in residual renal function. Growth data from these and other studies must be collected at optimal times and are best presented as growth velocity standard deviation scores (GVSDS). As Barrett et al<sup>1</sup> clearly demonstrate, GVSDS are most likely to demonstrate whether GH or any other therapy is associated with normal or catch-up growth in children with CRF.

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### In Future Issues

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# Lipodystrophy

William L. Clarke, M.D.  
Associate Editor  
Growth, Genetics, and Hormones

Lipodystrophy is a term used interchangeably with lipatrophy to describe a group of rare disorders characterized by hyperlipidemia, marked insulin resistance, and the absence of subcutaneous fat. Three lipodystrophic syndromes have been described: congenital or total lipodystrophy, total acquired lipodystrophy, and partial acquired lipodystrophy (Table). These are categorized on the bases of genetic inheritance, distribution of fat loss, and the age of onset.

*Congenital or total lipodystrophy* is an autosomal recessive disorder characterized by a generalized lipodystrophy that is present from birth. *Total acquired lipodystrophy* occurs sporadically and is not present at birth. *Partial acquired lipodystrophy* also occurs sporadically, but fat loss is confined to a single area of the body. Associated findings in all of these disorders include advanced height and bone age in childhood, hepatomegaly, and acanthosis nigricans. In addition, some individuals exhibit enlarged genitalia, and females may have polycystic ovarian disease. Systemic cystic angiomas, idiopathic hypertrophic subaortic stenosis, glomerulosclerosis or glomerulonephritis, pancreatitis, and thyroiditis also have been reported. Although statistical analyses are lacking, the prognosis for persons with these disorders is poor, with most affected individuals dying of the complications of liver disease in early adulthood.

Exemplary of congenital total lipodystrophy are three siblings whom we have had the opportunity to study over a period of 12 years at the University of Virginia Children's Medical Center. The oldest sibling, a female, presented at 3 years of age with hepatomegaly, total absence of subcutaneous fat, acanthosis, hyperlipidemia, and an

advanced bone age. Absence of fat and a protuberant abdomen had been noted by the mother when the child was 3 months old. By 13 years of age, she had developed insulin-resistant diabetes mellitus, virilization with clitoromegaly, polycystic ovarian disease, thyroiditis, proteinuria, and cystic angiomas. By age 20 she had also developed idiopathic hypertrophic subaortic stenosis.

The next-born sibling, also female, presented at 9 months of age with fat wasting, hepato-

extreme heterogeneity of the disorders make it difficult to characterize or rigorously evaluate the etiology or pathogenesis of these syndromes.

If a primary absence of fat cells were etiologic, then excess carbohydrate would be stored as glycogen, metabolized to lactate or triglycerides, or circulated in the bloodstream as glucose.<sup>1</sup> This, however, does not explain the presence of insulin resistance, virilization, or other associated findings.

Table. Lipodystrophic syndromes

| Type             | Inheritance         | Distribution of fat loss |
|------------------|---------------------|--------------------------|
| Congenital       | Autosomal recessive | Total                    |
| Total acquired   | Sporadic            | Total                    |
| Partial acquired | Sporadic            | Confined to single area  |

megaly, acanthosis, and hyperlipidemia. During her teenage years, she also developed virilization, polycystic ovarian disease, insulin-resistant diabetes, thyroiditis, cystic angiomas, and idiopathic hypertrophic subaortic stenosis. The third affected sibling, a male, presented at 6 years of age with subcutaneous fat wasting, acanthosis, and advanced height and bone age. Currently 18 years of age, he has yet to develop diabetes or hyperlipidemia.

## Pathogenesis and Etiology

The pathogenesis of the lipodystrophic disorders remains unclear. Various studies have suggested a variety of possible etiologies including a primary absence of fat cells, the presence of a lipolytic or insulin-antagonizing factor, hypothalamic/pituitary disease, and insulin-binding abnormalities. However, none of these can explain all of the abnormalities seen in these disorders. In addition, the limited numbers of patients available for study and the

Taton et al<sup>2</sup> extracted both a lipid-mobilizing factor (Chalmers factor) and an insulin-antagonizing factor (Louis factor) from the urine of a patient with total acquired lipodystrophy. When injected into normal mice, these factors produced hyperlipidemia and fatty infiltration of the liver as well as insulin resistance. In addition, injection of Chalmers factor produced hyperglucagonemia, which is known to be lipolytic and associated with carbohydrate intolerance. In contrast, Louis factor prevented the storage of triglycerides in adipocytes. Studies by other investigators, however, have not confirmed the existence of these factors in all patients.

Data from several studies led to the suggestion that lipodystrophy might be caused by a disturbance of the hypothalamic/pituitary axis.<sup>3</sup> Lack of growth hormone (GH) release following pharmacologic stimuli or during sleep, an acromegalic appearance, detectable levels of hypothalamic-releasing factors in the peripheral plasma, hyperprolactinemia, pres-

ence of both elevated basal and nonsuppressible adrenocorticotrophic hormone, and hyperresponsiveness of thyroid-stimulating hormone to thyrotropin-releasing hormone have been reported. In addition, Louis factor has been thought to be of pituitary origin. However, hypophysectomy has not reversed the metabolic or physical abnormalities associated with lipodystrophy.<sup>4</sup>

The lipodystrophic disorders share common features of extreme insulin resistance, virilization, and acanthosis in both obese and nonobese women with polycystic ovarian disease. In these syndromes, androgen levels correlate directly with the degree of insulin resistance. However, the etiologic relationship between the two findings remains unclear.

Kahn et al,<sup>5</sup> studying insulin binding in patients with extreme insulin resistance and acanthosis nigricans, have described two types of binding abnormalities and used them to classify patients with insulin resistance and acanthosis. Type A patients demonstrate a decrease in number of receptors or possibly a post-receptor defect and are characterized by onset of symptoms in childhood, with virilization and polycystic ovarian disease occurring typically. Type B patients display antibodies against the insulin receptor. These patients acquire their disorder later in life than do those with type A insulin resistance. Androgen excess in type B patients is unusual. Harrison et al<sup>6</sup> have recently reported the presence of a circulating inhibitor of insulin's action after it has been bound to its receptor in a patient with insulin resistance, acanthosis, and polycystic ovaries without lipodystrophy. How these studies in patients without lipodystrophy, but with many of the same clinical findings of the three lipodystrophic syndromes described above, are related to the lipodystrophies remains to be determined.

Oseid et al,<sup>7</sup> however, have demonstrated a decrease in binding affinity of insulin to the receptors on monocytes in congenital

lipodystrophy, and Kriaciunas et al<sup>8</sup> recently demonstrated a 50% decrease in insulin binding in the three patients with congenital lipodystrophy who were presented above. Using restriction fragment endonucleases, Kriaciunas et al have also demonstrated a unique defect in the portion of the insulin receptor gene that codes for the receptor's alpha subunit in these three siblings, but not in one of their nonaffected siblings nor in more than 100 normal controls or patients with diabetes. How this finding relates to lipodystrophy, acanthosis, virilization, polycystic ovaries, and the myriad of other abnormalities associated with lipodystrophy is not known, but the discovery of a unique genetic defect in this family raises the prospect of being better able to understand the etiology of the lipodystrophic syndromes.

### **Skeletal Maturation and Growth Velocity**

The etiology of advanced skeletal maturation and accelerated growth velocities in children with lipodystrophy has not been clarified. Undoubtedly, the elevated prepubertal androgen levels described in some individuals with lipodystrophy contribute to the advanced skeletal maturation and accelerated growth. However, the acromegaloïd appearance of some of these individuals has led to investigations of GH secretion in lipodystrophy. In our patients, the mean 24-hour integrated GH levels were within the normal range for our laboratory (1.7 to 3.9 ng/mL).<sup>9</sup> Since these studies were performed prior to the advent of GH pulse amplitude analyses, and since somatomedin-C/insulin-like growth factor I concentrations have not been reported in these disorders, it is obvious that further studies are needed.

### **Treatment**

Treatment of patients with lipodystrophy remains difficult and inadequate. Insulin requirements rise to 9,000 U/day and even this dose may fail to normalize blood glucose levels.<sup>10</sup> Caloric restric-

tion to 1,200 or fewer calories/day has been shown to increase insulin sensitivity, improve carbohydrate tolerance, and reduce lipid levels in patients with congenital and acquired lipodystrophy.<sup>11</sup> However, it is rarely possible to achieve the prolonged compliance with dietary therapy that is necessary to evaluate the long-term effects of such therapy on either carbohydrate tolerance or hyperlipidemia.

As stated previously, hypophysectomy fails to restore metabolic or physical abnormalities. Pimozide, an inhibitor of hypothalamic-releasing factors, has been used with limited success.<sup>4,12</sup> Plasmapheresis also has been used to reduce hypertriglyceridemia, but rebound hypertriglyceridemia has been found to occur within seven days.<sup>3</sup>

Fenfluramine, a serotonin inhibitor that was originally marketed to control weight, has some insulin-like activity, which includes reduction of blood glucose concentrations in type II diabetes and elevation of glucose uptake both in the forearm *in vivo* and in adipocytes *in vitro*.<sup>11</sup> In addition, fenfluramine lowers serum triglycerides by inhibiting their absorption and synthesis. Trygstad<sup>13</sup> stated that fenfluramine improved carbohydrate tolerance in patients with congenital lipodystrophy but noted that its use was accompanied by a decrease in caloric intake. In our laboratory, Wilson et al<sup>11</sup> compared the separate effects of fenfluramine and caloric restriction in congenital lipodystrophy. Carbohydrate tolerance improved initially in one of three patients, but the effect was sustained for only four to six weeks. Caloric restriction to 1,200 calories/day produced a much greater improvement in carbohydrate tolerance, but patients did not adhere to the regimen well.

In a recent trial, a dietary medium-chain triglyceride substitution for long-chain fatty acids successfully reduced serum lipid levels, hepatomegaly, and carbohydrate intolerance in a patient

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## Lipodystrophy

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with acquired total lipodystrophy.<sup>14</sup> Medium-chain triglycerides reportedly decrease hepatic glucose production and may directly stimulate insulin secretion. The patient reported in this trial experienced improved carbohydrate tolerance, an 83% decrease in insulin concentrations, a 37% decrease in plasma glucagon levels, and decreases in chylomicron and triglyceride levels, xanthomata, and liver size. This therapy may prove to be effective in others with lipodystrophy, but the long-term effects of a diet that fails to provide essential fatty acids and possibly accelerates premature atherosclerosis remain to be evaluated.

Typically, patients with lipodystrophy present early in childhood with few of the manifestations of the disorder and gradually develop the full constellation of abnormalities by adulthood. Frequently, the loss of subcutaneous fat is the only obvious initial physical finding. Pediatricians are encouraged to examine patients with subcutaneous fat loss carefully and to be aware of the many manifestations of the complicated multisystem lipodystrophic disorders. Until a registry of such patients is initiated and a protocol designed for the systematic evaluation of their physical and biochemical abnormalities, it may not be possible to understand the complex nature of these disorders or to design rational treatment strategies for affected individuals.

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## Letter From the Editor

Dear Colleagues:

Recently, several cases of leukemia in hypopituitary children receiving growth hormone (GH) were reported in *The Lancet* (1988;1:159). The pediatric endocrinologists of the world and the parents of children receiving GH were most fortunate to have strong leadership from the Lawson Wilkins Pediatric Endocrine Society. The officers appointed a senior committee of pediatric endocrinologists to serve on an ad hoc committee that would speedily accumulate and evaluate worldwide data in this regard and present recommendations to the scientific community.

Drs. Delbert Fisher and Louis Underwood convened a meeting of the committee members. Joining them were Drs. J.C. Job of Paris, who represented the European Society for Pediatric Endocrinology; S. Watanabe of the Cancer Institute in Japan; G. Antony of Australia; H. Dean of Canada; J. Fradkin, R. Miller, and J. Mills of the National Institutes of Health; L. Robison of the Pediatric Oncology Study Group in Minneapolis; and representatives from Eli Lilly, Genentech, Kabi Vitrum, and Sero Corporation. The meeting was cosponsored by the Human Growth Foundation.

After two days of intensive study and consideration, the ad hoc committee concluded that there possibly is a very small increase in the incidence of leukemia in GH-deficient patients treated with GH. On the basis of current evidence, however, the committee could not conclude that GH therapy was responsible for this possible increase. Current estimates indicate that if there is any risk to an individual patient, it is small; patients can be told this. Nevertheless, all patients receiving GH should be followed closely during and after therapy.

It is not my purpose to discuss the data that prompted these conclusions. Readers are encouraged to read the articles and discussions in *The Lancet*. It is my purpose (1) to reassure readers that, with the possible exception of patients in Japan, the increased incidence of leukemia in patients receiving GH is very small, and (2) to publicly express the gratitude of many endocrinologists, parents, and patients to the ad hoc committee members for their tremendous concern, logic, and efficiency.

Obviously, continued observations and collection and reporting of data are essential. However, we have reason to be optimistic in predicting that no significant increase in the incidence of leukemia will be found in association with GH therapy in the future.

For the Editorial Board,  
Robert M. Blizzard, M.D.,  
Chairman

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## Growth Without GH: The "Invisible" GH Syndrome

Four children with normal growth velocity, relatively low growth hormone (GH) concentrations as measured by radioimmunoassay (RIA), increased GH concentrations by radioreceptor assay (RRA), markedly increased RRA:RIA ratios, and normal somatomedin C assays were described. These unusual findings suggest the presence of a biologically active GH that is not detected by the usual RIA for GH. Therefore, the structure of the GH molecule in these children is believed to be unusual.

The authors postulate that the

GH molecule(s) secreted by these patients may be a product of the human growth hormone (hGH)-V gene rather than a product of the hGH-N gene, which is normally responsible for GH production. This gene was previously reported by other authors to be unexpressed, since no messenger RNA (m-RNA) derived from it could be detected in human cells.

Bistrizter T, Lovchik JC, Chalew SA, et al. *Lancet* 1988;i:321.

**Editor's comment**—The hypothesis expressed by the authors is tenable on the basis of the data presented, although not proven. To prove the hypothesis, m-RNA

for the hGH-V gene would have to be demonstrated in the patient(s). The findings are intriguing regardless. Assay measurements in these patients are the opposite of patients previously described by Kowarsky et al (*J Clin Endocrinol Metab* 1978;47:401); those patients had very low RRA:RIA ratios and slow growth, and were believed to secrete biologically inactive but immunoreactive GH. The four patients reported in the current article resemble some acromegalic patients (reported by Hizuka et al in *J Clin Endocrinol Metab* 1982;55:545) who also had significantly higher GH concentrations on RRA than on RIA.

Robert M. Blizzard, M.D.

## Placental Chromosomal Mosaicism Is Responsible for Variations in Growth Rates: Three Reports

If the placentas of children with unexplained intrauterine growth retardation (IUGR) are examined for a chromosomal aneuploidy, a surprisingly large number (perhaps as many as one third of cases of IUGR) will have chromosomal mosaicism confined to the placenta, with a normal cell line and an abnormal cell line. Now that chorionic villus sampling is being done on a regular basis for prenatal diagnosis, it has been found that about 5% of placentas have placental mosaicism with one normal cell line and another with a variety of different chromosomal aneuploidies. These findings suggest that there is a common explanation for IUGR that cannot be attributed to other causes such as maternal smoking or a syndrome: namely, the presence of cytogenetic abnormalities confined to the placenta. Most interesting are the new reports that mosaicism confined to the placenta may also be responsible for allowing fetuses with certain types of chromosomal problems to be carried to term.

Kalousek and McGillivray have recently reported the presence of

a normal cell line in all of the placentas recovered from fetuses with trisomy 18 and trisomy 13 that were born alive. By contrast, trisomic fetuses that are miscarried spontaneously as abortions or stillbirths do not have mosaicism or cytogenetically normal cells in their placentas. It appears that the normal cell line allows such fetuses to survive long enough to be born alive at term.

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**Editor's comment**—Since the placenta is fetal in origin, we have assumed that it has the same chromosomes as the baby. The work described in these reports suggests that 5% of placentas have some cells that are cytogenetically different from those of the baby. When the placenta has abnormal cells but the baby has only normal cells, the baby may have IUGR. When the baby has abnormal cells but the placenta has some normal cells, the abnormal fetus may survive to term.

Judith G. Hall, M.D.

## Mapping and Screening in Families With Multiple Endocrine Neoplasia Type 2A: Four Reports

Recently, multiple endocrine neoplasias type 2A have been mapped to chromosome 10. A number of polymorphic DNA markers around the gene allow prediction in most families of those individuals who are carriers of the gene. In addition, prospective screening annually for manifestations of the disease appears to be effective in prevention of morbidity and mortality. For example, provocative tests to guarantee the release of calcitonin can be used to monitor whether or not "medullary" thyroid carcinoma is present, and 24-hour urine screening for both epinephrine excretion and the ratio of urinary epinephrine to norepinephrine allows detection of proliferation of the adrenal medulla before life-threatening manifestations occur.

An 18-year follow-up study of a large family by Gagel et al suggests that total thyroidectomy, when done at the first appearance of increased calcitonin secretion, is curative since there were no recurrences or metastatic diseases in their patients. Parathyroid dis-

continued on page 8



## Multiple Endocrine Neoplasia Type 2A

continued from page 7

ease seemed to occur only in those patients with well-established medullary thyroid carcinoma or pheochromocytoma. Because more than 50% of affected individuals within the family eventually developed adrenal medullary abnormalities, screening in such families is mandatory.

Sobol H, Salvetti A, Bonnardel C, et al. *Lancet* 1988;i:62.

Gagel RF, Tashjian AH, Cummings T, et al. *N Engl J Med* 1988; 318:478-484.

Mathew CGP, Chin KS, Easton DF, et al. *Nature* 1987;328:527-528.

Simpson NE, Kidd KK, Goodfellow PJ, et al. *Nature* 1987;328: 528-530.

**Editor's comment**—*The potential for malignancy of multiple endocrine neoplasia type 2A is frightening, but these new chromosomal and metabolic screening techniques allow us to recognize family members at risk. The screening techniques also suggest clear and reliable methods to be used in following at-risk individuals.*

Judith G. Hall, M.D.

## Clinical Findings in Twelve Patients with Mucopolysaccharidosis IV A (Morquio Syndrome): Further Evidence of Heterogeneity

### (I) Clinical and Biochemical Findings

Morquio syndrome has long been known as a distinct mucopolysaccharidosis (MPS) characterized by short trunk dwarfism with skeletal radiographic changes quite distinct from those of the other mucopolysaccharidoses, as well as corneal clouding, enamel dysplasia, and urinary excretion of keratin sulfate. In recent years, heterogeneity in Morquio syndrome has been delineated, with three main types described: MPS IV A, associated with N-acetylgalactosamine-6-sulfate sulfatase deficiency; MPS IV B, with beta-galactosidase deficiency; and MPS IV C, with mild manifestations in which the enzyme defect has not been determined.

The authors describe the clinical findings in 12 cases of MPS IV A and document markedly variable clinical presentations, with some cases only mildly affected. Nevertheless, all cases show deficiency of N-acetylgalactosamine-6-sulfate sulfatase in fibroblasts. The patients with the mildest clinical presentation showed a high residual enzyme activity, although several

had markedly diminished enzyme activity.

The urinary glycosaminoglycans (GAGs) were also examined in all patients by a two-dimensional electrophoresis technique that proved to be highly reliable and efficient. In particular, no false-negative results were obtained, which is often a problem with routine screening methods.

The authors divided MPS IV A into three subgroups: the severe "classical" type, an intermediate type, and a mild type, all caused by N-acetylgalactosamine-6-sulfate sulfatase deficiency. Residual enzyme activity may be an important prognostic indicator for each subgroup.

### (II) Dental Findings

Dental changes associated with Morquio syndrome have been recognized for some time. They are characterized by a thin enamel layer, with tooth surfaces marked by numerous, minute, irregularly distributed pits. The teeth, which are smaller than normal, are separated by spaces and the enamel appears to be structurally weak. Radiologic examination confirms

the thinness of the enamel and abnormalities of cusp formation. These dental abnormalities are unique to Morquio syndrome and are not found in any of the other mucopolysaccharidoses or spondyloepiphyseal dysplasias.

The authors studied the clinical and radiographic dental changes in 12 patients with MPS IV A and found varying degrees of the characteristic dental changes in all. These dental changes, however, are not present in either MPS IV B (beta-galactosidase deficiency) or MPS IV C (enzyme defect unknown). Although these dental abnormalities are present in all cases of MPS IV A, they may only be demonstrable radiologically in some clinically mild atypical cases. The dental changes are highly specific and can be extremely useful in the diagnosis of clinically atypical cases of MPS IV A.

### (III) Odontoid Dysplasia

Spinal cord compression in the upper cervical region related to odontoid dysplasia is a major complication of Morquio syndrome. In addition to the odontoid hypoplasia, spinal cord compression is thought to be due to the associated ligamentous laxity and hypertrophy of the posterior longitudinal ligament. The pectus carinatum and sternal protrusion invariably found in these patients might act as a protective mechanism in some cases by limiting neck flexion.

The authors studied the cervical spine radiographically in 12 patients with Morquio syndrome; all showed evidence of odontoid dysplasia. In seven, it was defined as minor and in none of these was there evidence of instability. In five patients, the odontoid dysplasia was defined as major, with evidence of atlantoaxial instability in all five. The five patients with severe dysplasia and instability had classical Morquio syndrome, while the seven with minor dysplasia had milder atypical forms.

Long-term follow-up, with detailed neurological assessment, is essential in patients with Morquio syndrome. Any suggestion that the upper cervical cord is compromised by atlantoaxial instability should be investigated further by computerized tomography (with contrast dye) or by magnetic resonance imaging (MRI) so that the possibility of posterior fusion of the upper cervical spine can be considered in patients likely to benefit from this procedure. The degree of odontoid hypoplasia correlates well with the overall clinical severity of the condition, although the patients were of different ages when studied. Indeed, age-related variation in the dysplasia is another factor that must be taken into account.

Nelson J, Broadhead D, Mossman J. *Clin Genet* 1988;33:111.  
Nelson J, Kiniron S. *Clin Genet* 1988;33:121.

**Editor's comment**—These papers clearly demonstrate that, in addition to the known genetic heterogeneity in Morquio syndrome, there is significant clinical variability within individuals having the same enzyme deficiency state (N-acetylgalactosamine-6-sulfate sulfatase). These findings are similar to those that have been described in the other mucopolysaccharidoses: ie, in MPS I, deficiencies of alpha-1-iduronidase can be associated with typical Hurler syndrome, the very mild Sheie syndrome, or a variety of intermediate clinical states known as "compound heterozygotes"; the mild and severe forms of the Hunter syndrome associated with iduronate sulfate sulfatase deficiency; and the mild and severe forms of the Maroteaux-Lamy syndrome (MPS VI) associated with deficiency of arylsulphatase B. Thus, there appears to be both inter- and intramolecular heterogeneity in these disorders. Deficiencies of different enzymes due to mutations in different genes may produce similar clinical features:

ie, the San Filippo syndrome (MPS III A, B, C, and D) and Morquio syndrome (MPS IV A, B, and C). In contrast, different mutations along the same gene, resulting in variable deficiencies of the same enzyme, can produce marked clinical variability with severe and mild forms of the same phenotype.

The authors found that the enamel hypoplasia characteristic of Morquio syndrome is seen in all patients with MPS IV A, but is not present in MPS IV B or C. It therefore may be of diagnostic potential in cases of MPS IV, although in the mild forms of the disease, radiographs may be necessary to detect the enamel dysplasia. It is of interest that it is the enamel that is involved in Morquio syndrome, which is characterized by lysosomal vacuolization in epithelial-like cells. In osteogenesis imperfecta, where collagen is involved, it is the dentin that is abnormal.

Finally, the authors describe the variability in odontoid hypoplasia and atlantoaxial instability in pa-

tients with Morquio syndrome. Odontoid hypoplasia is characteristic of numerous skeletal dysplasias, including Morquio syndrome, the spondyloepiphyseal dysplasias, and metatropic dysplasia. Although it had been considered that all patients with Morquio syndrome have C1/C2 fusion of the spine because of the inevitability of atlantoaxial instability and spinal cord compression, the authors here demonstrate that in the mild forms of MPS IV A, there was no evidence of instability despite minor evidence of odontoid dysplasia. Nevertheless, all patients with Morquio syndrome must be followed longitudinally and with careful neurological and radiographic assessment of the C1/C2 area and cord. MRI, CT scanning, and neurophysiological studies should be done if there is any evidence of instability. If any evidence of spinal cord compression exists, fusion of the cervical spine is mandatory.

David L. Rimoim, M.D., Ph.D.

### Genomic Imprinting— Genes Inherited From the Father May Act Differently Than the Same Genes When Inherited From the Mother: Four Reports

Research on embryogenesis in the mouse, utilizing transplantation and transgenic mice, indicates that maternally derived genes seem to play a greater role in the early development of the embryo, while paternally derived genes play a greater role in the development of the extraembryonic membranes. The pattern of DNA methylation is different and depends on whether the alleles on the mouse chromosomes are maternally or paternally derived. These observations have been interpreted to suggest that differential imprinting of the genome occurs during male and female gametogenesis. These

findings may help to explain why human diseases such as myotonic dystrophy and Huntington disease, both autosomal dominant disorders, may vary in severity depending on which parent passed on the gene.

Marx JL. *Science* 1988;239:352-353.

Solter D. *Trends in Genetics* 1987;3:23-27.

Reik W, Collick A, Norris ML, et al. *Nature* 1987;328:248-251.

Sapienza C, Peterson AC, Rossant J, et al. *Nature* 1987;328:251-254.

**Editor's comment**—This new work is startling, but "imprinting" has been observed by several groups. This suggests there are many such mechanisms at work in embryogenesis and early development that may be critical to normal growth.

Judith G. Hall, M.D.

## Chronic Intermittent Elemental Diet Improves Growth in Children With Crohn's Disease

Inadequate caloric intake over a prolonged period of time is considered the major cause of growth failure in children with Crohn's disease. However, appropriate nutritional therapy may reverse growth retardation and may even improve the clinical status of children with the disease. Seven boys and one girl, ages 9.8 to 14.2 years, with Crohn's disease and growth failure, were evaluated for a period of one year while receiving standard medical therapy. During the second year of the study, these children were given continuous feedings of an elemental diet (Vivonex) at night by nasogastric tube. These feedings were given for one month every four months for a total of three months of nutritional therapy. Four children, matched for age and disease, received standard medical therapy throughout the two years of the study. The parameters measured in all children were height, weight, triceps skinfold and mid-arm circumference measurements, Tanner stage of sexual development, the Crohn's disease activity index (CDAI), bone age, and prednisone intake. Hemoglobin, lymphocytes, serum albumin, iron, total iron-binding capacity, folic acid, and urinary creatinine were also evaluated.

Children receiving the intermittent elemental diet showed an increased annual mean growth velocity of  $7.0 \pm 0.8$  cm, as compared with their previous growth velocity of  $2.9 \pm 0.4$  cm. During the same period, children in the control group had a growth velocity of  $1.7 \pm 0.8$  cm ( $P < 0.01$ ). Significantly, patients treated with the elemental diet had greater weight gains, increased triceps skinfold thickness and arm muscle circumference, and increased creatinine excretion. In addition, the CDAI and prednisone

intake were reduced during the year of elemental diet feedings, compared with levels during the year of standard therapy and in the control group ( $P < 0.001$ ). However, no differences were noted in the advancement of bone age and pubertal development and in other biochemical and nutritional parameters between the groups.

Belli DC, Seidman L, Bouthiller AM, et al. *Gastroenterology* 1988; 94:603-610.

**Editor's comment**—This well-designed study provides further evidence that nutritional rehabilitation of children with Crohn's disease may reverse growth retardation and promote clinical improvement. In particular, it showed that intermittent elemental enteral feedings, given for one month out of every four months for one year, are sufficient to triple the growth rate. The mechanisms whereby elemental enteral feedings improved growth go beyond the provision of calories. The enteral feedings not only provided calories, but all necessary nutrients, while eliminating all other food intake. Additionally, children received supplemental vitamin K, folic acid, and more importantly, elemental iron. Any one or all of these nutrients could have contributed to the improved growth.

The effect of "bowel rest" while the children receive infusions of monomeric enteral feedings also may have played a role in reducing the antigen load and decreasing the disease activity. This may have reduced steroid needs, thereby allowing for more growth. The lessening of the inflammatory intestinal process may have also resulted in decreased energy needs by reducing the hypermetabolic effects of the diseased bowel and by alleviating anorexia. Whatever the mechanism, nutritional rehabilitation of Crohn's disease patients is essential, and should be attempted even before growth failure occurs.

The nutritional dwarfing of children with Crohn's disease is evident, even though they appear well adapted to decreased nutrient intake. Often, dietary intake is not reduced below the level needed to maintain body weight and height, but is insufficient for normal growth. Only stable isotope measurements may detect malnutrition in well-adapted Crohn's disease patients whose growth has slowed as an adaptive response to decreased nutrient availability. Treatment must include provision of all the necessary nutrients for growth, with surgery contemplated only if appropriate nutritional therapy fails.

Fima Lifshitz, M.D.

## Growth of Immigrant Children in the Newcomer Schools of San Francisco

This study evaluated the effects of migration and nutritional change on the heights and growth velocities of four groups of immigrant children (Chinese, Filipino, Hispanic, Southeast Asian) who were 5 to 12 years of age when they enrolled in one of three San Francisco schools. A low score on an English-language achievement test was the criterion for study entry. Education regarding nutrition was provided during the study.

At the initial examination, all four groups had mean heights and weights between the fifth and 25th percentiles, as calculated by United States reference population growth curves. In general, the Hispanic and Filipino children were above the mean when weight was compared to height (>50% of children exceeded the mean), and the majority of the Chinese and Southeast Asian children were underweight for their height. During 12 months of observation, the children in all groups showed catch-up growth, with the growth velocities being above the 50th

percentile of the Fels longitudinal growth velocity curves for U.S. children.

The authors concluded that the catch-up growth indicated a growth deficit as a direct consequence of their previous environment and that they were malnourished (see editor's comment). The authors also noted that interpreting these data was difficult because they knew little about these children prior to their arrival in the U.S. For example, many of the Southeast Asian children had been in refugee camps where they may have received only minimal food and medical care.

Schumacher LB, Pawson IG, Kretchmer N. *Pediatrics* 1987; 80:861-868.

**Editor's comment**—Readers are strongly encouraged to read the article entitled "Malnutrition: Definition, Incidence, and Effect on Growth" by Dr. David Seckler (Growth, Genetics, and Hormones, Volume 1, Number 2). After reading Dr. Seckler's article, readers may question, as I do, whether the conclusion of Schumacher et al that these immigrant children were malnourished is necessarily correct. Dr. Seckler suggests that children grow in accordance with their nutritional intake, but that bigger is not necessarily better. The implications of Seckler's article may have gone unnoticed by many. If so, that is unfortunate since that article is extremely pertinent to our concepts of growth and nutrition.

Schumacher et al may be right in concluding that malnutrition was responsible for the short stature of the patients evaluated in their study, but if so, the malnutrition had to be one that was related to a specific factor or factors and not to calories alone, since the Hispanic and Filipino children were overweight for height. The Hispanic and Filipino population negated that consideration.

Robert M. Blizzard, M.D.

## A Wide Variety of Different Mutations of Collagen Seem to Be Responsible for Most Cases of Osteogenesis Imperfecta

Collagen is responsible for much of the strength of connective tissue. It consists of a triple helix of polypeptides, each with repeating segments of amino acids, with every third amino acid being a glycine molecule. The three polypeptides are tightly wound together, and glycine occupies the axial position. Thus, when mutations occur at a glycine site, the molecule is greatly weakened. Each collagen molecule (more than ten have been described) may be constructed of the same or combinations of different polypeptides.

Most cases of osteogenesis imperfecta (OI) are associated with mutations in type I collagen. Clinical effects depend on the particular position and domain of the collagen molecules in which a mutation or substitution occurs;

the closer the mutation occurs to the carboxyl terminus of the polypeptide of type I collagen, the more severe the clinical defect. Specific mutations have been identified in most cases of the lethal type of OI and for many non-lethal types. Each family has a distinct mutation that is different from mutations found in other families. Identification of a specific alteration or mutation can be used to recognize carriers in a particular family and to assist in prenatal diagnosis.

Sykes B. *Nature* 1987;330: 607-608.

**Editor's comment**—OI, a relatively common bone disorder that leads to short stature and multiple fractures in most cases, has several clinical subtypes. Recent research on collagen demonstrates the molecular basis for the disease and allows prenatal diagnosis. Recently, Byers brilliantly reviewed the subject of OI in this publication (vol. 4, no. 2). Readers are encouraged to read Dr. Byers' article.

Judith G. Hall, M.D.

## Prenatal Diagnosis of Congenital Adrenal Hyperplasia

The use of a combination of DNA probes for polymorphisms both within the 21-hydroxylase gene and in and around the closely linked HLA region permits reliable prenatal diagnosis of 21-hydroxylase deficiency in more than 95% of families at risk for having a child with congenital adrenal hyperplasia. Chorionic villus sampling during the first trimester permits early detection of the defect, and also provides the option of using intrauterine therapy in families where there is severe salt loss and significant masculinization of females.

Since masculinization is likely to have occurred prior to the prenatal diagnostic procedure, the current

recommendation is to treat the mother with dexamethasone as soon as the first menstrual cycle is missed. Treatment is continued until the results of chorionic villus sampling determine whether the fetus is male or female and whether it is affected or not. If the fetus is female and affected, treatment is continued throughout pregnancy.

Dreno B, Meignier M, Bignon JD, et al. *Lancet* 1987;ii:1272-1273.

**Editor's comment**—Although many families will opt for prenatal treatment of 21-hydroxylase deficiency, others will opt for termination of affected pregnancies. The new linkage techniques allow accurate detection and, therefore, offer more options to family and physicians.

Judith G. Hall, M.D.



## Increase of Serum Lipids and Serum Lipoproteins in Girls on Therapy With Estrogen and Norethisterone for Height Reduction

Weninger et al measured the levels of serum cholesterol and triglycerides in 23 tall girls, ages 11.2 to 15.5 years (mean  $12.7 \pm 1.1$ ) whose height was 2 to 4.2 standard deviation scores above the mean for age, prior to and during therapy with a combination of ethinyl estradiol (0.5 mg/day orally) and norethisterone (10 mg/day on days 21 to 25). These patients were followed for 11 to 24 months.

Serum cholesterol and triglyceride levels rose significantly (cholesterol,  $4.27 \pm 0.93$  mmol/L v  $5.33 \pm 0.65$ ,  $P < 0.001$ ; triglyceride,  $0.95 \pm 0.28$  mmol/L v  $1.82 \pm 0.57$ ,  $P < 0.001$ ) after three months of therapy. In a subgroup of 11 girls, low-density and high-density lipoprotein levels were determined; both rose significantly above baseline values after three months of treatment.

These investigators also evaluated the effect of norethisterone on the serum cholesterol and triglyceride concentrations immediately before and after five days of administration. No immediate effects of norethisterone were evident. After the cessation of all therapy, elevated lipid levels returned to pretreatment levels within 3 to 12 months in all but two patients.

The authors point out that the observed increase in lipids and lipoproteins during therapy for tall stature is in contrast to the physiological decrease in lipids that occurs during adolescence. Therefore, they conclude that therapy rather than sexual maturation is responsible for the lipid and lipoprotein changes they observed.

Weninger M, Frisch H, Schober E, et al. *Acta Paediatr Scand* 1987; 76:500-503.

## Pubertal Development in Male Hypopituitarism

In hypopituitary adolescents, puberty takes a different course than it does in normal individuals, patients with hypogonadotrophic hypogonadism, and those with isolated growth hormone deficiency (IGHD).

Martinez et al analyzed the course of sexual maturation in 65 male patients with hypopituitarism who were treated at the Hospital de Niños in Buenos Aires between 1965 and 1982. Eighty-two percent of the children with IGHD and 32.5% of those with multiple pituitary hormone deficiencies (MPHD) experienced spontaneous puberty. Thirty-six patients

were followed longitudinally. Fifteen showed spontaneous sexual development commencing at a chronological age (CA) of 15 years, 3.2 years later than in normal Argentinian boys. Mean bone age (BA) was 10.4 years. There was no difference in BA between patients with IGHD and MPHD. Plasma testosterone was slightly but not significantly higher in these patients than in the controls.

Eleven of the 15 patients received human growth hormone (hGH) continuously; four could not be treated. Peak height velocity was  $7.49 \pm 1.7$  cm/year in the treated subjects and  $6.67 \pm 2.3$  cm/year in the untreated ones. Mean CA of the 21 subjects with MPHD whose sexual maturation

**Editor's comment**—*Treatment of tall stature with estrogen has been used for several decades. Usually, the therapy is reserved for girls who are experiencing significant psychological problems because of tall stature. It has been stated that the earlier treatment is begun, the greater the reduction in adult stature. It has been well documented that height reduction can be achieved. However, there are numerous side effects associated with treatment. Do the benefits of treatment justify the risks?*

*Weninger et al have observed a marked increase in serum lipids and lipoproteins during combined estrogen and norethisterone treatment for height reduction in tall girls. Although this increase is transient, reverting to pretreatment values once therapy is concluded, the long-term effects of elevated lipids cannot be known at this time. Pediatric endocrinologists are cautioned that hormonal therapy for height reduction in tall girls may not be without significant risk and should be reserved for patients for whom counseling is not effective. The side effects described here should be discussed with the parents before initiating therapy.*

William L. Clarke, M.D.

## Long-Term Monitoring of Treatment With r-hGH by Serial Determinations of Type III Procollagen-Related Antigens in Serum

The measurement of type III procollagen (P-III-NP) by two radioimmunoassays before and during treatment with human growth hormone (hGH) in 20 patients with GH deficiency was reported. One assay (RIAgnost assay) recognized the intact propeptide predominantly. The second (FAB assay) recognized both the intact propeptide and a smaller monomeric peptide.

In childhood, P-III-NP levels vary with age and correlate significantly with the growth velocity curves. In the 20 GH-deficient patients studied by the authors, P-III-NP and somatomedin-C (Sm-C) levels increased after three days of recombinant hGH (r-hGH) therapy (2 IU/m<sup>2</sup>, six or seven days per week). The values increased within one month to those usually seen in healthy children, but Sm-C levels did not increase above the values seen at three days.

Only two parameters—lower

was induced by gonadotropin was  $19.04 \pm 2.2$  years at start of treatment, while BA was  $12.94 \pm 0.8$  years. Plasma testosterone reached physiological values quickly, except in two patients who showed no response. Mean growth velocity of the patients who simultaneously received treatment with hGH was  $6.11$  cm/year and only  $4.91$  cm/year in those who did not receive hGH.

Martinez AS, Heinrich JJ, Rivarola MA, Bergada C. *Eur J Pediatr* 1986;145:384-388.

**Editor's comment**—The observations confirm the often-published experience that the less global the central lesion is, the more

frequently spontaneous puberty occurs. The retardation of spontaneous puberty in the non-hypogonadotropic patients amounted to 3.2 years over healthy Argentinian boys. BA, however, corresponded to the standard of normal boys at start of puberty. Growth velocity was within the normal range in these boys. However, the observation that patients who did not receive GH also had high growth velocities appears to be remarkable. Their mean height velocity amounted to  $6.67$  cm/year, a value slightly but not significantly below that of the hGH-treated boys. For patients with GH deficiency, this value is surprisingly high.

Jürgen R. Bierich, M.D.

skeletal age and higher basal P-III-NP values as determined by the RIAGnost assay—studied during the pretreatment period proved to be associated with height increase after six months of therapy. Both seemed to have predictive relevance to growth velocity. Levels of basal Sm-C, alkaline phosphatase, or P-III-NP (by FAB assay) did not have predictive relevance for growth velocity during this period. The increases of P-III-NP levels following three days or one month of GH treatment did not correlate with the increase observed in growth velocity during the six months of treatment.

P-III-NP serum antigens measured by both assays correlate with each other but provide different information. The RIAGnost method recognized intact propeptide and may be more sensitive than the FAB assay in reflecting unstimulated basal collagen metabolism. The authors postulate that the correlation between the normal P-III-NP values obtained by the RIAGnost assay before treatment and the subsequent growth response indicates that reasonable normal collagen synthesis may be a prerequisite for a satisfactory growth response to

hGH. The authors further postulate that this is important because hGH is proposed as possible therapy for diseases in which disturbances of connective tissue or bone metabolism are suspected, such as in Turner syndrome. In patients with Turner syndrome, low basal levels of P-III-NP are reported, and the growth response to hGH is not as striking as that observed in patients with GH deficiency.

Danne T, Gruters A, Schnabel K, et al. *Pediatr Res* 1988;23:167.

**Editor's comment**—A marker to determine the propensity of various patients to grow well when hGH is administered is very much needed. A better understanding of the roles of various types of collagen in the growth process and their controlling factors, such as hGH, is also much needed. Danne et al have the methodology that permits them to begin the study of such phenomena. The importance of this article remains unknown, but it is presented because of its potential therapeutic implications and its succinct review of what is currently known about the effect of hGH on collagen synthesis.

Robert M. Blizzard, M.D.

## Growth of 519 Small-for-Gestational-Age Infants During the First Two Years of Life

Tenovo et al have carefully followed the physical growth of 519 small-for-gestational-age (SGA) infants for a period of two years. SGA infants were defined as those below the tenth percentile on growth curves generated at the authors' institution. These infants were compared to 4,517 term infants whose length and weight were appropriate for gestational age. The authors used the Rohrer's Ponderal Index (PI)

$$\frac{\text{weight (g)}}{\text{length}^3(\text{cm})} \times 100$$

to classify the SGA infants.

Infants who were small with respect to weight and length (Type I intrauterine growth retardation [IUGR]) tended to have a normal PI, while those who were small with respect to weight only or who had disproportionate growth had a low PI. The latter infants have type II, or disproportionate, IUGR.

Approximately 92% of the SGA infants and 94% of the control infants took part in the follow-up study two years after birth. In addition to the comparison studies between the two groups, the authors utilized stepwise logistic regression analysis for determining variables, such as maternal smoking and toxemia, that might best explain the small neonatal size.

The findings demonstrate that SGA infants with a low PI were taller and had a larger head circumference at age 24 months than the term infants with a normal PI. Among preterm SGA infants, the degree of IUGR appeared to have no effect on later growth. The catch-up growth in SGA infants occurred in the first three months after birth although they remained significantly smaller than the control infants.

The type of IUGR affects later growth. Type II IUGR infants (PI

*continued on page 14*

**Growth of 519 SGA Infants***continued from page 13*

below the tenth percentile) grow better than type I SGA infants. In many cases, infants with type II IUGR have some nutritional deficit during the late stages of gestation and their growth potential is thus not permanently affected. Other studies have confirmed the finding that prolonged IUGR (which would result in a normal PI) is associated with poor growth in infancy. Later growth, however, could be predicted by the degree of weight retardation. By the age of 2 years, one in every four SGA infants, regardless of PI, still had a weight below the tenth percentile. The authors also demonstrated that the risk factors most often related to poor intrauterine growth are maternal toxemia, maternal smoking of more than ten cigarettes a day, multiple pregnancies, and the birth of a previous SGA infant. Unfortunately, these data were not analyzed with respect to the type of IUGR.

### Predictive Value of Minor Anomalies: Association With Major Malformations

The report is part of an ongoing study of congenital anomalies in white newborns. In this study, in which 4,305 babies were scored for 114 minor physical findings and for all major anomalies, these data confirm the previous hypothesis that infants with three or more minor anomalies are at increased risk of having a major anomaly. In this study, the risk for a major anomaly in the presence of multiple minor anomalies was only 20%; previous studies found a much higher incidence. Less than 4% (3.76%) of the 4,305 babies had a major malformation. Approximately five sixths of these major malformations were considered significant and required intervention; the remaining one sixth were

Tenovuo A, Kero P, Piekkala P et al. *Acta Paediatr Scand* 1987;76:636.

**Editor's comment**—Although the article describing this study is somewhat difficult to read, the information presented is significant. The study demonstrates the difference between prolonged and short-term IUGR on future growth. Similar studies of infants from different populations should be carried out to confirm these findings. In addition, long-term follow-up studies of childhood growth and final adult height in these infants are required to provide better predictive information for pediatricians who counsel parents concerning their child's growth, and to help design studies directed at increasing our knowledge about growth reduction in these children. Parents of SGA infants should be advised that these infants will most likely remain smaller than average throughout the first two years of life.

William L. Clarke, M.D.

thought to require no special care or treatment. The 3.2% incidence of major malformations requiring intervention is higher than previously reported. With regard to minor anomalies, 28% of the babies studied had one such anomaly, 8% had two, and 3.1% had three or more.

Leppig KA, Werler MM, Cann CI, et al. *J Pediatr* 1987;110:531-537.

**Editor's comment**—This study indicates that the presence of multiple minor anomalies is a good predictor of a major malformation and that children with minor anomalies should be studied more intensively. It also indicates that minor anomalies are very common in the general population and that the presence of one or two minor anomalies should not cause great distress for the parents or the physician.

Judith G. Hall, M.D.

### Effects of Testosterone Therapy for Pubertal Delay

Wilson et al reviewed the charts of 50 adolescent boys treated with testosterone enanthate in oil to determine the long-term effect of testosterone therapy on growth and sexual development. Each boy received a total of four 200-mg injections, each given at three-week intervals. The authors also reviewed the charts of 38 adolescent boys who did not receive treatment.

Follow-up data were requested from subjects whose baseline visit was at least two years earlier. Nineteen (58%) of eligible treated subjects and 11 (52%) of eligible untreated subjects responded. A height Z score (the number of standard deviations away from the mean height for age) was calculated for each boy, and bone ages were obtained and read using the method of Greulich and Pyle. Adult height was predicted by a computer program based on the method of Bayley and Pinneau. The mean bone age delay, height Z score, average Tanner stage, predicted adult height, growth rate, serum testosterone, and somatomedin C concentrations, as well as midparental heights, were not significantly different between the treated and control groups.

Initial response to treatment at four months showed a significantly greater increase over baseline in the height Z score. At 12 months, however, only the mean increase in sexual maturation was significantly greater in the treated group. To minimize the statistically confounding effect of potential additional growth, data on final growth were obtained from subjects who were over 17 years of age. There was no significant difference in final absolute height Z scores between the treated and untreated groups, but the mean increase of final height Z scores from baseline was significantly greater among treated subjects because of dif-

ferences in the standard deviation of the final height Z scores between the two groups. Although not statistically significant, the actual mean height of the treated group was 4.9 cm greater than that of the untreated group. There was no significant correlation between baseline predicted adult heights and the actual heights at the time of the last visit. This study demonstrates that four courses of 200 mg testosterone enanthate at three-week intervals do not appear to compromise adult height in boys with delayed puberty.

Wilson DM, Kei J, Hintz R, et al. *Am J Dis Child* 1988;142:96-99.

**Editor's comment**—Although others have looked at the long-term effects of different androgen preparations, control populations with which to compare results are often not available. In addition, most other studies have utilized long-term (9- to 12-month) treatment regimens. Wilson et al also looked at patient satisfaction with therapy, and 95% of the treated subjects indicated that they believed the treatment had been helpful.

The authors correctly point out that patients who fail to show signs of pubertal regression a year after therapy should be carefully re-evaluated for hypogonadism. We have previously reviewed other reports in this publication (Vol. 3, No. 4 and Vol. 4, No. 2) of the long-term effects on height of testosterone injections for pubertal delay. The present study corroborates the findings of those studies and presents additional useful data for pediatric endocrinologists who treat this common problem.

William L. Clarke, M.D.

#### Letter to the Editor

I write in reference to "Catch-Up and Catch-Down Growth: A Review" by Dr. Tanner (*Growth, Genetics, and Hormones*, Volume 3, Number 4). I found the article provocative and enjoyable. I must say, though, that I do not like the term "catch-down growth" because it does not convey the appropriate meaning to students. The term seems to imply that the children get shorter and/or lose height potential, which of course is not the case.

The phenomenon is essentially another form of catch-up growth. Perhaps "catch-up growth, type II" would be a better term. What really happens is that height ages are trying to catch up with bone ages. When linear growth is abnormally stimulated, as by a virilizing disorder, and the disorder is then alleviated, the growth velocity drops for a while—but only for as long as it takes the height age to catch up to the bone age. Bone length tends to catch up with bone maturation. The result is restoration of height potential. Then normal growth resumes.

"Compensatory deceleration" is an accurate term for the phenomenon, but it does not convey the concept that the process is preserving rather than reducing height potential. Perhaps the term "catch-up growth, type II" better conveys this concept.

Robert L. Rosenfield, M.D.  
Professor of Pediatrics and Medicine  
University of Chicago School of Medicine  
Chicago, Illinois

#### Dr. Tanner's reply

I agree that "catch-down" growth is not a perfect term. In the formal setting, auxologists use the phrase "compensatory deceleration" or, preferably, "homeorrhetic deceleration." This expresses precisely what one is after; namely, a deceleration that restores the child to his programmed growth chart.

In the original paper on catch-up growth, Prader et al (1963) pointed out that this phenomenon was simply a special case of the principle of homeorrhexis described by Waddington in his classic book *The Strategy of the Genes* (London, 1957). Homeostasis is a well-known term and describes the tendency of an organism to return to a balanced position when pushed away from it. "Homeorrhexis" describes the same tendency, but in relation to an organism moving through time. Rhexis signifies flow as opposed to stasis.

I am not so sure that Dr. Rosenfield's explanation of the mechanism of homeorrhexis is correct in the general case. It is possible to advance both height growth and bone maturation, for example, by overfeeding (or even by providing a nice roomy uterus) and the stimulus is terminated when the animal slows down in both respects. We do not understand the mechanism of this at present, nor even whether the control is chiefly central or chiefly peripheral. There seems to be a size-for-age mismatch involved, and perhaps maturation-for-age and height-for-maturation mismatches as well.

I admit that the term "catch-down growth" has its disadvantages, but "catch-up growth, type II" sounds like a rare disease, probably with chromosomal deletion.

#### Address for Correspondence

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## MEETING CALENDAR

**October 7-8** International Growth Hormone Symposium. Vienna, Austria. Contact: Dr. H. Frisch, Scientific Secretary, Universitäts Kinderklinik, Allgemeines Krankenhaus der Stadt Wien, Wahringer Gürtel 18-20, A-1090 Vienna, Austria

**October 15-20** 57th Annual Meeting of the American Academy of Pediatrics. San Francisco, California. Contact: American Academy of Pediatrics, 141 Northwest Point Boulevard, PO Box 927, Elk Grove Village, IL 60009 (800-433-9016, outside Illinois; 800-421-0589, in Illinois)

**October 27-31** 40th Postgraduate Assembly of The Endocrine Society. Franklin Plaza Hotel, Philadelphia, Pennsylvania. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**November 30-December 2** 8th Annual Bristol-Myers Symposium on Nutrition Research: Enteropathy of Infantile Malnutrition, Diagnosis and Management. Children's Nutrition Research Center, Houston, Texas. Contact: Vicki L. Forgac or Lila K. Lerner, Office of Continuing Education, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030 (713-799-6020)

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# GROWTH

## Genetics & Hormones

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### The Role of Confined Chromosomal Mosaicism in Placental Function and Human Development

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*Canada*

Chromosomal mosaicism has long been recognized in clinical genetics practice as a cause of abnormal development. By definition, constitutional chromosomal mosaicism is the presence of two or more cell lines with different chromosomal complements in one individual. It has been described for both autosomes and sex chromosomes. Diagnosis of a mosaic chromosomal syndrome is usually based on the finding of both a normal diploid cell line and an aneuploid cell line in cultured lymphocytes and/or skin fibroblasts.

Mosaicism may originate in early embryonic development through nondisjunction, anaphase lag, or structural rearrangement. The resultant mosaic pattern in the conceptus depends on many factors, such as the number of blastomeres at the time of mutational event, the cell lineage affected by the mutational event, and cell selection based on the viability of mutant cells.

Only recently has the importance of the timing of the mutational event and the cell lineage involvement been realized, and this has led to the recognition of

two types of constitutional chromosomal mosaicism: (1) generalized, and (2) confined to the placenta or to the embryo (Figure 1).

#### Generalized Chromosomal Mosaicism

Generalized mosaicism originates from a mutational event in the first or second postzygotic division. All tissues of the conceptus are affected. This type of mosaicism has been described for most autosomal trisomies and for both monosomy and trisomy of the sex chromosomes. Study of mosaic trisomy 21 in children has shown that the selection against a trisomic cell line occurs in blood lymphocytes, but the ratio of trisomic to diploid cells remains the same in skin fibroblasts.<sup>1</sup> More severe selection is found in the Pallister-Killian syndrome, where aneuploid cells eventually disappear completely in peripheral blood but remain present in fibroblasts.<sup>2,3</sup> In other sporadic dysmorphic cases, in which mosaicism can only be detected in fibroblast cells and has never been documented in lymphocytes,

the distinction between the evolution of generalized mosaicism and confined mosaicism within the embryo and fetus becomes difficult.<sup>4-6</sup>

#### Confined Constitutional Mosaicism

The existence of confined forms of constitutional mosaicism is less well known. It is the pattern of cell lineage differentiation during early embryonic development that supports confinement of mosaicism arising during cleavage and blastogenesis to either the placenta or the embryo/fetus. Significant confined *placental mosaicism* results from viable mutations occurring in trophoblast or extraembryonic mesoderm progenitor cells, while significant confined *embryonic mosaicism* originates after early implantation and initiation of development of the embryo proper from a designated small number of embryoblasts.<sup>7-9</sup> Only mosaicism confined to the placenta will be discussed in this article.

#### Confined Placental Mosaicism

Confined placental mosaicism (CPM), defined as a dichotomy between the chromosomal constitution of placental tissues (both cytotrophoblasts and villous stroma) and embryonic/fetal tissues, is usually detected on chorionic villus sampling (CVS) at 9-12 weeks of gestation.<sup>10,11</sup> Its existence has

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## The Role of Confined Chromosomal Mosaicism

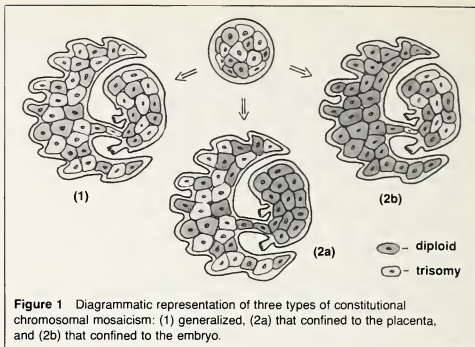
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also been demonstrated in term placentas.<sup>12,13</sup>

Discrepancies between the cytogenetic findings in cytotrophoblasts and villous stroma and fetus reported in 2% of pregnancies studied by CVS can assume three different forms, as shown in the table below.

Although such findings are designated in the current literature as CVS discrepancies or pseudo-mosaicism,<sup>11,14</sup> they exemplify constitutional mosaicism confined to the placenta.

The existence of CPM—with its expression restricted only to the cytotrophoblast, the extraembryonic mesoderm, or both of these lineages—and a complete absence of mosaicism in the embryo reflect the complexity of preimplantation and early post-implantation placental development. Placental development starts at day 6 postfertilization when the implanting blastocyst invades the endometrium in the area of polar trophoblast as two cell populations, cytotrophoblasts and syncytiotrophoblasts. These cells form primary villi between days 6 and 9, with the syncytiotrophoblast externally located and the cytotrophoblast internally situated (Figure 2). Secondary villi develop between days 9 and 18 by migration of the cells from both extra-embryonic mesoderm and primitive embryonic streak into the villous core. Tertiary villi are characterized by the appearance of primitive capillaries in the villous core at about day 18.



**Figure 1** Diagrammatic representation of three types of constitutional chromosomal mosaicism: (1) generalized, (2a) that confined to the placenta, and (2b) that confined to the embryo.

After 3 weeks of development, chorionic villi have the structure of tertiary villi and are derived from three different cell lineages: polar trophoblast, extraembryonic mesoderm, and primitive embryonic streak. Although both extra-embryonic mesoderm and the embryo proper originate from the inner cell mass (ICM), it is possible that, in the case of mosaic blastocysts, the cells of the ICM that give rise to extraembryonic mesoderm may have a different karyotype from the cells migrating from the primitive streak of the embryo proper. The reason for this is that only a small number of cells (3 to 8) from the ICM become progenitors of the embryo proper.<sup>5</sup>

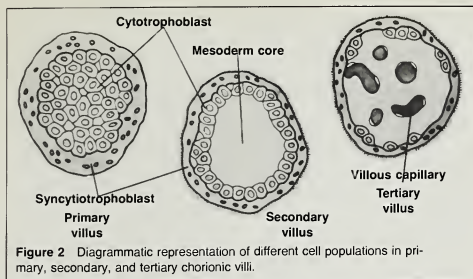
CPM can be understood by being aware of the complex embryonic derivation of placenta from three different lineages as well as by further development of the placenta. Cytotrophoblast, the cell

type utilized as a source of dividing cells in direct preparation of villi, is the predominant cell in primary placental villi and is an actively dividing cell in the secondary and young tertiary villi. However, by the fourth month of pregnancy, the cells of the cytotrophoblast become attenuated, lose their appearance as a continuous layer, and become difficult to demonstrate histologically. The existence of this cell type can be documented in term placentas by using special immunohistochemical techniques, but the cells appear inactive and sparse. Confirmation of the presence of an abnormal cell line in the cytotrophoblast that was identified at 10-12 weeks of gestation becomes technically more difficult at term, and it is not clear whether any selection against the aneuploid cell line takes place in mosaic placenta during the second and third trimesters, when the cytotrophoblast becomes less prominent.

All three cell lineages must be evaluated to study placental mosaicism. Trophoblast lineage is analyzed by direct preparation or by short-term incubation of the chorionic villi.<sup>15,16</sup> Fibroblasts in long-term cultures of chorionic villi and chorionic plate represent the extraembryonic and embryonic mesoderm lineages. The derivation of amnion is still controversial.

**Table.** Discrepancies between the cytogenetic findings in cytotrophoblasts and villous stroma

| Tissue           | Type I                         | Type II         | Type III                     |
|------------------|--------------------------------|-----------------|------------------------------|
| Cytotrophoblast  | Mosaic or nonmosaic aneuploidy | Normal diploidy | Mosaic or nonmosaic diploidy |
| Placental stroma | Normal diploidy                | Mosaic          | Nonmosaic aneuploidy         |
| Fetus            | Normal diploidy                | Normal diploidy | Nonmosaic aneuploidy         |



**Figure 2** Diagrammatic representation of different cell populations in primary, secondary, and tertiary chorionic villi.

### Role of CPM

Although the effects of CPM on placental function are not yet fully understood, some data documenting its significance exist.

Type I CPM is the most common. The aneuploid line in the cytotrophoblast detected on CVS has been shown to persist throughout the entire gestation in the same proportion (Kalousek DK, unpublished observation). It is not known why certain chromosomal trisomies (such as trisomies 3 and 15) occur frequently in type I CPM, while others are completely absent in this type.<sup>11,14</sup>

Although many pregnancies with this type of confined chromosomal mosaicism progress to term uneventfully and result in the birth of a normal live infant, some pregnancies result in unexplained intrauterine fetal death, intrauterine growth retardation (IUGR), or perinatal morbidity.<sup>16,17</sup> Abnormal placental function that interferes with fetal development may be the cause of pregnancies with complications. Not enough cases have been studied to make a correlation between a specific aneuploidy involved in mosaicism and pregnancy outcome.

Chromosomal mosaicism confined only to the chorionic villous stroma represents type II CPM. It is less common than mosaicism confined to the cytotrophoblast, and its effect on fetal intrauterine survival is largely unknown. It has been described in both normal pregnancies and pregnancies with fetal IUGR.<sup>12,13</sup>

In type III CPM, the presence of the diploid cell line confined to the cytotrophoblast in nonmosaic aneuploid conceptions appears to provide a protective effect and facilitate their intrauterine survival. It has been shown that all analyzed placentas from live newborns and terminated pregnancies with trisomies 13 and 18 were mosaic.<sup>19</sup> Prenatal diagnosis of this type of CPM by CVS is of concern, as it may lead to a false-negative diagnosis when only direct preparations are analyzed.<sup>10</sup>

### Role of CPM in Human Development

There are many unanswered questions regarding the effects of CPM on human development.

Although extensive information is available from studies of population cytogenetics data with respect to spontaneous abortuses, stillbirths, and live births, there are no data for their placentas. Although type III CPM facilitates intrauterine survival of embryos and fetuses with trisomies 13 and 18, the role of other types of CPM in the embryonic and fetal development is less clearly defined.

CPM has not been reported in spontaneously aborted conceptions, except for two cases involving type III mosaicism with the normal diploid cell line in the cytotrophoblasts and the tetraploid cell line in cultures of amnion, chorion, and villous stroma.<sup>17,18</sup>

It is obvious that further studies of placentas involving both direct preparations from trophoblast and

cultures from villous stroma, chorion, and amnion at different stages of intrauterine development are necessary to understand the chromosomal mutational events taking place during the cleavage and implantation period and the effect of these events on intrauterine development of both placenta and embryo/fetus. Studies at term that confirm the finding of CPM diagnosed by CVS are especially needed; only in confirmed cases can obstetrical findings such as IUGR or unexplained intrauterine fetal death be meaningfully associated with CPM.

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# Anabolic Steroid Hormones for Athletes: Efficacy or Fantasy?

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 Pharmacology  
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Scientific interest in drugs is usually focused on their therapeutic effects—prevention, diagnosis, or treatment of disease—or on their abuse potential. Little attention has been given to the use of drugs for the following purported benefits: increase in physical strength, delay in the onset of fatigue, increase in exercise endurance, prevention of anxiety that could interfere with performance, enhanced attention and concentration, and development of a more satisfactory competitive attitude. The use of chemical or medicinal substances with the deliberate intention of altering athletic performance is generally considered unethical; for this reason, drug use by athletes is usually covert.

## Metabolic Actions

All anabolic steroids are derivatives of testosterone, the natural male sex steroid hormone that is responsible for the androgenic

and anabolic effects noted during adolescence and adulthood in males. Androgenic effects are those biologic activities that relate to the growth of the male reproductive tract and to the development of male secondary sexual characteristics. In the pubertal male, these effects are responsible for increases in the length and diameter of the penis, development of the prostate and scrotum, and the appearance of pubic, axillary, and facial hair. Anabolic effects are those occurring in the somatic or nonreproductive tract tissues, that is, those that promote nitrogen retention. Anabolic effects include an acceleration of linear growth before bony closure, enlargement of the vocal cords, the development of libido and sexual potential, and an increase in muscle mass and strength.

During normal male pubertal development, testosterone is responsible for accelerated linear growth, in part by augmenting the amount of growth hormone secreted, increasing muscle bulk and strength, and decreasing the percentage of fat as body mass. This androgen is also probably responsible for the increase in aggressive and sexual behavior in

young males, although its role in these traits is controversial.

The androgens that produce predominantly anabolic (as opposed to androgenic) effects are esters of natural androgens or derivatives of 19-nortestosterone. Orally active agents are alkylated at the C-17 position of the androgen steroid nucleus. This chemical modification retards the hepatic metabolism of these agents. The parenterally effective compounds are mainly esters of the C-17 oxygen function and have an extended duration of action due to delayed systemic absorption from intramuscular sites. Methandriol is an exception, for it is a C-17 alkylated derivative. Oxandrolone is an anabolic steroid with a particularly favorable anabolic-to-androgenic activity ratio. In addition, there has been probably more experience with this compound than with any other for the appropriate stimulation of delayed growth in male and female adolescents, and girls with Turner syndrome.

The potencies and usual replacement dose ranges of many of the anabolic steroids taken by athletes in the United States are listed in the table below.<sup>1-3</sup>

**Table.** Anabolic steroid drugs

| Orally Active                       |  |                  |  |
|-------------------------------------|--|------------------|--|
| Drug                                | Trade name   | Relative potency | Usual dose range for replacement therapy |
| Ethylestrenol                       | Maxibolin  | ≈ 8              | 4-8 mg/day                               |
| Methandrostenolone                  | Dianabol   | ≈ 2.5            | 5 mg/day                                 |
| Oxandrolone                         | Anavar   | 13               | 5-10 mg/day                              |
| Oxymetholone                        | Anadrol-50   | 5-10             | 5-15 mg/day                              |
| Stanozolol                          | Winstrol   | ≈ 6              | 6 mg/day                                 |
| Parenterally Active                 |  |                  |  |
| Nandrolone phenpropionate           | Androlone<br>Durabolin<br>Nandrolin                          | ≈ 4              | 25-50 mg/week                            |
| Nandrolone decanoate                | Androlone D<br>Deca-Durabolin                                | ≈ 3              | 50-100 mg, 3-4 ×/week                    |
| Methandriol<br>(also orally active) | Anabol<br>Durabolic<br>Methabolin<br>Methydiol<br>Steribolic | ≈ 6              | 50-150 mg/day (oral)                     |

## Therapeutic Role

Anabolic steroids are medically unquestioned as replacement therapy in men with hypogonadism of central or peripheral origin and in some young males with marked delay of pubertal development. The amounts used, either orally or parenterally, are within severalfold of the normal male testosterone production rate. At these levels, normal pubertal progression and the maintenance of adult male strength and sexual function are attained, and untoward effects are usually minimal. Somewhat increased amounts are used to stimulate anabolism or to produce positive metabolic effects in some patients with neoplastic diseases. These agents may also be prescribed for patients with certain hormone-dependent tumors, such as breast cancer.

## Can Anabolic Steroids Improve Athletic Performance?

The most desirable effect of anabolic steroid use by athletes is an increase in muscle bulk, which is purported to give them more strength and power. One must realize, however, that objectively measured size and strength of muscle are not the sole determinants of athletic performance. Some athletes are very pragmatic and success oriented. Training methods that include the use of chemical compounds and drugs are chosen if the athlete thinks they will help. They may be needed

merely for their placebo effect, or they may allow the athlete to train more diligently and/or more aggressively. The significant untoward effects (see following section) are most often quite removed in time from their use. As in any other clinical study, it would be important to follow athletes long term and to know precisely what agents were taken, how much, and for how long. Such data are unavailable at present and probably will always remain so because of the covert nature of most anabolic steroid use.

Although a large number of studies of the effects of anabolic steroids on athletic performance have been completed, there are very few that have been well controlled and randomized. Of those studies that have shown increases in muscle strength, virtually all evaluated highly trained athletes whose performance relied heavily on explosive muscular power to overcome the inertia of implements; for example, weight lifters or field event throwers.<sup>4</sup> The gains, based on relative increase in muscle strength, were small and statistically significant in less than half the studies. When evaluating relatively untrained subjects, it is very difficult to distinguish the results of training from those attributed to anabolic steroid use because of the very significant benefits of training itself. The aerobic performances of athletes treated with anabolic steroids do not exceed those expected from aerobic training itself.<sup>5</sup>

## Are Anabolic Steroids Harmful?

Some men taking anabolic steroids will experience prostatic hypertrophy, acne, and gynecostasia. At higher doses, they can expect more pronounced changes in these physical signs; priapism and edema are also common because steroids promote the retention of salt and water. This last effect explains, in part, the rapid weight gain that occurs after the administration of high doses of anabolic steroids and is probably the reason why

athletes taking these compounds can lose enormous amounts of weight very quickly after discontinuing these agents before certain competitions.

In women, anabolic steroids produce virilization, as indicated by enlargement of the clitoris, increased pubic, axillary and body hair, and libido. Menstrual dysfunction is common.

Abnormal hepatic function test results and mild jaundice are regularly seen in persons taking compounds that carry a 17-methyl (alkyl) group. Jaundice may also occur as a secondary phenomenon to other drug-induced liver diseases, such as peliosis hepatis (the pooling of blood within the sinusoids) and/or hepatocellular carcinoma. More commonly, however, there is a lack of sperm production despite the heightened sexual drive in men receiving high doses of anabolic steroids. Although androgens are required for spermatogenesis, in high dosages they feed back upon the hypothalamus and pituitary to decrease concentrations of luteinizing hormone and follicle-stimulating hormone. The latter is an absolute requirement for the maturation of spermatozoa within the seminiferous tubules of the testes.

Some laboratory test results can be influenced by the levels of circulating androgenic hormones, and the results of certain tests—gonadotropin and thyroid hormone levels, for example—may become abnormal in persons taking anabolic/androgenic agents. Serum lipid levels correlate with the incidence of coronary artery disease—directly for total and low-density lipoprotein cholesterol (LDL-c) and inversely for high-density lipoprotein cholesterol (HDL-c). Anabolic steroids profoundly lower HDL-c levels, elevate LDL-c concentrations, and severely depress the HDL-c:LDL-c ratio. Thus, they are indirectly atherogenic.

The training regimens of body builders are often associated with a more favorable lipid profile than those used by power-lifters who are more likely to be in the cata-

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*continued on page 6*

## Anabolic Steroid Hormones

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bolic state after strenuous work-outs. The different metabolic states may be important when attempting to verify drug activity.

Prepubertal and peripubertal children show disturbances in growth and sexual development, the most serious being the rapid advancement in bony epiphyseal closure. Children with diseases associated with androgen excess (eg, virilizing adrenal hyperplasia) are tall, muscular, and prematurely sexually developed as youngsters but relatively short as adults, since the maturational effects of the excessive hormones are potent. More profound actions are seen in men, and, by implication, in women and children who receive increasingly supraphysiologic doses of anabolic steroids. Great mood swings are common to those taking anabolic steroids, but frank psychosis, although reported a number of times, is quite rare.

Certainly, not all effects occur in all persons, nor are they necessarily obvious. In addition to the dosage, one must factor in the length of time that these compounds have been used to arrive at a total dose.<sup>6</sup> However, there have been a number of deaths

from hepatocellular carcinoma in young male weight lifters and body builders whose only risk factor was the long-term use of high doses of anabolic steroids.<sup>7,8</sup>

### Should Adolescent Athletes Take Anabolic Steroids?

The easiest recommendation to make is to say that no athlete should ever take these potent compounds for any reason. But as noted above, many athletes are supreme pragmatists who will not heed that advice, especially when faced with an immense amount of anecdotal information from their colleagues and competitors. Moreover, since many of the side effects have not occurred in the athlete or his/her companions who take anabolic steroids, there are only glowing stories of their efficacy. Many at this stage of life feel invincible, and have unrealistic views of dangerous practices.

Whether it is fair or allowable to take anabolic steroids before competition is a philosophical issue. In weight-related sports such as wrestling, the gain in weight might just put one into a higher weight class—not particularly advantageous unless one competed in the heaviest class.

I believe that the use of anabolic steroids ought to be condemned

except for therapeutic purposes. It is abundantly clear to me that prepubertal and peripubertal children of either sex should not have access to the anabolic/androgenic compounds because of their adverse side effects upon the growing and developing adolescent. Likewise, the potent androgenic effects of anabolic steroids upon the female reproductive system warrant their condemnation except in therapeutic situations. For the adult male competitor, I strongly feel that the potential side effects of these drugs far outweigh their purported benefits, and athletes should not use them.

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## Special Report: Seminar and Workshop on Limb Lengthening, May 23-24, 1988, San Francisco, California

Judith G. Hall, M.D.  
Associate Editor  
*Growth, Genetics, and Hormones*

This seminar was held to provide information to North American pediatric orthopedists on the remarkable new techniques that have been developed in Russia and Italy for lengthening of limbs. One section was devoted specifically to the lengthening of limbs in persons with disproportionate short stature. The history of leg lengthening and the techniques that have been used were reviewed. Professor Wagner from Germany discussed the Wagner technique, Professor Ilizarov from Russia described the Ilizarov

technique, and Professor De-Bastiani from Italy described "calotaxis" using the orthofix fixator.

A number of technical changes and improvements in leg lengthening in the past 5 years have led to lower complication rates and dramatically better results. The essence of the new lengthening procedure involves fixing a long bone (femur, humerus, tibia, fibula) at both ends by percutaneous pins or wires. An osteotomy is made midshaft. As the callus begins to form at the site of the osteotomy, the two ends of the bone are distracted from each other by the pins at the rate of about 1 mm/day. The newest research indicates that if that millimeter is divided into

fourths (ie, four smaller distractions a day) there is less pain and more rapid healing. The distraction process takes place over a 3- to 4-month period, after which the long bone is held in place to allow full strength and healing of the newly formed bone for another 4-month period (total, 8 months per limb segment). Patients are permitted to walk during the lengthening procedure. Most patients require only 5 or 6 days of hospitalization for the initial phase of the procedure, although weekly appointments for adjustments are required.

Leg-lengthening procedures should not be performed by those without experience or by those

who intend to do it on a one-time basis, since it is quite clear that many adjustments are required during the entire lengthening process. However, in the hands of experienced surgeons, patients can anticipate as much as 25 cm of increased length in a limb with two procedures (ie, femur and tibia). Use of the Ilizarov technique permits two osteotomies to be performed in the same limb segment, with two areas of lengthening.

Complications include stiffness of the joints, nerve palsy, skin infections, and very rarely, nonunion or fractures to the area of lengthening. Although minor adjustments and complications are frequent, the incidence of major complications appears to be less than 3% in experienced hands. Much higher complication rates

and much less lengthening were reported in earlier studies.

Limb lengthening is used not only for lengthening, but for non-unions and pseudoarthroses as well. Although the technique was primarily developed for unilateral lengthening and for the treatment of amputations, it seems appropriate as a treatment for disproportionate short stature. It has been used in patients with hypochondroplasia and achondroplasia, but the results are much less promising in those with spondyloepiphyseal dysplasia. There are no reports of its use in those with other chondrodysplasias, although a few individuals with Turner syndrome and familial short stature have been treated in Europe.

Limb lengthening holds great

promise as symptomatic therapy for those with disproportionate short stature. At the same time lengthening is accomplished, bowing and other abnormalities can be treated. The ideal time for this treatment is adolescence so that the teenager with disproportionate short stature can have a growth spurt along with his or her peers. Moreover, by waiting until adolescence, the affected individual can participate in making the decision to undergo the procedure.

There is a great need for collaborative research to study carefully the outcomes of limb-lengthening procedures in different types of chondrodysplasias. Information about the long-term outcomes and complication rates of these procedures is simply not available at this time.

## Special Report: 48th Annual Meeting of the American Diabetes Association, June 12-14, 1988, New Orleans, Louisiana

William L. Clarke, M.D.

*Associate Editor*

*Growth, Genetics, and Hormones*

Although there were no presentations devoted specifically to growth in children with diabetes, there were several presentations on growth hormone (GH) pulsatility, insulin-like growth factor I (IGF-I), insulin resistance, and the relationship between GH and proliferative diabetic retinopathy that should be of interest to readers of this publication.

Cohen and Frohman (Cincinnati) characterized GH pulsatility in type I diabetic men. Utilizing both DETECT and PULSAR computer programming and 20-minute sampling for 24 hours before and after 10-14 days of improved glycemic control, they demonstrated an increased number of GH pulses (compared with controls) that did not change over the short term with improved glucose control. The diurnal rhythm of GH secretion was markedly abnormal in poorly controlled diabetics, with  $44 \pm 6\%$  of the pulses occurring between 8:00 A.M. and 8:00 P.M. After improved glucose

control, only  $26 \pm 4\%$  of pulses occurred during these hours.

The authors postulated that the time available for target tissues to recover from prior GH exposure is reduced in diabetics, particularly in those with poor glycemic control. They also postulated that these changes in GH secretory patterns may alter the nature of time-dependent GH metabolic effects in persons with diabetes.

Moxley et al (Rochester, New York) evaluated the effects of IGF-I infusions in adult rats using 2-hour euglycemic infusions. Measurements of 2-deoxyglucose uptake and hepatic glucose production were also performed. With infusion of low-dose IGF-I (21 U/kg/min), or high dose IGF-I (83 U/kg/min), whole body glucose disposal was similar to that seen when low and high doses of insulin were infused (2 mU/kg/min and 40 mU/kg/min, respectively). However, glucose disposal during the first hour of IGF-I infusion was significantly lower than that associated with insulin at each dose. At the low-dose infusion, IGF-I was less effective than insulin in suppressing hepatic glucose output. The authors con-

clude that IGF-I has insulin-like activity in vivo that results in part from crossover effects on the insulin receptor. They also demonstrated that IGF-I produces a fall in serum insulin. The physiologic significance of these findings is not clear.

Lager et al (Sweden) infused a tritiated glucose accompanied by either a placebo, propranolol, or somatostatin to evaluate glucose turnover following hypoglycemia. Hypoglycemia was shown to produce a prolonged insulin resistance (up to 7 hours). Propranolol did not prevent the insulin resistance, but somatostatin, which completely abolished GH release, significantly reduced this insulin resistance. The authors conclude that the late insulin resistance seen after hypoglycemia is not, like early-phase insulin resistance, due to  $\beta$ -adrenergic stimulation and that GH significantly contributes to this observation.

Two teams of investigators evaluated IGF-I and stimulated GH release in adults with proliferative diabetic retinopathy. Dills et al (Madison, Wisconsin) determined IGF-I levels in a large group of

*continued on page 8*



## Special Report: 48th Annual Meeting of the American Diabetes Association

*continued from page 7*

patients with diabetes. IGF-I levels were negatively correlated with age, duration of diabetes, and glycosylated hemoglobin, but were positively correlated with proteinuria. Using logistic regression analysis and controlling for duration of disease, glycosylated hemoglobin, blood pressure, and proteinuria, the authors demonstrated that higher levels of IGF-I

were significantly associated with an increased risk of proliferative diabetic retinopathy.

Janka et al (Boston) prospectively evaluated GH release to arginine in 91 insulin-dependent patients who had diabetes for more than 15 years and who had minimal background retinopathy. After 4 years of follow-up, those individuals who exhibited severe proliferative and preproliferative retinopathy had significantly higher postarginine GH responses. These differences remained after adjustments were made for hemo-

globin A<sub>1c</sub>, insulin dose, and creatinine. The study demonstrated, for the first time in a prospective manner, that individuals responding to arginine with high GH levels might be at risk for developing severe eye lesions.

The findings described by Dills et al and Janka et al differ from previous reports since it has been previously shown that high GH levels in persons with diabetes are usually associated with lower IGF-I levels. It should be noted, however, that Dills et al did not determine GH levels in their patients.

## Special Report: Fifth International Auxology Congress, July 20-23, 1988, Exeter, United Kingdom

Robert M. Blizzard, M.D.  
*Chairman, Editorial Board  
Growth, Genetics, and Hormones*

This excellent international conference on the measurement, physiology, and pathophysiology of growth was arranged by Dr. James Tanner. Its strength and interest evolved from the great diversity of the participants, who included economists, embryologists, statisticians, chemists, pediatricians, pediatric endocrinologists, and educators.

The roles of insulin-like growth factors (IGF) were discussed by Drs. Underwood, Froesch, and Hintz. The gene for IGF-I has five exons that have at least two ways to splice into two prepro hormone variants. The prepro IGF-I (E peptide) is present in large quantities in the plasma of patients with renal failure. In addition to the various prepro IGFs and two separate IGFs, there are several binding proteins that may act as storage receptacles for IGF-I, which itself has a very short half-life when in the unbound state.

Much emphasis is currently placed on investigating the role of IGF-I in various nutritional states. In human beings on restricted diets, IGF-I falls significantly. Restoration to previous levels is dependent on adequate caloric replacement and replacement of protein requirements with essen-

tial amino acids. In contrast, the use of nonessential amino acids limits the increase of IGF-I to prediet levels.

IGF-I infusion into growth-retarded diabetic rats leads to near-normalization of growth, as does IGF-I infusion in hypophysectomized rats.

Short-term measurements using knemometry were discussed by several investigators. Dr. Hermans (Kiel, West Germany) reported data based on extensive observations of patients and refined statistical methodology to study mini growth spurts. Seventy percent of healthy children were observed to have sharp growth spurts alternating with periods of decreased growth velocity, with a peak-to-peak distance of 30-55 days. He concluded that these mini growth spurts seem to be a major reason why the differences of leg lengths, which are obtained at short intervals, are inadequate for long-term growth prediction. Thus, the investigation of short-term growth, starting from the traditional idea of convertibility of length increment to growth, is complex, and the problem is far from being solved satisfactorily.

Since measurements of the tibia may be of use in studying the physiology of growth patterns in long bones and the spine, Dr Cronk and associates (Philadelphia) designed a knemometer that

is smaller, more portable, and less expensive than the one previously designed by Dr. Valk. We may be hearing more concerning this type of bone study with the new machine if the limitations are understood and the equipment is truly less expensive.

Dr. Mosier (Irvine, California) presented data from studies of growth retardation and catch-up growth in infant rats following cranial irradiation or glucocorticoid administration. Mosier concludes that growth retardation and catch-up growth secondary to irradiation are under control of the central nervous system. The concept of central control requires that there be a mechanism for sensing current body size, a set point for target size (normal body size for age), and a means of stimulating increased growth rate. He believes that the set point is altered by radiation, and this change in set point is independent of nutritional or endocrine dysfunction as they can currently be assessed. The area of the dorsal medial hypothalamic nucleus is thought to be involved in the determination of the set point. However, all scientists interested in these phenomena realize that little is known as yet, and this difficult and important field of investigation needs extensive study.

The Sixth International Auxology Congress will be held in 1991 in Madrid, Spain.

### Three-Year Results of a Randomized Prospective Trial of Methionyl GH and Oxandrolone in Turner Syndrome

Seventy girls with Turner syndrome were divided into four groups to receive growth hormone (GH) (0.125 mg/kg, 3 times/week), oxandrolone (either 0.125 mg or 0.062 mg/kg daily), or a combination of both agents. Sixty-five subjects were evaluated after three years of therapy.

GH given alone over three years increased the growth velocity (GV) from  $-0.1$  standard deviations (SDs) on the Turner growth charts to  $+3.1$ ,  $+2.0$ , and  $+1.4$  SDs for the first, second, and third years, respectively. By the end of three years the mean height was  $+0.7$  SD, and five of 17 patients (29%) had heights above the 90th percentile. The increment in bone age over three years was  $2.73 \pm 0.72$  years. The  $\Delta$  Turner height age/  $\Delta$  bone age was  $1.6$  at the end of three years, and the  $\Delta$  predicted height was  $+4.5 \pm 0.9$  cm. After three years, four of 16 girls receiving GH alone achieved their projected adult height according to the Turner growth curves. All 16 were still growing at the end of the evaluation period. GV fell from a mean of  $6.6$  cm the first year of treatment to  $5.4$  and  $4.6$  cm during the second and third years, respectively. This compares to a value of  $4.5$  cm/year before therapy. The decrease in GV occurred although the insulin-like growth factor-I (IGF-I) values increased progressively over four years from  $0.55$  to  $2.46$  U/mL, indicating that the GV did not correlate over a long period of time with the IGF-I concentrations.

Combination therapy proved more effective than GH therapy alone. This was without apparent adverse effect on the rate of bone maturation or predicted adult height. The GV in this group increased from  $-0.1$  SD on the Turner growth charts to  $+6.6$ ,  $+4.3$ , and  $+1.4$  SDs for the first, second, and third years of treatment, respectively. By the end of

three years the mean height was  $+2.0$  SDs, and 11 of 16 patients (69%) had heights above the 90th percentile on the Turner growth curves. The increment in bone age over three years was  $4.0 \pm 1.2$  years. A  $\Delta$  Turner height age/  $\Delta$  bone age was  $1.6$  at the end of three years and the  $\Delta$  predicted height was  $+8.2 \pm 1.4$  cm for this group. After three years, ten of 16 girls receiving the combination therapy achieved their projected adult height according to the Turner growth curves, and all were still growing. GV fell from a mean of  $9.8$  cm during the first year of treatment to  $7.4$  and  $6.1$  cm for the second and third years, respectively. These compared to a pretherapy value of  $4.3$  cm/year. As in the group on GH therapy, the IGF-I values increased with therapy but did not correlate with GV.

Oxandrolone was used alone in 17 patients and for only one year. The mean GV increased from  $4.1$  to  $7.6$  cm/year. Since these patients then received combination therapy, the prolonged effect of oxandrolone could not be evaluated, but previously published data indicate that GV in such patients return to the pretreatment rate during the third year of treatment. Oxandrolone used alone resulted in no significant increase in IGF-I values.

The GV data from the patients receiving GH alone compare favorably with those reported in three other studies. The effect of therapy on adult height is more

difficult to assess, but all 65 subjects who completed the study continue to grow. However, the median  $\Delta$  height age/  $\Delta$  bone age values suggest a permanent increase in predicted adult height since 65% of those receiving combination therapy have already equalled or exceeded their projected adult heights.

Appropriately, the authors point out that GH and oxandrolone are potent anabolic agents and are capable of causing insulin resistance, virilization, and the clinical stigmata of acromegaly. Also, patients with Turner syndrome do not respond to GH as well as GH-deficient patients, and the expectations of the patient, family, and physician for increased growth must be realistic. The authors also point out that it still remains to be seen whether such treatment will permit a significant number of these girls to attain a "normal" adult height of  $>150$  cm.

Rosenfeld RG, Hintz RL, Johanson AJ, et al. *J Pediatr* 1988;113:393.

**Editor's comment**—This editor has avoided treating patients with Turner syndrome with GH, but the current evidence suggests that we may eventually see an increase in ultimate height. If patients with Turner syndrome are to be treated, I certainly agree with the authors' comments that the expectations of the patients and parents for increased growth must be realistic.

Robert M. Blizzard, M.D.

#### In Future Issues

The Morbid and Functional Anatomy of the Human Chromosome Map in Endocrine Disorders and Hormonal Genes by Victor A. McKusick, M.D.

Occult Celiac Disease: A Common Cause of Short Stature by Asaria Ashkenazi, M.D.

Growth, Sexual Precocity, and Treatment: Physiology and Pathophysiology by Paul Boepple, M.D., and William Crowley, M.D.

Reprints of the article abstracted above as well as selected slides based on the study findings can be obtained by writing to *Growth, Genetics, and Hormones*, c/o BMI/McGraw-Hill, 800 Second Avenue, New York, NY 10017. As part of its ongoing policy to provide readers with important information about growth and growth disorders, the Editorial Board of this publication will, in the months to come, develop slides to accompany other selected articles and abstracts. Announcements regarding these slides, which will also be provided with reprints on request, will appear in subsequent issues.

## Immunoreactive Sm-C/IGF-I in Urine From Normal Subjects, Pituitary Dwarfs, and Acromegalics

The authors report the development of a somatomedin-C (Sm-C) radioimmunoassay that permits measurement of minuscule quantities of Sm-C found in urine (1/1000 the quantity/mL in serum). The assay was used to measure Sm-C in the early morning urines of three acromegalics, 15 growth hormone (GH)-deficient children, and 25 normal adults, and in the urines of 230 normal infants, children, and adolescents. The total excretion was referred to the creatinine excretion (Cr) to gain more consistency than was otherwise possible.

The mean GH:Cr values for ten different age groups were:

Mean values of GH:Cr were high in the newborn (1.07) but much lower (0.34) in children 1 month of age or older. The values for the three acromegalics were 17.3, 1.52, and approximately 1.10. The values for the 15 children with GH deficiency (GHD) were all less than the mean for age, but only five were below -2 standard deviations for age. The test probably cannot be used for the diagnosis of GHD at this time.

The authors emphasize that no correlations have been made with serum GH concentrations or serum Sm-C determinations. The urinary concentrations of Sm-C are in most cases only 0.1-1 ng/mL,

much lower than one might have expected from plasma concentrations. The presence of binding proteins for Sm-C in plasma may contribute to the discrepancies observed between plasma concentrations and urinary excretion. It is possible that the Sm-C found in urine is secreted by the renal tubules. Further studies are needed to clarify the physiology involved.

Yokoya S, Suwa S, Maesaka H, et al. *Ped Res* 1988;23:151.

**Editor's comment**—Both Sm-C and GH are now measurable in very minuscule quantities in urine. The amounts of each are so small that it is difficult to believe that these assays will be pertinent to routine clinical studies. However, they may have applications in research. The reader is encouraged to read the abstract on page 15 on quantitation of urinary GH in children with normal and abnormal growth.

Robert M. Blizzard, M.D.

## A Triumph of Reverse Genetics: Characterization of Dystrophin in Duchenne and Becker Muscular Dystrophy

During the last five years, dramatic advances have been made in the study of Duchenne muscular dystrophy through DNA techniques in patients with visible X chromosome deletions on translocations through the Duchenne gene. It has been possible to isolate the gene and describe the protein (dystrophin) that is missing in Duchenne muscular dystrophy and abnormal in Becker muscular dystrophy. DNA analysis has demonstrated that these two diseases, which were thought to be separate, are in fact allelic.

The dystrophin gene, which is very large, has more than two million base pairs. The protein that has subsequently been isolated is also very large (400 kD) but it occurs in low amounts and repre-

sents only 0.002% of total muscle protein. The protein appears to be a subcellular component of the plasma-membrane system of normal muscle fibers. There are both qualitative alterations that lead to more severe muscular dystrophy and quantitative alterations that cause milder dystrophy.

It appears that at least one third of all cases of Duchenne muscular dystrophy represent new mutations, and at least half of them occur as deletions in or of the dystrophin gene. There still remains a great deal of work to be done in Duchenne muscular dystrophy. Defining the gene and the missing protein is only the beginning. Finding ways to treat affected individuals and replace the missing protein is the next step.

Hoffman EP, Fishbeck KH, Brown RH, et al. *N Engl J Med* 1988;318:1363-1368.

Rowland LP. *N Engl J Med* 1988;318:1392-1394.

**Editor's comment**—Research in molecular genetics is providing the information to work miracles, but the miracles begin with what can be found in the patients themselves. Patients with single-gene disorders who have visible cytogenetic alterations may well be the way to get to specific genes.

Judith G. Hall, M.D.

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## Long-Term Growth in Juvenile-Acquired Hypothyroidism: The Failure to Achieve Normal Adult Stature

Hypothyroidism was diagnosed and treated in 18 girls and six boys with a mean age of approximately 10.5 years and a mean bone age of 6.1 years. At diagnosis, heights were  $4.05 \pm 0.5$  standard deviations (SD) and  $3.15 \pm 0.5$  SD below the 50th percentile in girls and boys, respectively. Prior to deceleration of growth the mean height of all patients was less than  $\pm 0.3$  SD from the 50th percentile. The bone age at diagnosis closely matched the age at which deceleration of growth began, which suggests that the bone age at diagnosis corresponds well with the onset of severe hypothyroidism. L-thyroxine was given at  $3.4 \pm 0.3$   $\mu\text{g/kg/day}$  for treatment. Serial bone age determinations were available in most cases.

Mature heights were  $2.1 \pm 0.2$  SD below the 50th percentile. Differences between the predicted mature heights and the actual mature heights were  $7.7 \pm 6.0$  cm and  $6.7 \pm 5.5$  cm for females and males, respectively. The loss occurred primarily in the first 18 months of treatment and correlated significantly with the duration of hypothyroidism and the height SD at diagnosis. There was no correlation between the loss in mature height and the

chronologic, height, or bone ages at diagnosis.

The authors demonstrated that catch-up growth is incomplete after treatment of long-standing juvenile hypothyroidism. In brief, these patients rarely achieve their full genetic growth potential. The authors conclude that the possible etiologies for this deficit include: (1) overtreatment; (2) prolonged hypothyroidism, which diminishes the potential for catch-up growth; and (3) puberty coinciding with initiation of therapy, which results in completion of skeletal maturation prior to the completion of catch-up growth.

The thyroid function tests did not indicate overtreatment. Loss in predicted height during the first 18 months of treatment occurred in children who did not exhibit pubertal changes. The authors suggest that multiple factors may be involved but a delay in therapy is a critical factor in limiting catch-up growth that underscores the need for early recognition of hypothyroidism.

Rivkees SA, Bode HA, Crawford JD. *N Engl J Med* 1988;318:599-602.

**Editor's comment**—Failure to

reach expected adult heights in patients with prolonged juvenile hypothyroidism has been apparent to most pediatric endocrinologists but data documenting its occurrence and extent have been lacking. Rivkees *et al* have provided us with those data, thereby permitting postulations that can be tested to be made. They are to be congratulated for their contribution.

The decision to be made now is what to do for the next patient with prolonged hypothyroidism so that he or she can achieve the height inherent in his or her genetic potential. I would use a lower dose of thyroxine than that used by the authors, as  $3 \mu\text{g/kg/day}$  in older children may be more than is necessary to attain a euthyroid state. Alternative approaches might include the use of an analogue of leutinizing-hormone-releasing hormone(a) to block puberty and/or the addition of growth hormone. These latter approaches, if chosen, should be used within rigid protocol guidelines and, therefore, should not be considered by most of us unless we are willing and able to establish and follow such a protocol.

Robert M. Blizzard, M.D.

## Mechanism of the Adolescent Growth Spurt Induced by Low-Dose Pulsatile GnRH Treatment

Stanhope *et al* studied growth velocity and growth hormone (GH) secretion in 14 females and 12 males with pubertal delay during treatment with gonadotropin-releasing hormone (GnRH). The findings were then related to stages of sexual development. All 26 patients in this study had puberty delayed by two standard deviations or had puberty arrested for at least 18 months. The mean ages were 16.4 years for girls and 16.8 years for boys.

GnRH was administered subcutaneously in a pulsatile fashion via pump. Initially, GnRH was administered only at night to mimic the normal nocturnal pattern of gonadotropin secretion in early puberty. However, when girls attained breast stage 3 and boys attained a testicular volume of 8 mL, pulsatile GnRH was administered over the 24 hours of each day. The duration of treatment averaged 1.05 years in both boys and girls.

Serum GH levels were sampled every 15 minutes between 8 P.M. and 6 A.M. prior to the initiation of the study, at 1- to 3-month intervals throughout the study, and 1 month

after the cessation of treatment. GH profiles were analyzed using the PULSAR computer program. GH pulse frequency, the sum of the GH peaks, and the area under the GH pulses were calculated for each overnight GH profile, and then correlated with breast stage in girls and mean testicular volume in boys.

Twenty-four patients responded to pulsatile GnRH treatment with the normal sequence of sexual maturation. Peak growth velocity occurred between breast stages 2 and 3 in girls; GH secretion was increased at stage 2 but was significantly increased with its peak value at stage 3. GH secretion de-

*continued on page 12*



### Mechanism of the Adolescent Growth Spurt Induced by Low-Dose Pulsatile GnRH Treatment *continued from page 11*

creased at the attainment of stage 4, after which it was altered cyclically depending on the stage of the menstrual cycle. There were no significant changes in GH pulse frequency.

In boys who responded to pulsatile GnRH treatment, there was an initial significant fall in the sum of GH peaks and in the area under the GH pulses with the onset of treatment, although testicular volume increased to 5-6 mL and virilization was initiated. A rapid increase in growth rate occurred at a testicular volume of 9-10 mL and reached a peak value between 11 and 15 mL. Peak height velocity (between 11 and 12 cm per year) coincided with peak GH secretion. The mean change in GH secretion demonstrated a pattern similar to the change in growth velocity. Se-

rum testosterone concentrations rose progressively throughout puberty, with no dramatic rise at the onset of the growth spurt.

Based on their study findings, the authors suggest that the mechanism of increased GH secretion is not due solely to sex steroid secretion, since testosterone secretion and virilization in boys occurs during early puberty while the growth rate and GH secretion continues to decelerate. They suggest that the level of testosterone achieved at a testicular volume of 10 mL may be important in the etiology of the increase and amplitude of the GH pulses. They also suggest that there may be an interaction between GnRH and somatotroph function.

Stanhope R, Pringle P, Brook C. *Clin Endocrinol* 1988;28:83-91.

**Editor's comment**—This study adds important information to the understanding of the mechanism

and timing of growth acceleration in puberty and the relationship to testicular volume and serum testosterone. Unfortunately, serum testosterone values are not reported in this paper. Others have demonstrated the relationship between testosterone and the increase in GH secretion and have demonstrated that testosterone increases mean GH secretion and the amplitude of GH pulses in prepubertal boys. The authors of this article have presented longitudinal data on a small group of patients, half of whom were later determined to have hypogonadotropic hypogonadism and half to have constitutional delay of puberty. They correctly point out that there are no longitudinal data available on physiologic GH secretion during normal puberty with which to compare their findings. Such studies should shed further light upon the mechanism of growth acceleration during puberty.

William L. Clarke, M.D.

### Diagnosis of GH Deficiency and GH Treatment

The availability of recombinant growth hormone (GH) has directed much attention to the diagnosis and treatment of GH deficiency (GHD). Rose et al of the NIH recently published in the *New England Journal of Medicine* an extensive analysis comparing the use of pharmacologically stimulated GH levels with spontaneous GH secretion—as determined by the GH levels over 24 hours—to diagnose GHD.

Three pharmacologic stimuli were used in 54 children with severe short stature. In 23, all GH values were  $\leq 7$  ng/mL, and these children were classified as GH-deficient on this basis. These results were compared with the mean integrated GH concentrations (ICGH). All 31 children who responded to pharmacologic tests with values  $>7$  ng/mL had ICGH

values in the range found in 46 normal-statured prepubertal children. Therefore, the authors conclude that no additional patients with GHD were detected and that the timely and costly measurement of ICGH in short children is of little diagnostic value.

The correlation between the results of the pharmacologic tests and ICGH levels in the GHD patients was poor, as only 57% of the 23 patients had ICGH levels below the range found in the 46 controls. Therefore, the authors recommend that the use of pharmacologic stimuli is sufficient to diagnose GHD.

The authors explain that the inclusion of more appropriate control subjects accounts for the discrepancy between their studies of ICGH with those of others. They postulate that the 43% of children with GHD, who had ICGH levels in the lower 20% of normal range, were children who require a higher GH level than most to grow nor-

mally; some defect reduced the spontaneous secretion of GH until it was in the lower normal range, and the defect was revealed after pharmacologic testing. Rose et al readily point out that further studies are indicated to determine how to best diagnose GHD.

They also report that the ICGH levels in the 46 controls did not correlate significantly with age, sex, height, weight, insulin-like growth factor (IGF-I) level, or growth velocity for age, although the IGF-I levels in the 31 short children without GHD were between the values seen in controls and GHD children.

In an editorial in the same journal, Grumbach addressed the use of GH therapy in GHD and short stature. He states that the criteria of Rose et al for the selection of short children for treatment with GH was rigidly defined and straightforward.

In the past, treatment was restricted to children with growth

velocities below the 25th percentile for age and GH levels  $\leq 14$  ng/mL on at least two provocative tests. However, conventional tests to define GHD have important limitations, including a paucity of standards that are related to age and sex in normal children. Also, there is growing concern about the variation in GH concentrations when kits from various commercial suppliers are used to measure GH levels.

Grumbach agrees that the observations of Rose et al are important and have practical implications. He agrees that the 24-hour GH profile remains a useful research tool but probably should not be used as a routine diagnostic procedure or in the selection of children with idiopathic short stature (ISS) for trial therapy with GH. He emphasizes that not one of the tests of GH secretion is, in fact, a useful discriminant in the selection of short healthy children for a trial therapy with GH. After excluding frank GHD by provocative tests, one acceptable approach is to categorize children with ISS as either responsive or unresponsive to GH over a 6-month period of treatment. This raises a most important question: Will the treatment increase the predicted height or merely lead to the attainment of adult height at an earlier age?

Ethical and economic issues must also be considered. For example, how will abuse of GH be avoided? Grumbach emphasizes that long-term, well-controlled studies to resolve the issues must be done promptly. He draws attention to the usefulness of oxandrolone, low-dose estrogen, and low-dose testosterone, all of which can be used as alternate therapeutic agents in certain short children (eg, those with constitutional delay of growth and Turner syndrome). His astute conclusion is that in considering GH treatment in children with ISS, we should recognize that the problem lies not in the GH profiles, but in the role of "heightism" in our society and the

psychosocial disadvantage it confers.

Rose SR, Ross JL, Uriarte M, et al. *N Engl J Med* 1988;319:201.

**Editor's comment**—Whoever says that life and the practice of medicine are easy has not visited the offices of doctors who treat short children. Although all agree that GHD is present in the patient with no response above 5 ng/mL (as determined by the GH assay utilizing the reagents and standards of the National Hormone Pituitary Agency), there are other short children who have partial GHD who will not be diagnosed in 1988 by pharmacological stimuli that test for GH adequacy. As pointed out by Reiter et al (*J Clin Endocrinol Metab* 1988;66:68), the results of GH concentrations vary widely with different assays. In our laboratory, the same serum specimen will yield a value of 4.0 ng/mL by the Hybritech assay and 8-12 ng/mL by the Nichols' kit assay. Some clinics using the same assay consider abnormal only values  $\leq 7$  ng/mL in response to a pharmacologic test; other clinics classify only values  $\leq 14$  ng/mL as abnormal. In addition, some of the short children with "normal" test results by accepted pharmacologic testing criteria will have low IGF-I concentrations and/or markedly delayed bone ages and/or low or low-normal ICGH levels. These patients may have GH inadequacy,

but not necessarily GHD, if GHD is interpreted as decreased GH production. GH inadequacy can encompass the production of a biologically inactive but immunologically active hormone or a partial resistance in generating IGF-I, which might be overcome with increased exposure to GH. The latter is comparable in concept to vitamin D dependency, a condition in which pharmacologic amounts of vitamin D are required to produce normal amounts of  $1\alpha,25$  dihydroxycalciferol.

Are these very short patients with possible GHD or inadequacy, whose physicians cannot agree on a uniform level of GH for interpretation of normalcy or on the GH assay to be used, to be deprived of GH? Rose et al and Grumbach have written that "further elucidation of what comprises GHD (and inadequacy) will need to be clarified, and the long-term effect of GH on ultimate height in ISS will have to be determined." In my opinion, a humane approach permits the occasional prescribing of GH on a trial basis for children with extreme short stature with possible deficiency of GH or inadequacy. However, all of us must prescribe judiciously to prevent abuse of GH. Most importantly we must be sympathetic, considerate, and supportive of those who are affected by the "heightism" in our culture.

Robert M. Blizzard, M.D.

## Body Composition of Peruvian Children With Short Stature and High Weight for Height

Chronic undernutrition frequently occurs among children from underdeveloped countries. When combined with infectious diseases, it can result in a low height-for-age ratio and/or nutritional dwarfing. Paradoxically, nutritional dwarfing may also be seen in children with excess weight for height.

One hundred and thirty-nine Peruvian children, ages 6 months to 5 years, with nutritional dwarfing

but excess weight for height, were studied using both total body water measurements and detailed anthropometric assessment. Results of this study were compared with the National Center for Health Statistics (NCHS) Reference Standards. The mean weight-for-length/height of children in the study sample was above the 50th percentile and appeared to increase with age. In contrast, the

*continued on page 14*

### Body Composition of Peruvian Children

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mean length/height-for-age fell from the 35th percentile by age 18 months and remained consistently low. Although arm muscle area, based on age and body weight, was not altered, anthropometric assessment revealed a lower body fat content (based on triceps skinfold, subscapular skinfold, and arm fat measurements). Additionally, greater values on assessment of total body water suggested a lower body fat content in these children.

Trowbridge FL, Marks JS, deRomana GL, et al. *Am J Clin Nutr* 1987;46:411.

**Editor's comment**—This interesting paper identifies nutritional dwarfing among children with ex-

cess body weight for length or height. The data show that undernourished children may cease to grow even before fat stores or lean body mass is depleted. This cessation may occur without weight loss or even when body weight continues to increase with age. Unfortunately, the authors did not provide data on progression of weight for age. It is possible that weight increments also slowed and the rate of advancement decreased, but since these changes were not as marked as those for length or height, a higher body weight for height continued. Additionally, these data emphasize the importance of repeated measurements of growth over time as indicators of normal health and nutrition. Any single weight-for-height measurement may fail to identify children with nutritional dwarfing.

The authors did not explain the causes of nutritional dwarfing in children without weight deficits. Dietary inadequacies beyond caloric or protein deficits may be implicated. For example, deficiencies of specific nutrients such as zinc and iron could lead to nutritional dwarfing. Additionally, obese children on weight-reduction diets also exhibit impaired growth despite continued excessive weight for height. Therefore, nutritional dwarfing in these Peruvian children may have resulted from a period of inappropriate diet following adequate nutrition. Stunting of growth without loss of body weight, but with reduction in body fat content, may occur as a gradual adjustment to inadequate dietary intake. Growth slows as an adaptive response to balance energy intake and expenditures.

Fima Lifshitz, M.D.

### Adolescent Growth and Pubertal Progression in the Silver-Russell Syndrome

Davies et al categorized the pattern of growth and development of 18 adolescents with Silver-Russell syndrome. All exhibited the classic features of the syndrome, including clinodactyly, triangular facies, and low-set ears. They had grown less than 1 cm in the previous year and had had their growth measured for at least 3 years prior to the onset of puberty. When attempting to describe a mean growth curve for these individuals, the authors paid special attention to the phase effect, ie, variability in timing, duration, and intensity of the adolescent growth spurt. Mathematical modeling utilizing the method of Preece and Baines was applied to the longitudinal data for each child. The age of attainment of pubertal stages was reported for each child as well.

In both males and females, the adult height was well below the third percentile for normal British

children. The standard deviation scores for adult height were  $-3.61$  for boys and  $-3.58$  for girls. However, subjects of both sexes exhibited height velocity curves that were well within the range of normal British children. No abnormal pattern or timing of pubertal events, including the pubertal growth peak height velocity, was observed. The actual peak height velocity was 8.3 cm per year in boys and 8.0 cm per year in girls. In addition, the age at the beginning of the adolescent growth spurt and the velocity at that time were also within normal ranges. The actual growth curve for these individuals with Silver-Russell syndrome demonstrates that there is little catch-up growth during childhood and adolescence, and that growth essentially proceeds normally in childhood. The mean age for the attainment of each stage of pubertal development and the mean age of menarche were also within normal range for British children. Thus, the authors conclude that there is normal pubertal development of sexual characteristics in those with the

Silver-Russell syndrome.

Davies P, Valley R, Preece M. *Arch Dis Child* 1988;63:130-135.

**Editor's comment**—This report is one of the first that carefully evaluates a number of individuals with Silver-Russell syndrome who have reached physical maturity. The growth curves that have been constructed for these children should be reviewed by pediatric endocrinologists. They are remarkably parallel to those for normal British children and the velocity curves fall well within the normal height velocity curves for British children as well. This careful characterization of children with Silver-Russell syndrome reemphasizes their poor prognosis with regard to adult stature but reassures that puberty is essentially normal in terms of the adolescent growth spurt and the development of sexual characteristics. Future long-term trials of growth-promoting agents will be required to see if there is to be any hope of catch-up growth in these children.

Robert M. Blizzard, M.D.

## Quantitation of Urinary GH in Children With Normal and Abnormal Growth

Albini et al have reevaluated the use of urinary growth hormone (GH) determinations as a screening test for GH deficiency (GHD) in children. Previous studies attempting to quantitate GH in the urine had not been successful because the assays were not sufficiently sensitive, and interfering substances found in the urine led to overestimation of GH excretion. In their study, the authors used a modification of the Hanssen procedure in which after urine is dialyzed and then lyophilized, GH is measured in a double-antibody radioimmunoassay (RIA).

This RIA uses polyclonal antibodies and standards obtained from the National Hormone and Pituitary Program. The intraassay and interassay coefficients of variation for GH antibodies and standards are 2.1 and 4.0, respectively, and the lower threshold of sensitivity of the assay is 0.15 ng/mL. High-pressure liquid chromatography (HPLC) studies confirmed the authenticity of urinary GH, as the elution profile of urinary GH was identical to both biosynthetic and pituitary GH standards. Furthermore, the HPLC fractions were assayed using a double-monoclonal immunoradiometric assay (IRMA) technique that recognizes only intact GH. The immunoreactive GH profiles defined by the two assays RIA and IRMA were identical. Recovery experiments were performed by adding known amounts of standard human GH to 50-mL aliquots of urine from GH-deficient subjects. The recovery of exogenous GH ranged from 80% to 100%.

Clinical studies were then performed to determine GH excretion in 82 children. These children were divided into three groups. Group 1 included 31 healthy children (ages 3-17 years) whose height was between the 5th and 95th percent-

iles. Nineteen were prepubertal and 12 were pubertal. Group 2 was composed of 21 children (ages 5-15 years) with GHD that had been determined by standard stimulation tests. Eleven of these children were prepubertal and ten were pubertal. Group 3 was composed of 30 children (ages 10-18 years) with idiopathic growth failure. Fifteen of these children were prepubertal and 15 were pubertal. Their heights were more than two standard deviations below the mean for age, and their growth rates were less than 4 cm/year. However, their peak GH responses to two or more stimulation tests were greater than 8 ng/mL. Overnight urines (6 P.M.-8 A.M.) were collected and refrigerated prior to GH analysis. GH excretion was standardized for body weight and expressed as ng/kg/12 hours as well as in terms of body surface area (ng/m<sup>2</sup>/12 hours). In addition, GH excretion was standardized in terms of creatinine excretion (ng/g of creatinine).

When urinary GH excretion was expressed in terms of body weight or body surface area, the secretion was significantly greater in group 1 than group 2 or group 3. In addition, children in group 2 excreted significantly lower amounts of GH than those in group 3. However, when the data were expressed in terms of creatinine excretion, the differences in GH excretion between group 2 (GH-deficient subjects) and group 3 (children with idiopathic growth failure) were not significant. Prepubertal and pubertal children in each of the three groups excreted similar amounts of GH regardless of the method of standardization. The authors conclude that measurement of urinary GH may prove to be useful in screening patients with suspected GHD. This clinical methodology is significantly easier for staff and patients and less costly than serial blood sampling over 24 hours in determining GH neurosecretory dysfunction. However, approxi-

mately 50% of the children with idiopathic growth failure had urinary GH values that were similar to those of children with classic GHD, leading the authors to suggest that these children may have GHD.

Albini C, Quattrin T, Vandlen R, et al. *Pediatr Res* 1988;23:89-92.

**Editor's comment**—The studies reported above were carefully performed and show significantly more precision than previously reported evaluations of urinary GH excretion. The documentation of the authenticity of urinary GH by this very sensitive assay is reassuring. However, the fact that 50% of the children with idiopathic growth failure had urinary GH values similar to those of children with classic GHD suggests that further studies are still required in approximately half of the children who had abnormal urinary GH values prior to the initiation of therapy with exogenous GH. In addition, since there were no differences in the excretion of urinary GH between prepubertal and pubertal children in each of the three groups, the question remains as to whether some of the children with idiopathic growth failure and abnormally low urinary GH values, in fact, had constitutional delay of growth and adolescence. The present study, however, should encourage others to obtain more data utilizing the reported procedure in an attempt to fully define its utility as a screening process for GHD. Clearly, the availability of a noninvasive, low-cost screening procedure for GHD would be welcomed by most pediatric endocrinologists.

William L. Clarke, M.D.

### Address for Correspondence

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## MEETING CALENDAR

**April 12-16, 1989** 15th Training Course on Hormonal Assay Techniques. Holiday Inn, Bethesda, Maryland. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**April 27-30, 1989** Lawson Wilkins Pediatric Endocrine Society Review Course in Pediatric Endocrinology. Hyatt Regency Washington on Capitol Hill, Washington, D.C. Contact: Beverly Wellman, Sero Symposia, USA, 280 Pond Street, Randolph, MA 02368 (800-225-5185)

**April 27-30, 1989** Biennial Meeting of the Society for Research in Child Development. Kansas City, Missouri. Contact: Kathleen McCluskey-Fawcett, Ph.D., Department of Psychology, University of Kansas, 426 Fraser Hall, Lawrence, KS 66045-2160 (913-864-4131)

**May 1-5, 1989** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Washington Sheraton, Washington, D.C. Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2650 Yale Boulevard S.E., Suite 104, Albuquerque, NM 87106 (505-764-9099)

**May 5, 1989** Annual Meeting of the Lawson Wilkins Pediatric Endocrine Society. Washington Sheraton, Washington, D.C. Contact: Gilbert August, M.D., Department of Endocrinology, Children's Hospital, National Medical Center, 111 Michigan Avenue N.W., Washington, D.C. 20010 (202-745-2121)

**June 3-6, 1989** Annual Meeting of the American Diabetes Association. Detroit, Michigan. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22314 (703-549-1500 or 800-ADA-DISC)

**June 21-24, 1989** 71st Annual Meeting of the Endocrine Society. Seattle Convention Center, Seattle, Washington. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**September 26-28, 1989** 30th Annual Meeting of the American College of Nutrition. Omni International Hotel, Norfolk, Virginia. Contact: Kay Balun, Administrative Assistant, American College of Nutrition, 345 Central Avenue, Suite 207, Scarsdale, NY 10543 (914-723-4247)

**October 11-14, 1989** 41st Annual Postgraduate Assembly of The Endocrine Society. Fairmont Hotel, New Orleans, Louisiana. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**October 29-November 3, 1989** Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology (scientific sessions, October 30-November 1). Jerusalem Hilton, Jerusalem, Israel. Contact: Zvi Laron, M.D., Beilinson Medical Center, Petah Tikva 49 100 Israel

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# GROWTH

## Genetics & Hormones

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### Sexual Precocity, GnRH Analogs, and Growth

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Exposure of the skeleton during childhood to pubertal levels of sex steroids profoundly increases linear growth and epiphyseal maturation. This is exemplified in the normal state of adolescent sexual development and occurs also in the pathological state of sexual precocity. In the latter, one sees marked acceleration of linear growth, but subsequent epiphyseal fusion results in early cessation of growth. Thus, many children with sexual precocity fall short of their genetic potential for adult height.

The initial goal of this article is to review the normal physiology of the normal role of gonadotropin-releasing hormone (GnRH), which is also known as luteinizing-hormone-releasing hormone (LHRH). A discussion of the impact of pituitary-gonadal suppression on growth and final height in children with central precocious puberty (CPP) with the use of suprapotent GnRH agonist-analogs (GnRHa) will follow.

#### GnRH, LH, and FSH in Normal and Central Precocious Puberty

Pubertal maturation and adult reproductive function are dependent on a precisely integrated neuroendocrine-gonadal axis. The same neuroendocrine changes that underlie normally timed pubertal maturation are active in CPP. Normal puberty and CPP are both initially characterized by nocturnal, sleep-entrained, pulsatile luteinizing hormone (LH) secretion. This pulsatility probably results from a pulsatile secretion of GnRH. With progression of pubertal maturation, secretion of LH is established in the daytime, and 24-hour patterns of pulsatile LH release, which are characteristic of the adult state, then evolve. The maturation of gonadal function that is induced by LH and follicle-stimulating hormone (FSH) results in the spectrum of physical changes observed at puberty. These changes are the result of both direct and indirect effects of sex steroids.

It is important to remember that the development of normal puberty consists of both gonadarche and adrenarche. The sex steroids produced by the gonads are responsible for gonadarche, which is under

the control of GnRH, LH, and FSH. The control of adrenarche is not understood, but it is not under the control of the gonadotropins, adrenocorticotrophic hormone (ACTH), or prolactin. Adrenarche is characterized by a significant increase in the secretion of adrenal sex steroids, best indexed by dehydroepiandrosterone sulfate (DHEAS). Interestingly, in CPP, gonadarche is always present, but adrenarche may or may not be present. The reason for this is not understood since the control of normal adrenarche is not understood.

In vitro and in vivo studies in animals and humans have shown that the dose and pattern of GnRH stimulation play critical roles in the pituitary's release of LH and FSH. Pulsatile secretion or administration of GnRH, which produces intermittent stimulation of the pituitary, is an absolute requirement for the generation of pulsatile secretion of gonadotropins. Continuous GnRH stimulation of the gonadotropes induces an initial release of LH and FSH, which subsequently wanes during continued exposure.

#### Pituitary Desensitization with GnRH Agonist-Analogs

If the level of continuous GnRH exposure is sufficiently high, the pituitary is desensitized and will no longer have the capacity to respond to superimposed boluses of GnRH. The pituitary can be even more significantly desensitized with potent analogs of GnRH. Interestingly, chronic desensitization is not associated with depletion of cell stores of

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## Sexual Precocity

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hormone, nor with a decreased number of GnRH receptors on the pituitary cell membrane. Responsiveness of the gonadotropes to pulsatile GnRH is fully and rapidly restored when continuous GnRH or analog exposure is withdrawn.

Critical experiments were performed several years ago to chart the physiologic actions of GnRH. The metabolic fate of the native decapeptide *in vivo* was also determined. These data helped to establish the basis for the synthesis of GnRH analogs that retained biological activity and were relatively resistant to *in vivo* degradation. The GnRH molecule was modified at the position of the sixth amino acid and the C-terminus. These modifications succeeded in creating a family of compounds with suprapotent agonist properties and the ability to desensitize the pituitary to GnRH pulsation. Thus, the availability of long-acting, suprapotent GnRHs and the emerging knowledge of the action of GnRH were combined. As a result, a role for these analogs was suggested as a possible therapeutic agent in a number of clinical settings in which reversible suppression of the pituitary-gonadal axis is desirable (eg, contraception, prostate cancer, endometriosis, uterine fibroids, and CPP). These compounds and their comparable potencies are listed in Table 1.

Since the specific potency and *in*

*vivo* clearance rates of GnRH agonists vary considerably, careful attention to dose, frequency of administration, and route of administration is essential. For example, only 5% of intranasally administered analog is absorbed. The reader should understand that utilizing an agonist of low potency, choosing too low a dose, administering doses at an insufficient frequency, or providing a combination of these factors, may result in a failure to induce complete desensitization of the pituitary. In this situation, the administered GnRHs may not act as an inhibitor but as a potent stimulator of pituitary-gonadal function. If this occurs for a prolonged period, the patient's ultimate height may be inadvertently diminished from that expected either normally or with complete suppression of the pituitary-gonadal axis by adequate GnRH therapy.

The availability of depot formulations of GnRHs that are able to maintain consistent pituitary suppression may serve to obviate some of the above concerns. Regardless of whether a depot or short-acting preparation is used, one can only be sure that the clinically desired suppression has been induced by determining with careful monitoring that the pituitary no longer continues to release LH. The response to a GnRH challenge—therefore, the release of gonadotropins—must be suppressed.

Monitoring is especially critical since one is hypothesizing that the observed endpoints, such as linear

growth and maturation of the skeleton in children with CPP, are being measured in a patient who has no sex steroids present. The concentration of gonadal sex steroids must fall to prepubertal levels and pulses must be eliminated even when LHRH is given intravenously. The documentation of prepubertal-sized ovaries and, therefore, gonadal "inactivity" by ultrasound also is of extreme value in demonstrating complete suppression of pituitary and ovarian function in girls with CPP.

A complication of evaluating the suppression of LH sometimes occurs when the radioimmunoassay kits that are used to measure LH also measure the  $\alpha$ -subunit of the glycoprotein-tropic hormones of the pituitary. Very interesting, and as yet unexplained, is the fact that the  $\alpha$ -subunit continues to be released in patients who are receiving GnRHs. The  $\alpha$ -subunit is biologically inactive, but in some LH assays the  $\alpha$ -subunit crossreacts with intact LH. In these assays it may appear that the patient does not have total suppression of the pituitary because  $\alpha$ -subunit measurements may be mistaken for LH measurements. However, the release of LH itself may be totally inhibited. Evaluation of the results of the LH assay requires knowledge as to whether the  $\alpha$ -subunit is measured, as well as intact LH, or whether just LH is measured. The assays used to measure LH following GnRH stimulation in the analog-suppressed patients should be those that measure

Table 1. GnRH and GnRH agonists

| pGlu<br>1 | His<br>2 | Trp<br>3 | Ser<br>4 | Tyr<br>5 | Gly<br>6           | Leu<br>7 | Arg<br>8 | Pro<br>9 | NH <sub>2</sub><br>10 | Relative Potency<br>1 (Standard) |
|-----------|----------|----------|----------|----------|--------------------|----------|----------|----------|-----------------------|----------------------------------|
|           |          |          |          |          |                    |          |          | NEt      | (Fujino)              | 4                                |
|           |          |          |          |          | D-Ala              |          |          | NEt      |                       | 3                                |
|           |          |          |          |          | D-Ala              |          |          | NEt      |                       | 14                               |
|           |          |          |          |          | D-Tyr              |          |          | NEt      |                       | 68                               |
|           |          |          |          |          | D-Trp              |          |          | NEt      | (Salk)                | 144                              |
|           |          |          |          |          | D-Ser (t-Bu)       |          |          | NEt      | (Hoechst)             | (20-40)                          |
|           |          |          |          |          | D-Leu              |          |          | NEt      | (TAP)                 | (20-30)                          |
|           |          |          |          |          | D-Nal <sup>2</sup> |          |          |          | (Syntex)              |                                  |
|           |          |          |          |          | D-His (ImbzI)      |          |          | NEt      | (Ortho)               | 140                              |

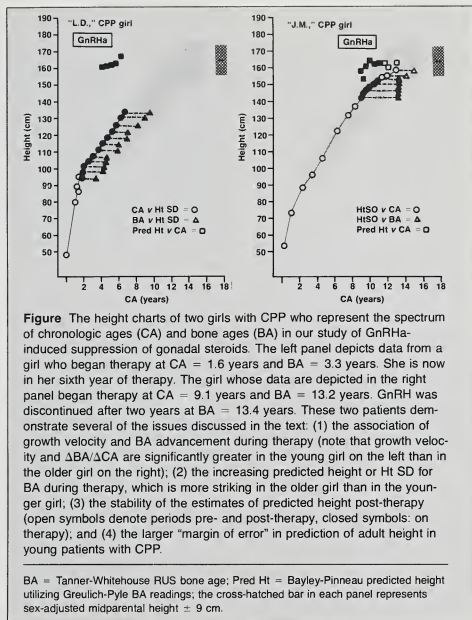
intact LH, and the antibodies in these assays should not crossreact with the  $\alpha$ -subunit.

## The Effect of GnRHa Therapy in CPP on Growth and Skeletal Maturation

Premature exposure of the child to sex steroids causes an acceleration in skeletal maturation that exceeds the acceleration of linear growth. Consequently, in the child with CPP the  $\Delta$ bone age (BA)/ $\Delta$ height age (HA) is greater than 1.0, and the ultimate height is reduced when this persists. The hypothesis that GnRHa therapy of CPP will increase the ultimate heights implies that suppression of the sex steroids will result in just the reverse: a more pronounced slowing of skeletal maturation than of linear growth ( $\Delta$ BA/ $\Delta$ HA will, therefore, be significantly less than 1.0). In this instance, the absence of sex steroids should delay epiphyseal fusion and result in an adult height greater than that which would have been expected if sexual precocity had continued unabated. Several studies, including our own in collaboration with investigators at Children's Hospital in Boston and at the University of Virginia in Charlottesville, have provided preliminary evidence and support of this hypothesis that patients with CPP who are treated with GnRHa may end up taller than they otherwise would have. The crux of success relates to the relative slowing of the advancement of BA in relation to the advancement of HA, and not to the actual increase in linear height each year. If the  $\Delta$ BA/ $\Delta$ HA is  $<1.0$  over an extended period, the predicted height and ultimate height should be increased.

In our clinical studies we demonstrated very early that most patients treated with the analog have decreased linear growth compared with their pretreatment growth rates. The Figure, in conjunction with the following explanation, clarifies the variations observed according to the pretreatment skeletal age.

Young children (as exemplified in the left panel of the Figure)



whose pubertal development was arrested when their BAs were still in the prepubertal range (ie,  $\leq 10$  years) have continued to grow at prepubertal rates. Other children whose therapy began when their skeletal maturations were advanced to late pubertal stages and whose BAs were  $\geq 13$  years (right panel), have grown at significantly slower rates than normal children who were matched for chronologic age (CA). The explanations for these observations are as follows: When one considers that the BA of patients in the latter instance is well past the age of peak height velocity growth, the slow growth velocities that are observed can be viewed in an appropriate developmental context. These individuals are growing less rapidly, as expected in relation to normal growth velocity curves. This slow

growth rate is not necessarily detrimental to the ultimate height, as it must be viewed along with changes in skeletal maturation that determine whether there ultimately is an increased increment in growth. As predicted, growth velocity during sex steroid suppression is greater in children with BA  $<10$  years than in patients with older BAs.

In a group of 43 girls with CPP who have completed three years of GnRHa therapy in our study, the mean growth velocity fell from 10.9 cm/year prior to therapy to 5.8 cm in the first, 4.8 cm in the second, and 4.2 cm in the third year. In comparing the patients with a pre-therapy BA  $\leq 10$  when therapy was started with those whose BAs were  $\geq 13$  years, one notes that the rate of growth was 6.9 cm/year v 4.2 cm the first year. In year two, the com-

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## Sexual Precocity

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parable growth velocities were 6.2 v 3.2 cm/year, and in year three, the comparable rates were 5.7 v 2.7 cm/year. Regardless of the slow growth velocity in the group with advanced BAs, if the BA remains fixed, as it often does (as exemplified in patient J.M. in the Figure), the increase in growth may equal nearly 9 cm while the BA stays essentially stationary.

The explanation for the slowing of growth when patients with CPP are given GnRHa may relate to the interrelationship of sex steroids and the production of growth hormone (GH). Both estrogen and testosterone have been shown to increase GH secretion in prepubertal individuals. We and others have investigated the effect of GnRHa and, therefore, the decrease of estrogen and testosterone on GH secretion and insulin-like growth factor-I (IGF-I) levels. GH secretion, as determined by integrated concentrations and peak height amplitudes, is diminished in these patients. IGF-I determinations also tend to fall with GnRHa administration, although they usually do not fall into the prepubertal range. Interestingly, adrenarche progresses normally for CA during GnRHa therapy, providing further evidence that adrenarche is not under the control of LH or FSH.

### GnRHa Therapy of CPP and the Possible Effect on Final Height

There are no published reports addressing actual final heights in children with CPP who were treated with GnRHa. Consequently, predictions of final height and the impact of therapy upon them must be used. Predictions of adult height using the Gruelich-Pyle BA with the Bayley-Pinneau tables have shown general agreement with subsequent final heights of untreated children with CPP. The use of the Tanner-Whitehouse and Roche-Wainer-Thissen methods of predicting height are not as accurate in chil-

**Table 2.** Final height prognosis: ht SD for BA

| Pretherapy BA | Pretherapy | After 1 year of therapy | After 2 years of therapy | After 3 years of therapy |
|---------------|------------|-------------------------|--------------------------|--------------------------|
| <10 years     | -1.3       | -1.5                    | -1.3                     | -1.1                     |
| 10-12 years   | -1.9       | -2.0                    | -1.7                     | -1.7                     |
| 12-13 years   | -2.8       | -2.3                    | -2.1                     | -2.1                     |
| >13 years     | -3.7       | -3.1                    | -2.6                     | -2.1                     |
| All           | -2.3(80*)  | -2.1(83*)               | -1.8(68*)                | -1.6(43*)                |

\*Number of patients.

dren with CPP. Although not ideal, the method of Bayley-Pinneau permits a standardized evaluation of BA (SD for BA) and the probable effectiveness of therapy.

During long-term therapy with GnRHa, reports of improvement in the prediction of final heights in children with CPP have been published. Changes in the final height predictions based on SD for BA over three years of therapy are presented in Table 2. These data reveal that patients whose BAs were  $\leq 10$  years when therapy was started had only a marginal statistical improvement (the SD score over three years of improvement changed only from -1.3 to -1.1). However, one must, in spite of the marginal statistical improvement, realize that complete suppression of sexual precocity apparently provides tremendous gains for patients in this group. This conclusion is based on the height SD for BA (-3.7) in patients who began therapy at BA > 13 years. The group with the younger BAs could be expected to end up with a comparable SD (-3.7) if they had received GnRHa.

For those whose BAs were  $\geq 13$  years when treatment was initiated, the height SD for BA increased from -3.7 to -2.1, a very significant increase. In all groups, the predicted height increased significantly.

Is it advantageous to use the analog until there is complete epiphyseal fusion or is it more advantageous to stop GnRHa therapy earlier than this? One must know the patterns of growth and skeletal maturation during the reactivation

of gonadarche to accept that the increments in predicted height during therapy truly reflect an impact on final height. Theoretically, cessation of analog therapy in children with CPP whose BA was  $\leq 11$  years (ie, prior to normal peak height velocity) could produce an increased growth rate and a greater ultimate height than if the analog were continued. On the other hand, if the rate of BA advancement was excessive, the patient might lose ultimate height by stopping the analog at such an early BA. Monasco et al have reported data in patients who were followed for one year after the discontinuation of GnRHa therapy. In their report and in our experience, increments in predicted height observed during therapy have not diminished in girls with BA > 13 years.

In a group of such girls whom we

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have followed post-therapy, pubertal function returned when they were far advanced on the declining slope of the pubertal growth spurt. (This was because the BAs were at a mean of 13.6 years.) The average growth velocity in the first year post-therapy in this group neither increased, as might be expected with the resumption of puberty, nor decreased (3.6 cm/year during therapy, 3.7 cm/year post-therapy). The rate of BA advancement with the return of pubertal gonadal function accelerated, with a resultant  $\Delta\text{BA}/\Delta\text{HA}$  of 0.7 in the first year after therapy. However, significant variability in both growth velocity and BA advancement existed within individuals in the group such that the optimal timing of the discontinuation of GnRHa therapy even in this group is still not known.

Theoretically, children who begin therapy at a young age, and whose BAs are prepubertal when the analog is stopped, might develop an adverse ratio of the  $\Delta\text{BA}/\Delta\text{HA}$  and lose predicted height. Children in this category who have been withdrawn from therapy at this writing are very few. As these children approach the normal age of puberty, a pubertal growth spurt will be necessary for them to attain the increased height that usually is predicted with the use of GnRHa. It is especially true that in treating this group, we must await the results of further longitudinal studies before the impact of GnRHa therapy on final height in CPP can be fully appreciated. The ideal time to stop the analog therapy remains unknown.

## Summary and Conclusions

During suppression of gonadal steroids with GnRHa in patients with CPP the growth velocity correlates inversely with the degree of underlying skeletal maturation. This velocity is similar to that in other hypogonadal states, such as in constitutional delay of puberty with GnRH deficiency. Skeletal maturation proceeds chronologically, year for year, in children with "prepubertal" BAs despite com-

plete suppression of gonadal steroids. However, since gonadal steroids are required for epiphyseal fusion, skeletal maturation virtually ceases in children with CPP whose BAs are in mid-late puberty (therefore, >13 years). Given this ability to forestall epiphyseal fusion, reversible suppression of gonadal steroids permits significant growth to continue at advanced degrees of skeletal maturation.

Like growth rates during gonadal suppression, growth rates during the reactivation of gonadarche correlate with the degree of underlying skeletal maturation. The rate of skeletal maturation following the return of pubertal gonadal function is not accelerated. The improvements in the prognosis for the mean final height of the group of patients whose BAs were >13 years that were observed during GnRHa administration are not lost following the discontinuation of therapy. It is unknown at present whether patients whose BAs are less than this will lose predicted height with discontinuation of GnRHa.

Adrenarche, when present in patients with CPP, is not suppressed by administration of GnRHa. The premature secretion of gonadal steroids in CPP, and the subsequent suppression by GnRHa, does not influence the onset, timing, or pace of adrenal androgen secretion. In the majority of patients with CPP, adrenarche develops according to a CA-appropriate secretion of adrenal androgens. Long-term suppression of the pituitary-gonadal axis

by GnRH agonists therefore permits investigation of the discrete impact of adrenarche on linear growth, skeletal maturation, and changes in body composition.

The reversible suppression of pituitary-gonadal function, which is induced by GnRHa, permits a clear-cut dissection of the factors affecting childhood and pubertal growth. Therapeutically, GnRH-induced suppression of gonadal sex steroid secretion appears capable of partially reversing the adverse effect that precocious puberty has upon final height. However, close monitoring to assure complete suppression is essential.

An improved understanding of the physiology of growth coupled with the clinical availability of various growth modulators (eg, GnRH, GH, IGF-I) will permit further investigation of the therapeutic implications of the ability to delay the introduction of gonadal steroids in a variety of clinical settings.

## Suggested Readings

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## In Future Issues

The Morbid and Functional Anatomy of the Human Chromosome Map in Endocrine Disorders and Hormonal Genes  
by Victor A. McKusick, M.D.

Assessment and Management of the Psychological Aspects of Short Stature  
by Heino Meyer-Bahlburg, Ph.D.

Occult Celiac Disease: A Common Cause of Short Stature  
by Asaria Ashkenazi, M.D.

## Letters to the Editor

### Diagnosis of GHD

This letter is a commentary on the article by Rose et al<sup>1</sup> on the measurement of stimulated  $\nu$  spontaneous growth hormone (GH) levels in the diagnosis of growth hormone deficiency (GHD) (abstracted in *Growth, Genetics, and Hormones*, 1988; Volume 4, Number 4).

A hormone, by definition, is "an internal secretion of the endocrine glands, carried by the blood to other organs, where it stimulates them to physiological activity." An unexpected conclusion one may draw from the Rose report is that GH is not a real hormone, since the authors found that normal growth could be documented in children who do not secrete GH; indeed, ten of their normally growing children had integrated GH concentrations (ICGH) (mean GH plasma concentration) as low as the ICGH in GH-deficient children.

The authors pointed out that their results did not agree with two previous studies, one of them by our group,<sup>2</sup> which reported that the 24-hour ICGH in normal children was higher than and did not overlap with that of the ICGH-deficient children. Rose et al stated that we studied only ten prepubertal children and that our observations may "reflect the difference in the pubertal status between the (control and GHD-deficient) groups." This statement is in error. Rose et al ignored our study on the effect of age on the 24-hour ICGH.<sup>3</sup> This is the only study of the ICGH in normal children that included only subjects of normal height as well as normal weight. In this study, we demonstrated that there was no significant difference between the ICGH of normal children who were in stages P1, P2, P3, and P4 of puberty. Only after reaching stage P5 did our normal controls have an increase in the mean ICGH from  $5.7 \pm 1.4$  ( $n=23$ ) to  $7.4 \pm 2.0$  ( $n=23$ )  $\mu\text{g/L}$ .<sup>3</sup> We believe that the most likely reason for the difference

between our results and those of Rose et al is their unfortunate use of unsatisfactory methods for measuring and deriving the ICGH. They have reported ICGH in the normal control subjects that are often too low and ICGH of GH-deficient children that are always too high.

There are presently two different methods for measuring the ICGH. In the continuous blood withdrawal method, which we introduced in 1971,<sup>4</sup> blood is withdrawn slowly through a non-thrombogenic catheter and collected every half hour over a 24-hour period. Our method has since been used by numerous investigators in the United States and abroad.<sup>5-19</sup> The ICGH is derived by measuring the concentration of GH in a pooled sample from plasma collected during the 24-hour period. The other method, which was used by Rose et al, requires multiple blood withdrawals at short intervals and derives the ICGH by measuring and averaging the concentration of GH in each of the 72 plasma samples collected over a 24-hour period.

We would like to point out several causes for error in the multiple sampling method used by Rose et al:

1. The multiple sampling technique is very stressful to most children, and certainly interferes with their sleep. Donaldson et al<sup>20</sup> studied a group of children by the multiple blood sampling technique on two consecutive nights. They found significant differences between the two measurements in each subject. Two children who did not sleep the first night, but slept soundly during the subsequent night, had second measurements of the ICGH much higher than the first.

We previously reported the results of two consecutive measurements of the ICGH in 36 subjects<sup>3</sup> and we found no significant difference between the two measurements in each subject. We therefore believe that the

continuous withdrawal method is less stressful.

2. We have previously demonstrated that the multiple sampling technique underestimates the integrated concentration of any blood constituent that exhibits peaks of concentration at unpredictable times.<sup>21</sup> The reason for inherent error in this technique is that the blood samples may be taken before or after the true peaks. We have also demonstrated that the continuous withdrawal method yielded the true ICGH.<sup>21</sup>

3. We also believe that Rose et al have inadvertently introduced an upward bias to their calculated ICGH in the GH-deficient children. It should be stressed that the secretion of GH is episodic. Several short-lived peaks rise above a basal level, which is usually at the undetectable level of the immunoassay. Most of the 72 blood samples collected from each GH-deficient patient represent such a low baseline. It should be appreciated that at the 0 level of GH, the intraassay variability of any immunoassay is very high. The unbiased results of the immunoassay at the baseline must have a symmetrical distribution around 0. However, the distribution of the GH level used by Rose et al was asymmetrical, since any GH level below 0.5  $\mu\text{g/L}$  was taken as if it were 0.5  $\mu\text{g/L}$ . The result of such a manipulation is an artificially positive ICGH, with a minimum of 0.5  $\mu\text{g/L}$ , even in children who secrete no GH. We believe, therefore, that the ICGH of the GH-deficient patients was overestimated.

4. Rose et al included in their GH-deficient group all children who did not respond to GH stimulation, even those whose growth rate was in the normal range. These children may not have been GH deficient, since we have previously reported cases of children with normal ICGH who were unresponsive to the

GH stimulation tests.<sup>13</sup>

We conclude that the multiple sampling method used by Rose et al was inaccurate. Our continuous withdrawal method has proved to be clinically useful because we have avoided the artifacts that caused the overlapping of the ICGH in the normal and GH-deficient children. We have minimized the stress of the procedure. Because we measure the average concentration of GH in a 24-hour pooled sample, our results in GH-deficient children do not include the upward bias. In normal children, we assay GH at a concentration that is most accurate for the immunoassay.

We also believe that the multiple blood sampling method often leads to a dilution error. The technique requires the emptying of a dead space in the indwelling catheter, which is filled with a heparin solution. A blood sample is then taken from the catheter, which at this point contains undiluted blood. Subsequently, a heparin solution is reintroduced to fill the catheter. To minimize total blood loss, the nurses are instructed not to exceed the dead space of the catheter during the initial emptying of the catheter. If the dead space is too close to the volume taken by the nurse,

some diluted blood may remain in the catheter from initial mixing of blood and heparin solution, leading to an artificial lowering of the concentration of hormone in the blood sample. It is possible that the unexpectedly low ICGH readings in the normal control subjects reported by Rose et al were due to unintentional dilution of some of the blood samples.

Finally, Rose et al pointed out the high cost of their intermittent blood withdrawal method. It is important to realize that the high cost was mostly due to the measuring of GH in 72 distinct plasma samples. A considerable cost savings is inherent in our continuous withdrawal method, since we can measure the ICGH in one immunoassay of the pooled sample of plasma that incorporates the continuous blood withdrawal over 24 hours.

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## To the Editor:

The interesting article by Rose et al<sup>1</sup> proposes that in prepubertal short children the diagnosis of growth hormone deficiency (GHD) be based on abnormal growth hormone (GH) secretory responses to provocative GH-releasing stimuli rather than upon spontaneous GH secretion. We suggest that the provocative and/or endogenous GH secretory data need to be interpreted in the context of the clinical setting, ie, short stature, poor growth velocity, delayed bone age, etc. For example, one might question the diagnosis of GHD in some of their patients who were growing as rapidly as 10 cm/year.

Although a subnormal GH secretion was reported in response

to provocative tests, such responses may occur in normal children. Using the same stimulation tests as Rose et al, Root and Russ<sup>2</sup> reported that 2 out of 20 (10%) nonhypopituitary short children had peak GH responses  $\leq 7.0$   $\mu\text{g/L}$ , and 4 of 20 (20%) had peak GH responses  $< 10$   $\mu\text{g/L}$ , evidence that false-positive results may occur despite using two or more stimulation tests of GH secretion.

More information about the child or children in the Rose et al study with "relative" tall stature and normal growth velocity(ies) up to +1.5 SD in height and +1.1 SD in growth rate would be of interest. How many GH-deficient children in their study

had such normal growth velocities?

Ideally, patients with the absence of the gene for GH or with radiographic evidence of structural disease of the hypothalamic-pituitary axis or multiple anterior pituitary hormone deficiency should be classified as clearly GH-deficient subjects for comparison with other short children. It would have been of interest if Rose et al had included provocative testing of the normal-statured children because, in the past, "short control" children have been the basis for almost all control data.

We also would be interested to learn whether using the statistical methodology of logarithmic trans-

*continued on page 8*



## To the Editor

### continued from page 7

formation in regard to provocative testing, as Rose et al have done for endogenous testing, would have altered the sensitivity and discriminatory ability of provocative tests. Hindmarsh et al<sup>3</sup> emphasized the overlap of peak GH secretion after insulin-induced hypoglycemia.

Rose et al measured the number of GH pulses and mean GH-pulse amplitude. Were there any abnormalities observed in the pattern of pulsatile secretion of the six children with abnormal provocative tests who had "normal" mean 24-hour GH concentrations? Measurement of a mean 24-hour GH concentration without assessment of the pulsatile pattern of GH secretion may obscure neurosecretory abnormalities.<sup>4</sup>

Our interest in examining endogenous GH secretion and describing GH neurosecretory dysfunction as part of the spectrum

of GHD<sup>5-7</sup> results from the observations of many investigators<sup>8-10</sup> who demonstrated that provocative testing, using an arbitrary GH peak cutoff of 7 or 10  $\mu\text{g/L}$ , did not identify all children in whom there was acceleration in growth velocity after exogenous GH therapy.<sup>11</sup> We recognize that endogenous GH secretion studies are not practical in many clinical settings, but they do offer a valuable research tool for assessment of endogenous GH secretion and its regulatory factors. The diagnosis of GHD is difficult, but to exclude studies of endogenous GH secretion in the appropriate patient in the pertinent clinical situation is premature.

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## Authors' Replies

In response to Dr. Kowarski's letter:

Dr. Kowarski draws the conclusion that growth hormone (GH) is "not a real hormone" because some of the normally growing children in our report<sup>1</sup> had mean 24-hour GH levels that overlapped those of GH-deficient children. However, our observation is entirely analogous to the observation of primary hypothyroidism, confirmed by plasma thyroid-stimulating hormone (TSH) elevation, with plasma thyroxine and free thyroxine levels that are within the normal range. The fact that a hormone level is within the normal range for the population does not imply that the level must be normal for a particular individual. For example, a patient whose free thyroxine level is ordinarily at the 80th percentile for the normal population may develop clinical and biochemical evidence of hypothyroidism when this level is reduced to the 20th percentile by

thyroid disease.

Dr. Kowarski's data concerning the timing of increased GH secretion during puberty conflict with our own observations.<sup>2</sup> In a study of 145 pubertal normal volunteers who were of normal height and weight, we observed a significant increase in the mean plasma 24-hour GH level by pubertal stage 2 in the girls and by pubertal stage 3 in the boys.

We agree with certain of the differences that Dr. Kowarski notes between the integrated and multiple sampling procedures.<sup>3</sup> However, we find it difficult to believe that these factors would cause both an underestimate of the GH levels in normal subjects and an overestimate of the levels in GH-deficient subjects. Additionally, these technical factors would not explain the difference between our results and those of Bercu et al,<sup>4</sup> who also used a multiple sampling procedure. We conclude that a more likely explanation for the different obser-

vations is our emphasis on rigorous matching of pubertal stage between patient and control subjects.

We do not agree with all of the points raised by Dr. Kowarski concerning multiple sampling methodology. First, we are not aware of any direct comparison between the two techniques, in the same children, that has established one approach to be more or less stressful than the other. In our unit, the children do sleep through the procedure. Sampling is done without a tourniquet and does not ordinarily disturb a sleeping child unless he or she is lying on top of the withdrawal site. We purposely chose not to monitor sleep by EEG because this procedure itself disturbs sleep and requires a period of adjustment.

Second, we disagree that the multiple sampling technique has a bias toward underestimation. This technique samples all concentrations in direct proportion to

the probability of their occurring during the total period of blood withdrawal. The fundamental equivalence of these approaches is evident by considering the situation in which the sampling frequency approaches infinity. We do agree, however, that the mean GH level by multiple sampling may be greater or less than would have been observed by the continuous withdrawal method over the same 24-hour period. Further study would be

required to determine whether these differences are clinically significant.

Third, the decision to treat undetectable levels as if they were 0.5  $\mu\text{g/L}$  causes a similar upward bias for the normal as well as for the GH-deficient series, since the majority of samples in both groups have GH levels below the assay detection limit.

Fourth, our sampling procedure allows for additional blood (0.1 mL) beyond the dead space vol-

ume to prevent sample dilution. Finally, we question the accuracy of single GH radioimmunoassay measurements near the low end of the dose-response curve. We agree that cost savings could be achieved by fewer measurements on pooled samples, but for research purposes we chose to measure all samples to preserve information about the frequency and amplitude of GH peaks.

#### *In response to Dr. Bercu's letter:*

We agree that the diagnosis of growth hormone deficiency (GHD) should be made in the context of the clinical setting. All but three of the GH-deficient children were growing at a rate of less than 4 cm/year. The remaining three children fully met other clinical criteria for GHD; for example, the one child with GHD who was growing at 10 cm/year was 13 months old and had deficiencies of all pituitary hormones. Analysis of data led to the same results and interpretation with or without these three patients.

We disagree that only GH-deficient children who lack the GH gene or have panhypopituitarism should be used to evaluate tests for GHD. Such children represent the most severely GH-deficient subjects and are therefore easiest to diagnose. Many short children with isolated GHD have some persisting GH secretion, and it is these children who present the most difficult diagnostic challenge.

We agree that better normative data for the stimulation tests are needed. However, the principal conclusions of our study were not altered by the use of different criteria for normal response to a GH stimulation test.

We found no difference between the pulsatile GH pattern in normal children and in the pulsatile GH pattern in children with decreased stimulation tests and

normal mean 24-hour levels.

Three of the patients had one, two, or three peaks at night but so did three of the normals. Seven of the patients had peak amplitudes less than 4, but so did four of the normals.

We originally pursued studies of spontaneous GH secretion in the hope that it would have greater sensitivity for the diagnosis of GHD. However, we observed markedly reduced diagnostic sensitivity of the mean 24-hour GH level compared with the GH stimulation tests. Thus, unless future research identifies settings in which this difficult and expensive procedure is more useful, we conclude that its routine use as a method to diagnose GHD is unwarranted.

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#### Dr. Blizzard's Reply:

The issue regarding whether there is value in performing an integrated concentration in diagnosing GHD remains contestable. Rose et al, as presented in their article, believe not, while Kowarski et al and Bercu et al believe so. Readers of *Growth, Genetics, and Hormones* will have to decide for themselves whether the existing information about this topic is adequate for them to make a diagnosis.

I am sure that there is agreement regarding the role of integrated concentrations in research studies. In such studies, pulse analyses should accompany analysis of the mean value of GH concentration.

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## Subject Review: GH Neuroregulation and Its Alterations in Disease States

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Dieguez et al have recently reviewed the neuroregulation of growth hormone (GH) secretion in great detail. Their article (*Clin Endocrinol* 1988;28:109-143) does not introduce new data but stresses the many advances in the understanding of GH regulation made since the discovery of GH-releasing hormone (GHRH).

A discussion of GH regulation by neuropeptides includes a re-

view of the interaction of GH with somatostatin, opioid peptides, thyrotropin-releasing hormone, and other neuropeptides. A section on peripheral feedback signals describes the influence of growth factors, thyroid hormones, and glucocorticoids that act at the hypothalamic and/or pituitary level. A section on neurotransmitter regulation of GH secretion summarizes a great deal of information concerning the roles of acetylcholine, catecholamines, serotonin and melatonin, histamine, and  $\gamma$ -aminobutyric acid.

The article concludes with a discussion of the diagnostic and therapeutic implications of this information. Acromegaly and its treatment, and GH deficiency and its treatment with GHRH are described. Studies reporting GH secretion and its response to GHRH in diabetes, obesity, psychiatric illness, and other diseases are also explored.

This long review article is current and well documented; it is recommended reading for those interested in the mechanisms of GH secretion in normal and disease states.

## Special Report: David W. Smith Workshop on Malformations and Morphogenesis, August 3-7, 1988, Oakland, California

Judith G. Hall, M.D.  
*Associate Editor*  
*Growth, Genetics, and Hormones*

The theme of this year's workshop was the relationship between dysmorphology and metabolic disease. Inborn errors of morphogenesis are likely to arise from alterations in the biochemistry of development, and it is therefore expected that inborn errors of metabolism will provide clues to developmental processes. The search for these clues has been fueled by the recently recognized association between many peroxisomal disorders (ie, deficiencies of enzymes found in peroxisomes) and developmental anomalies such as renal cysts, retinal dysplasia, malmigration of neurons, stippled epiphyses, and unusual facies.

Drs. Kay Johnson and Stephen Bamforth presented evidence that many, if not all, infants with pyruvate dehydrogenase deficiency, nonketotic hyperglycemia, and congenital lactic acidosis have structural anomalies of the central nervous system, including agenesis and hypoplasia of the corpus callosum, ventriculomegaly with loss of white matter, and appar-

ently nonspecific malmigration of neurons. Drs. John Graham and Cynthia Morris demonstrated cardiac anomalies, nonspecific facial anomalies, and hypotonia with secondary changes in bones in some individuals with defective fatty acid oxidation. These types of anomalies should prompt aggressive metabolic workup.

While it is well recognized that recreational use of cocaine, which has been increasing in North America, can lead to a number of health problems for adults, Drs. Gilberto Chavez and Eugene Hoyme showed that maternal use at any time during fetal development can also lead to vascular accidents in the embryo or fetus. They reported an increase in genitourinary or gastrointestinal and limb reduction anomalies secondary to vascular compromise in utero. The exact incidence and dose relations in humans have not yet been defined.

Molecular approaches to dysmorphology are beginning to yield new information. Dr. Holly Ardinger found a significant association between particular alleles of transforming growth factor alpha (TGFA) and nonsyndromic cleft lip and cleft palate. Dr. Jon

Zonana demonstrated close linkage of X-linked hypohydrotic ectodermal dysplasia to a particular restriction fragment length polymorphism (RFLP) marker on Xq1. Interestingly, Dr. Jim Bartley failed to find a detectable molecular deletion in five families with the X-linked Norrie disease even though the exact location of the deletion has been identified.

Dr. Kathy Sulik reviewed the embryology of the abdominal viscera, pointing out that major migrations from the branchial arch system and the cranial endoderm accounted for these organs. She noted that the formation is complete by eight weeks. The growth factors that play a role locally have just begun to be investigated and defined.

Many new syndromes and methods to define them were described. Dr. Roger Williamson described the use of magnetic resonance imaging (MRI) to define the fetus in utero, and Dr. Andy Poznanski elaborated on new uses for hand pattern profile analysis. He explained how the test could be used to evaluate age-associated hand changes in specific syndromes.

At one session, the Rubenstein-

Taylic Syndrome Symposium, Drs. Rubenstein and Taylic presented a historical overview of the syndrome; the natural history was described by several groups. As with other syndromes, this one "changes" with age and looks somewhat different clinically in dif-

ferent ethnic groups. By definition, affected individuals have broad toes and/or thumbs. Perhaps the most useful facial feature is a long columella that protrudes and attaches below the alae nasi. Dr. Roger Stevenson presented a tantalizing case of 7q21-3 deletion

that was associated with Rubenstein-Taylic syndrome in one family. Although it has generally been hypothesized that the syndrome is most likely due to a small chromosomal deletion, no consistent evidence of this has been found as yet.

#### Abstracts From the Literature

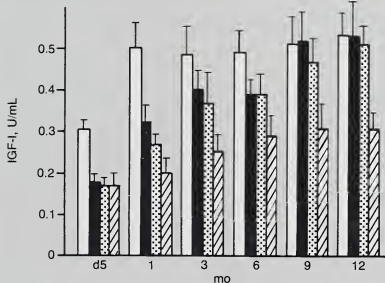
### Serum IGF-I and Serum Growth-Promoting Activity During the First Postnatal Year in Infants with IUGR

Twenty-one infants with intrauterine growth retardation (IUGR) were followed from birth to age 12 months. Serum insulin-like growth factor-I (IGF-I) was measured by radioimmunoassay. The bioassayable growth-promoting activity of the serum was measured as the thymidine activity (TA) on lectin-activated lymphocytes at ages 5 days and 1, 3, 6, 9, and 12 months, and was compared with control values. Based on their length at age 12 months, the infants with IUGR were divided into three groups: at or above the average (group A,  $n = 8$ ); between the mean and  $-2$  SD (group B,  $n = 7$ ); and less than  $-2$  SD (group C,  $n = 6$ ). No differences in nutritional indices or in head circumference were found among the three groups.

IGF-I levels (Figure 1) were significantly lower at 5 days of age in IUGR infants than in controls. Levels increased slowly in groups A and B and reached the control values at ages 9 and 12 months. Levels in group C remained significantly subnormal at 12 months of age.

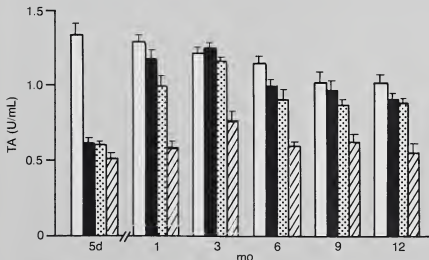
TA (Figure 2) was also significantly lower at age 5 days in infants with IUGR compared with controls. It increased sharply at age 1 to 3 months in groups A and B, but remained significantly lower in group C through 12 months.

Individual values of IGF-I and TA were closely correlated; the increase in body length during the first postnatal year correlated sig-



**Figure 1** Somatomedin C /IGF-I mean and SEM values from 5 days to 12 months in controls (open bars), group A (closed bars), group B (dotted bars), and group C (hatched bars) IUGR patients.

Reprinted with permission from *Pediatric Research*.



**Figure 2** TA mean and SEM values: symbols are the same as in Figure 1.

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continued on page 12



### Serum IGF-I and Serum *continued from page 11*

nificantly with the TA levels at 1 and 3 months, but not with the IGF-I levels at 1, 3, and 6 months. These data may be of some physiologic significance in understanding the postnatal catch-up growth that occurs after IUGR.

Thieriot-Prevost G, Boccara JF, Francoual C, et al. *Pediatr Res* 1988;24:380-383.

**Editor's comment**—These authors contributed significantly to

our understanding of what happens to IGF-I and growth-promoting activity as measured by TA during the first year of life. Their goal was to follow the evolution of IGF-I and growth-promoting activity concentrations and determine whether they correlated with growth. Their findings will be useful in other studies. For example, could failure of growth-promoting activity to increase at 1 and/or 3 months—as seen in group C—indicate responsiveness or unresponsiveness to growth hormone given under a standardized protocol?

Robert M. Blizzard, M.D.

### Copper Deficiency Impairs Growth of Infants Recovering from Malnutrition

Malnourished infants who receive milk-based formulas with low copper content and who experience episodes of diarrhea are at risk for developing copper deficiency. However, the routine treatment modalities for malnourished infants often do not address the potential for copper deficiency and its effect on growth and nutritional rehabilitation.

To evaluate the effect of copper deficiency on growth, the investigators studied 11 copper-deficient infants who were inpatients at a nutrition recovery center in Chile. All had low plasma copper levels ( $<70 \mu\text{g}\%$ ) and ceruloplasmin ( $<200 \mu\text{g/L}$ ); three had neutropenia (neutrophil count  $<1,500/\text{mL}$ ). These infants were compared with "control" infants (with malnutrition, but no copper deficiency) who were matched for age, sex, birth weight, weight for length, and mean stay at the center. Growth was evaluated one month before and one month after copper supplementation in both groups. Copper sulfate was given at a dose of  $80 \mu\text{g/kg/day}$  for 30 days. Supplemental vitamins and  $1.2 \text{ mg/kg/day}$  of elemental iron were also administered. Infants were fed ad libitum every four hours by

caretakers who were blinded to their copper status.

In the copper-deficient group, plasma copper and ceruloplasmin levels increased after one month of copper supplementation ( $P<0.001$ ) and all but one infant achieved normal plasma copper levels  $>90 \mu\text{g}\%$ . The control group maintained normal biochemical indices of copper status throughout.

Although weight-for-length and length-for-age values were similar in both groups, the copper-deficient infants had a lower weight-for-age ( $P<0.05$ ) at the onset of the study. After copper supplementation, weight-for-age and weight-for-length values were significantly improved in the copper-deficient infants. These infants also demonstrated a greater rate of weight gain after supplementation than before supplementation ( $4.8 \text{ v } 3.6 \text{ g/kg/day}$ ) and as compared with the control group ( $4.8 \text{ v } 2.4 \text{ g/kg/day}$ ). The acceleration in weekly weight gain occurred by the second week of copper supplementation and was maintained in the copper-deficient group. However, the control infants had a gradual decline in relative weight gain over time.

Castillo-Duran C, Uauy R. *Am J Clin Nutr* 1988;47:710-714.

**Editor's comment**—The authors interpreted the data to show that copper supplementation improves

Copies of back issues of *Growth, Genetics, and Hormones* are available and will be sent to readers who request them. Simply note the issue number(s) on a postcard (or your letterhead) and mail to Ms. Patti Galati, McGraw-Hill Healthcare, 800 Second Avenue, New York, NY 10017.

growth of copper-deficient infants recovering from malnutrition. However, all infants in the study, regardless of their copper status, demonstrated improved weight gain while receiving  $>150 \text{ kcal/kg/day}$  for catch-up growth. Even the copper-deficient infants gained weight without copper supplementation—as did the control infants—during the two-month evaluation period at the nutrition recovery center ( $P<0.1$ ,  $t$  test; editor's statistic). The fact that the control group did not maintain an accelerated weight gain, as observed in the copper-deficient group, may be attributed to differences in the degree of malnutrition. The copper-deficient infants were more severely malnourished than the controls; this explains their need to continue to gain weight at an accelerated rate for a longer period of time.

Although the authors report that these infants did not have zinc deficiency, no information is presented on mineral status before and after study entry. Also, one cannot exclude the impact of iron and/or vitamin supplementation on the recovery of these infants. Thus, the role of copper supplementation alone on improving growth is cloudy. However, the recognition and treatment of this problem are important because copper has other important physiologic implications that are not growth-related.

Fima Lifshitz, M.D.

## The Frequency of Genetic Disorders in Children and Young Adults

Utilizing the British Columbia Health Surveillance Registry, a survey was taken of more than one million consecutive births to determine the frequency of genetic diseases in individuals younger than 25 years of age. The survey revealed that 5.3% of individuals develop a disease with an important genetic component. Of the 5.3%, 0.36% are single-gene disorders and 4.6% are clearly defined multifactorial disorders such as cleft lip or diabetes.

The survey was carefully designed to exclude congenital anomalies that were thought to be nongenetic in origin. If data for all congenital anomalies were included in the tabulation, 7.9% of individuals could be identified as having either a congenital anomaly or a known genetic disorder. A further breakdown of the single-gene disorders gives an incidence of 1.4 per 1,000 for autosomal dominant disorders, 1.7 per 1,000 for autosomal recessive disorders, 0.5 per 1,000 for X-linked disorders, and 1.8 per 1,000 for chromosomal abnormalities. Adult-onset diseases were not included in this survey.

It is quite clear from the study that genetic disorders occur very frequently if grouped together, although each individual disorder is relatively rare.

Baird PA, Anderson TW, Newcombe HB, et al. *Am J Hum Genet* 1988;42:677-693.

**Editor's comment**—This survey is very useful because it gives a handle on the incidence and frequency of genetic disorders in the general population. It provides the kind of information that policy makers and healthcare planners need.

Judith G. Hall, M.D.

## Knemometry in Childhood: Accuracy and Standardization of a New Technique of Lower Leg Length Measurement

The authors carried out a study of the accuracy of lower leg length measurement by the apparatus introduced some five years ago by Valk and his colleagues. Ninety subjects between the ages of 2.4 and 17.1 years were involved; 46 were referred because of tall or short stature and 44 were normal. Six measurements were taken from each subject at each measurement session; the total number of measurement sessions was 2,200. Subjects and apparatus were displaced and repositioned between each measurement.

The ranges within the six measurements were 0.1 mm (rare) through 0.3 and 0.4 mm (very common) to 1.0 mm, with a few measurements beyond 1.0 mm. Using the median instead of the mean, the average SD of a single estimation was 0.16 mm. When differences between each of the six measurements and the medians of all six were examined, it was clear that the first of the six measurements was both biased and unreliable; when it was dropped, the SD decreased to 0.13 mm. Moreover, when only four measurements were taken and the first discarded, the results were as reliable as when the six measurements were taken and the first discarded. The procedure now recommended for future studies, therefore, is to take four measurements, discard the first, and average the next three.

Although its effect disappears in about two hours, exercise immediately before the lower leg length measurement is taken seems to reduce the measurement in children; strangely, it produces the opposite effect in adults. Therefore, vigorous physical activity should be avoided before measurements are taken. However, the child should be instructed to stand or walk slowly for five to ten minutes before being measured to

provide standardized loading to the lower leg; sitting should not be allowed.

Twelve children were measured on successive days. Over 24 hours the average gain in lower leg length was 0.67 mm, with a range of approximately 1.5 mm to -1.5 mm. Most of the children were also measured weekly and straight lines were fitted to each individual's series of measurements over a period of six months. Deviations among these lines greatly exceeded amounts that could be attributed to measurement error. Thus, the authors state that "estimates of growth velocities of the lower leg on the basis of two measurements taken some weeks apart...are barely reproducible." However, longitudinal series of measurements on individuals, taken weekly over long periods, are a very useful way to investigate growth.

Hermanussen M, Geiger-Benoit K, Burmeister J, et al. *Ann Hum Biol* 1988;15:1-16.

**Editor's comment**—This is the definitive study, to date, of the reliability of the Valk technique for lower leg length measurement. The results speak for themselves. The four-measurement technique, in which the first is discarded and the next three averaged, should be adopted as standard. The accuracy of a given measurement is then high; however, due to fluctuations in growth rates, differences between two short-term velocities are unreliable. This conclusion agrees with that of Wales and Milner (abstracted in *Growth, Genetics, and Hormones*, Vol. 4, No. 1). Anyone using the Valk knemometer or a similar apparatus should read this article before embarking on clinical studies.

James M. Tanner, M.D., D.Sc.

## Periodic Changes of Short-Term Growth Velocity ('Mini Growth Spurts')

Lower leg length was measured in 73 healthy children, ages 2.7 to 15.9 years, 18-106 times by the Valk machine once or twice a week. The standardized technique described in the abstract on knemometry (see p 13) was used. Straight lines covering three to four successive points were fitted to each 31-day period of each child. Rolling monthly average rates for each child were then computed (just as rolling annual velocities are calculated for stature when measurements are taken every three or six months). These rolling monthly rates were then plotted for each child on a day-by-day basis: the mean of the observations during days 1-31 was recorded, then the mean for days 2-32, and so on.

The deviations of the actual measurements from these individual curves showed significant clustering above or below the line in 38 of the 73 curves, and a characteristic up-and-down pattern of

growth velocity was found in 45 curves. The investigators referred to these variations as "mini growth spurts." The peaks have a velocity of about two to three times that of the troughs, and occur between 30 and 55 days apart. There was a significant correlation between the stature of the child and the frequency of his mini growth spurts.

Growth troughs coincided often with periods of intermittent infectious illnesses, but only a small percentage of the mini growth spurts could be explained as catch-up growth after this type of growth arrest. The reason for the majority of these spurts is unknown.

The authors conclude that "monitoring the pattern of short-term growth kinetics by multiple longitudinal knemometric measurements may provide an additional and very sensitive tool to control therapeutic regimes."

Hermanussen M, Geiger-Benoit K, Burmeister J, et al. *Ann Hum Biol* 1988;15:103-109.

**Editor's comment**—This paper

*extends the observations noted in the abstract on knemometry. Many perfectly healthy children seem to have fluctuations in the growth rate of their tibial epiphyses, with peak-to-peak periods of approximately 30 to 50 days. Elucidation of the physiology of this phenomenon will be awaited with the greatest interest. Do such spurts occur in all the long bones at once, or do they alternate from one long bone to another (as was alleged by Godin and others in the early years of this century)? Rats have a tibial epiphyseal cell cycle time of two to three days; humans, about 20 days. Do mini growth spurts occur several days apart in the rat tibia? From a clinical point of view, the authors throw cold water (not for the first time) on the short-term use of short-term velocities. But they very correctly point out that the long-term use of short-term velocities—a daunting task for patient and anthropometrist alike—might yield very valuable information. This phenomenon seems to be analogous to the pulsatile secretion of hormones.*

James M. Tanner, M.D., D.Sc.

## Uniparental Disomy as a Mechanism in Human Diseases

Although an individual normally inherits one of each pair of chromosomes from mother and one from father, very rarely there is an individual who has inherited both chromosomes of a pair from only one parent. This phenomenon is known as uniparental disomy. Although it has been previously demonstrated in animals, the report by Spence et al marks the first time that uniparental disomy has been demonstrated by DNA studies in a human being with normal chromosomes. The affected individual, a girl with cystic fibrosis, inherited both of her number 7 chromosomes from her mother. Nonpaternity was confirmed by numerous other markers. Thus, in that particular family, the probab-

ility of the proband's having a child affected with cystic fibrosis is reduced from the usual 25% to nearly zero.

Interestingly, this girl also had intrauterine growth retardation (IUGR). The observation of uniparental disomy suggests that it may occur for other chromosomes and for other diseases. The evaluation of families by studies in molecular genetics now makes it possible to recognize this type of mechanism. It seems likely that uniparental disomy is responsible for autosomal recessive diseases only rarely, probably accounting for less than 1% of individuals with

apparent autosomal recessive inheritance. However, the clue to such cases may be the presence of IUGR.

Spence JE, Perciaccante RG, Greig GM, et al. *Am J Hum Genet* 1988;42:217-226.

**Editor's comment**—Nothing is sacred anymore. Things as basic as Mendel's peas have exceptions. For the geneticist, however, exceptions serve as incentives to learn more about normal mechanisms of inheritance. At this point, we do not yet know how "normal" uniparental disomy is.

Judith G. Hall, M.D.

In Future Issues

Gonadotropin and Steroid Concentrations in the Fetus and Newborn, by Claude Migeon, M.D.  
The Remarkable Catch-up Growth in American Slaves by Richard H. Steckel, Ph.D.  
Growth in Late Adolescence, by Alex Roche, M.D.

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Kenneth L. Jones, M.D.
- "Human Placental Lactogen and Fetal Growth"  
Stuart Handwerger, M.D.
- "Creutzfeldt-Jakob Disease: Current Reports and Comments"  
Robert M. Blizzard, M.D.

### Special Reports:

- International Turner Syndrome Symposium—November 9-11, 1987, San Francisco, California
- The March of Dimes Clinical Genetics Conference—July 19-22, 1987, Minneapolis, Minnesota
- 26th Annual Meeting of the European Society for Pediatric Endocrinology (ESPE)—September 6-8, 1987, Toulouse, France
- 8th Annual Workshop on Malformation and Morphogenesis—August 15-19, 1987, Greenville, South Carolina

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- Statural Development Parallels IGF-I Levels in Subjects of Constitutionally Variant Stature
- A Longitudinal Study of the Relationship of Plasma Somatomedin-C Concentration in the Pubertal Growth Spurt
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- Risk of Hypoglycemia with Alternate-Day Growth Hormone Injections
- Short-Term Testosterone Treatment at Bone Age 12-13 Years Does Not Reduce Adult Height in Boys with Constitutional Delay of Growth and Adolescence
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- The Measurement of Stature

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### Letter From the Editor

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Dagmar K. Kalousek, M.D.
- "Anabolic Steroid Hormones for Athletes: Efficacy or Fantasy?"  
Alan D. Rogol, M.D., Ph.D.

### Special Reports:

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- 48th Annual Meeting of the American Diabetes Association—June 12-14, 1988, New Orleans, Louisiana
- Fifth International Auxology Congress—July 20-23, 1988, Exeter, United Kingdom

### Abstracts:

- Three-Year Results of a Randomized Prospective Trial of Methionyl GH and Oxandrolone in Turner Syndrome
- Immunoreactive Sm-C/IGF-I in Urine From Normal Subjects, Pituitary Dwarfs, and Acromegalics
- A Triumph of Reverse Genetics: Characterization of Dystrophin in Duchenne and Becker Muscular Dystrophy
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## MEETING CALENDAR

**April 12-16** 15th Training Course on Hormonal Assay Techniques. Holiday Inn, Bethesda, Maryland. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**April 27-30** Lawson Wilkins Pediatric Endocrine Society Review Course in Pediatric Endocrinology. Hyatt Regency Washington on Capitol Hill, Washington, D.C. Contact: Beverly Wellman, Sero Symposia, USA, 280 Pond Street, Randolph, MA 02368 (800-225-5185)

**April 27-30** Biennial Meeting of the Society for Research in Child Development. Kansas City, Missouri. Contact: Kathleen McCluskey-Fawcett, Ph.D., Department of Psychology, University of Kansas, 426 Fraser Hall, Lawrence, KS 66045-2160 (913-864-4131)

**May 1-5** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Washington Sheraton, Washington, D.C. Contact: Debbie Wogenrich, Executive Director, Society for Pediatric Research, 2650 Yale Boulevard S.E., Suite 104, Albuquerque, NM 87106 (505-764-9099)

**May 5** Annual Meeting of the Lawson Wilkins Pediatric Endocrine Society. Washington Sheraton, Washington,

D.C. Contact: Gilbert August, M.D., Department of Endocrinology, Children's Hospital, National Medical Center, 111 Michigan Avenue N.W., Washington, D.C. 20010 (202-745-2121)

**June 3-6** Annual Meeting of the American Diabetes Association. Detroit, Michigan. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22314 (703-549-1500 or 800-ADA-DISC)

**June 21-24** 71st Annual Meeting of the Endocrine Society. Seattle Convention Center, Seattle, Washington. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**June 25-28** 2nd International Pituitary Congress. Marriott's Desert Springs, Palm Desert, California. Contact: Grace Labrado, Department of Endocrinology, Cedars Sinai Medical Center, Room B-131, 8700 Beverly Boulevard, Los Angeles, CA 90048 (213-855-4691)

**September 23-25** 30th Annual Meeting of the American College of Nutrition. Omni International Hotel, Norfolk, Virginia. Contact: Kay Balun, Administrative Assistant, American College of Nutrition, 345 Central Avenue, Suite 207, Scarsdale, NY 10543 (914-723-4247)

**October 11-14** 41st Annual Postgraduate Assembly of The Endocrine Society. Fairmont Hotel, New Orleans, Louisiana. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**October 29-November 3** Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology (scientific sessions, October 30-November 1). Jerusalem Hilton, Jerusalem, Israel. Contact: Zvi Laron, M.D., Beilinson Medical Center, Petah Tikva 49 100 Israel

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# GROWTH

## Genetics & Hormones

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### Occult Celiac Disease: A Common Cause of Short Stature

Asaria Ashkenazi, M.D.  
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Director, Department of  
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Affiliated with the Medical School  
of the Hebrew University and  
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Failure to thrive in association with gastrointestinal (GI) symptoms is common in children with active celiac disease (CD). In two studies<sup>1,2</sup> of patients with GI symptoms due to CD, 36% and 55% were below the third centile for height, and 40% and 60% were below it for weight. A small proportion (~5%) of CD patients with short stature, however, have no GI symptoms. They are considered to have occult CD, which is quite important in the differential diagnosis of short stature.

#### **Etiology and Pathogenesis of CD**

Wheat gluten, specifically its gliadin fraction, is toxic to genetically susceptible individuals. In recent studies, we demonstrated the toxic effects of purified gliadin derived from peptides; it was obtained from intestinal mucosa cultures of CD patients ingesting a normal gluten-containing diet. The smallest toxic peptide had a molecular mass of 6,129 daltons and contained 53 amino acids.

Although gliadin-toxic peptides damage the intestinal mucosa in genetically susceptible individuals, the exact mechanism is not completely understood.<sup>3-5</sup> Current opinion favors the hypothesis that the harmful effects of gluten are mediated by immunologic processes. Immunologic reactions appear to play a part in the pathogenesis of CD, but the distinct mechanism—be it cellular immunity, humoral immunity, or a combination of the two—remains elusive.<sup>6</sup>

#### **Short Stature as the Presenting Symptom of CD**

Several investigators have reported short stature as the only manifestation in some cases of CD (Table).<sup>7-11</sup> In one cohort<sup>7</sup> of 796 children and adolescents seen for growth retardation, 14 had CD (1.8%). Although the children with CD had no current GI symptoms, eight had a history of diarrhea during infancy. In another study, 34 patients with short stature of unde-

termined origin, but no GI symptoms, underwent jejunal biopsy for exclusion of celiac disease.<sup>8</sup> Eight patients (24%) had subtotal or severe partial villous atrophy; seven of these patients showed significant increases in height and weight velocity after switching to a gluten-free diet.

In a group of 108 children with short stature of undetermined etiology referred to an endocrine clinic in Italy, 9 (8%) had CD.<sup>9</sup> All patients with CD were diagnosed by a biopsy of the small intestine, and the specimen was obtained via the oral route. The investigators found that although no single screening test or combination of tests identified all of these patients, an abnormal xylose test, the presence of antireticular and/or anti gliadin antibodies, and a history of diarrhea in the first 2 years of life identified most cases of CD. In a similar study,<sup>10</sup> 87 children with short stature (height > 2 SD below the mean for age and sex) underwent biopsy of the small intestine after other causes of growth retardation had been ruled out. Although none had GI symptoms, four (5%) had CD.

Another study, conducted in Israel, described short stature as a major manifestation of CD in older children.<sup>11</sup> In 11 of 23 (48%) patients referred to a gastroenterologist after an extensive negative endocrine evaluation, CD was diagnosed by small-bowel biopsy.

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## Occult Celiac Disease

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No clinical or laboratory clues enabled the investigators to differentiate between the cases of short stature due to occult CD and those due to other causes. Equally unhelpful in differential diagnosis was the age at diagnosis, length of follow-up prior to referral to the GI service, and degree of bone-age retardation. Although abnormal stool-fat excretion was found only in children with CD, sensitivity was low, and abnormal results were found in only four of nine patients tested. However, testing for the presence of microcytic anemia revealed that all of the children with CD, but none without the disease, were anemic. A positive history of past GI problems, particularly diarrhea, during early childhood also was very helpful.

The prevalence of CD is low in some studies (1.8%,<sup>7</sup> 8%<sup>9</sup> and 5%<sup>10</sup>) but high in others (24%<sup>8</sup> and 48%<sup>11</sup>). This disparity may indicate that CD can be recognized and treated in some areas of the world at a younger age, when the malabsorption symptoms are more clear-cut and before severe stunting of growth occurs; in other regions, however, it tends to be diagnosed later, when short stature is the more obvious problem. This varying prevalence may also reflect a real disparity between the incidence of CD and its mode of presentation.

### Is CD-Induced Short Stature Occult?

Detecting occult CD as a cause of short stature is a function of professional alertness insofar as the clinician considers this condition in a differential diagnosis. Data in the medical literature, as well as from our own experience, show that many CD patients with short stature have a history of diarrhea at an early age and/or have microcytic anemia. Other diagnostic clues that may indicate CD include increased stool fat, antigliadin and antiendomysial antibodies, and low folate and serum ferritin

**Table.** Reports of the prevalence of CD in children with short stature

| Study                                    | Year | Total no. studied | No. with CD |
|--|------|-------------------|-------------|
| Verkasalo et al., Helsinki <sup>7</sup>  | 1978 | 796               | 14 (1.8%)   |
| Groll et al., London <sup>8</sup>        | 1980 | 34                | 8 (24%)     |
| Cacciari et al., Bologna <sup>9</sup>    | 1985 | 108               | 9 (8%)      |
| Stenhammar et al., Sweden <sup>10</sup>  | 1986 | 87                | 4 (5%)      |
| Rosenbach et al., Tel Aviv <sup>11</sup> | 1986 | 23                | 11 (48%)    |

levels.<sup>3-5</sup> In a few cases, however, there may be no clue to CD other than abnormal intestinal mucosa diagnosed by an orally obtained jejunal biopsy.

### Pathogenesis of Growth Retardation

The cause of growth failure in children with CD has been the focus of many investigations. Initial studies suggested secondary hypopituitarism might be the basis for the retarded growth.<sup>1,12-14</sup> Subsequent assessments, however, failed to substantiate growth hormone (GH) deficiency in most growth-impaired children with CD. On continued investigation, it became increasingly evident that a gluten-free diet could reverse this complication. This finding further emphasized the importance of adequate and appropriate nutrition in maintaining normal linear growth.

The effect of gluten ingestion on the growth pattern of CD patients is still unresolved. Is growth retardation induced by the effect of the disease on the general nutritional status? That is, does malabsorption lead to malnutrition, which, in turn, leads to a decrease in weight and failure to gain height? Or, in addition, is there a direct effect of gliadin peptides on the GH-secreting apparatus, as well as on other hormones affecting growth, such as somatomedin?

### GH and CD

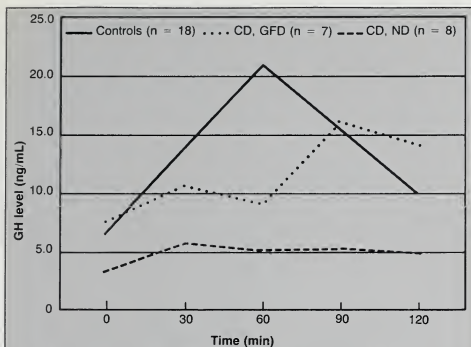
Inadequate response of serum GH levels to insulin stimulation was re-

ported by Hamilton in 1969 in one of five CD patients examined.<sup>1</sup> We have examined the plasma GH levels in response to insulin stimulation in CD patients on a normal gluten diet, in patients on a gluten-free diet, and in normal children of comparable age who served as controls (Figure).<sup>12</sup> The CD children showed a lower GH response, as a group, than the normal controls. Also, serum GH response to insulin stimulation in the CD group consuming a normal gluten diet was significantly lower than in the CD group on the gluten-free diet. Similar results have been reported in other studies.<sup>1,7,13,14</sup>

Somatomedin-C (Sm-C), a GH-dependent growth factor modulated by nutrient status, is variable in CD patients.<sup>15-17</sup> We were unable to find a correlation between serum Sm-C levels in CD children and the diet consumed. As would be expected, some CD patients who had low Sm-C levels while on the normal gluten diet had increased levels while on a gluten-free diet. On the other hand, other patients had normal serum Sm-C levels while consuming a normal gluten diet. Data are accumulating to suggest that Sm-C may be a useful marker of nutritional adequacy in patients with inflammatory bowel disease<sup>18,19</sup> but not in patients with CD.

### Enhanced Enteric Losses and Nutritional Dwarfism

Any one or a combination of the nutritional alterations may lead to nutritional dwarfing in CD patients. This may occur even in patients



**Figure 1** The mean GH responses to insulin are shown for CD patients on a normal gluten diet, CD patients on a gluten-free diet, and normal controls. All were given a standard insulin tolerance test and had an apparent drop in blood glucose levels. At each point, the SD of the GH levels varied from 0.5 to 6.5.

who have an increased body weight/height ratio (i.e., short and plump patients).

The basic defect in CD is destruction of the intestinal epithelial cell layer, with reactive production of intestinal cells up to seven times the normal rate. The net result is flattening of the intestinal mucosa; immature and damaged epithelial cells cover the surface. This blunting of the villi is instrumental in decreasing the absorptive surface of the intestine and producing malabsorption and malnutrition.<sup>3-5</sup> The nutritional status of these patients is further impaired by the defects in the intestinal epithelium that cause protein-losing enteropathy, and is aggravated by the desquamation of the epithelial cells and shedding of lymphocytes. The resultant loss of protein, DNA, and lymphocytes leads to hypoproteinemia and lymphopenia. CD patients also experience intestinal loss of zinc. There is a tendency toward lower serum zinc levels in the CD patients, but these levels are not low enough to produce clinical symptoms.

Children with CD have impaired iron balance as well. Serum ferritin levels in CD patients are often sig-

nificantly lower than in controls, and a gluten-free diet produces a definite rise toward normal levels. The low ferritin levels reflect impairment of iron absorption, as well as increased iron losses in the desquamating epithelial cells and lymphocytes. Additionally, the serum folate levels are significantly lower in the untreated CD patients and may result from a decrease in the folate absorption caused by the impairment of the deconjugase enzyme present in the brush border of the intestinal epithelial cells. This enzyme facilitates absorption by splitting glutamic acid residues from the folate heptaglutamate in foods and reducing them to mono- or diglutamates. However, it is well known that there is a sevenfold increase of cell turnover and in DNA synthesis in patients with CD. This regenerative effort by the crypt cells in active CD requires folic acid, an essential component of the enzymes involved in the synthesis of DNA. Thus, there is a decrease in folic acid absorption that parallels an increase in folic acid requirements for DNA synthesis in patients with active CD. The net result is decreased serum folate levels.

## Diagnosis of CD

Two factors are important to remember when considering occult CD in the differential diagnosis of short stature: 1) Studies show an increased incidence of CD among children hospitalized for examination of short stature; and 2) CD may be present even in the absence of GI symptoms. A small-bowel biopsy, "the gold standard" for the diagnosis of CD, should be performed in all children examined for short stature who show a decrease in the body weight/height ratio, have a history of diarrhea during the first year of life, or present with hematologic abnormalities such as anemia.

## Summary

CD is more common than idiopathic GH deficiency as a cause of short stature. Thus, it is important to rule out this disease in children in whom there is no endocrinologic cause of short stature. CD should be suspected in patients who 1) fail to grow at normal rates and have a bone age that is delayed, particularly if by more than 4 years; or 2) have a bone age below that expected for height. Because abnormal absorption may not be detected by common tests even in known CD patients, an intestinal biopsy may be needed for diagnosis. Confirmation of CD as the cause of delayed growth should come from documentation of catch-up growth in weight and height after institution of a gluten-free diet, the only treatment now available for CD.

*The original work was supported by a grant from DFG, West Germany.*

**Editor's comment**—One of the greatest enigmas in pediatrics today is why celiac disease is so prevalent in Europe and the Middle East (Israel, for example) but not in North America. There are many descendants of Europeans in North America, but the disease is rare. Readers who have a tenable theory, particularly one with supporting data, are encouraged to write to the editors of *Growth, Genetics, and Hormones*.

*continued on page 4*



## Occult Celiac Disease

continued from page 3

Even though celiac disease is less frequent in our clinics than in those of Europe, we must remain alert to the possibility that any child with short stature of unexplained etiology may have celiac disease.

Robert M. Blizzard, M.D.

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# The Remarkable Catch-up Growth of American Slaves

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## Editor's Note:

Dr. Steckel is an academic economist whose research relates cultures and environment to growth patterns. He has capitalized on the records kept by slave traders and the U.S. Army during the time of slavery to evaluate the growth of slave children. Examination of those records and a process of logical deduction fuel this fascinating account of how and why slave children grew at the rates they did in the early 19th century. The data and concepts presented will not be used in the clinical practice of medicine, but they do clarify how the children in one culture were exceedingly small until late childhood and then manifested significant catch-up growth. Dr. Steckel presented these data at the International Auxology Conference in England in August 1987, and a longer version of this article appeared in *Annals of Human Biology* (1987;14:111-132).

Robert M. Blizzard, M.D.

Much of our present knowledge of human growth has accumulated

by studying healthy children from quasi-experiments created by wartime starvation, poor children who live in developing countries, children who were born with malformations or who otherwise acquired maladies, and from animal experiments appropriately interpreted for parallels with human behavior. The diversity of past cultural and environmental conditions suggests that studies of historical populations may also provide insights into human growth. This article summarizes recent research on the unusual height-by-age profiles of American slaves, a line of work that sheds light on both biological and social processes.

## Origins of the Data

Measurements in this study were collected as a by-product of American legislation that, beginning in 1808, prohibited the African slave trade as an import activity, but permitted monitored slave trading to continue along the U.S. coast and inner waterways.<sup>1</sup> Slaves entered the coastal and waterways trade as part of the general westward migration of farming during the 19th century. The law required ship captains who left American ports to prepare duplicate manifests that described each slave by name, age, sex, height, color, and the name of the owner or shipper. The collector at the port of origin

retained one manifest, and the captain delivered the other for comparison against the "cargo" by the collector at the port of destination. Thus, the ship captains took measurements as part of an identification scheme designed to prevent smuggling of slaves. The data presented here are based on records of 10,562 manifests housed at the National Archives.<sup>2</sup> A total of 50,606 slaves were involved.

## Results

The Table gives the means, mean annual increments, sample sizes, and centiles of modern height standards for males and females in this population. The mean increments fell irregularly from early childhood, then rose during adolescence and reached a peak near age 15 years in males and near age 13 years in females. Before adolescence, boys and girls were about the same height, but earlier maturation propelled the girls beyond the boys at 13 to 14 years. Growth ceased at about age 21 years in men and about age 19 in women.

Young slave children were extraordinarily small, falling near or below the first centile of the Tanner, Whitehouse, and Takaiishi standards. Their relative size increased little before age 11, when boys began to exceed the third

**Table.** Mean, mean annual increment, sample size, and centile of modern standards applied to slave height

| Age<br>at last<br>birthday | Males        |           |       |         | Females      |           |       |         |
|----------------------------|--------------|-----------|-------|---------|--------------|-----------|-------|---------|
|                            | Mean<br>(cm) | Increment | n     | Centile | Mean<br>(cm) | Increment | n     | Centile |
| 4                          | 91.2         | —         | 195   | 0.3     | 91.2         | —         | 206   | 0.5     |
| 5                          | 97.2         | 6.0       | 169   | 0.3     | 99.0         | 7.8       | 200   | 1.6     |
| 6                          | 103.2        | 6.0       | 218   | 0.5     | 101.6        | 2.6       | 262   | 0.4     |
| 7                          | 110.8        | 7.6       | 200   | 1.5     | 110.0        | 8.4       | 241   | 1.8     |
| 8                          | 114.6        | 3.8       | 281   | 0.9     | 115.5        | 5.5       | 337   | 2.2     |
| 9                          | 121.0        | 6.4       | 266   | 1.7     | 119.6        | 4.1       | 306   | 1.4     |
| 10                         | 125.1        | 4.1       | 557   | 1.6     | 124.6        | 5.0       | 528   | 1.4     |
| 11                         | 131.9        | 6.8       | 347   | 3.6     | 130.4        | 5.8       | 443   | 2.1     |
| 12                         | 135.2        | 3.3       | 751   | 2.4     | 134.8        | 4.4       | 736   | 0.9     |
| 13                         | 141.1        | 5.9       | 470   | 3.0     | 142.0        | 7.2       | 556   | 0.9     |
| 14                         | 146.6        | 5.5       | 732   | 2.1     | 148.0        | 6.0       | 765   | 1.7     |
| 15                         | 153.0        | 6.4       | 571   | 1.3     | 152.4        | 4.4       | 812   | 5.6     |
| 16                         | 158.1        | 5.1       | 709   | 1.2     | 155.5        | 3.1       | 1,113 | 13.3    |
| 17                         | 163.2        | 5.1       | 655   | 4.6     | 157.4        | 1.9       | 871   | 21.5    |
| 18                         | 165.7        | 2.5       | 1,142 | 8.9     | 158.0        | 0.6       | 1,268 | 24.5    |
| 19                         | 167.7        | 2.0       | 900   | 14.5    | 158.6        | 0.6       | 594   | 27.4    |
| 20                         | 168.5        | 0.8       | 1,527 | 17.6    | 158.4        | -0.2      | 1,264 | 26.8    |
| 21                         | 170.5        | 2.0       | 944   | 26.1    | 158.8        | 0.4       | 337   | 28.4    |
| 22                         | 170.2        | -0.3      | 1,374 | 24.8    | 159.0        | 0.2       | 664   | 29.5    |
| 23                         | 170.6        | 0.4       | 795   | 26.8    | 158.8        | -0.2      | 404   | 28.4    |
| 24                         | 169.9        | -0.7      | 872   | 23.6    | 159.4        | 0.6       | 442   | 31.9    |
| 25-49                      | 170.6        | 0.7       | 8,725 | 26.8    | 159.8        | 0.4       | 6,552 | 34.5    |

centile and girls, the second centile. The heights then descended through the centiles, primarily because the reference population matured early. But the apparent growth retardation had disappeared and the slaves grew rapidly during and after adolescence. They emerged as adults near the 25th centile (in males) to the 30th centile (in females).

## Comparisons

Perspective on the growth profile of American slaves may be obtained in comparisons with poor populations in developing countries and with historical populations. According to worldwide data for the mid-20th century compiled by Eveleth and Tanner,<sup>3</sup> young slaves were among the smallest children ever measured.<sup>3</sup> At age 3, for example, slave children were smaller than children from the urban areas of Bangladesh, who reached the centile of 0.35, and those from the slums of Lagos, Nigeria, who attained centiles of 12.1 as boys and 6.4 as girls at the same age.<sup>4</sup> It is clear that the typical slave child had an

exceptionally poor start in life.

The Eveleth and Tanner data also indicated that children and adults of the same population tended to be in similar centiles. If environmental conditions were poor, for example, then both children and adults were correspondingly diminished in stature. This was not true of the slave children whose percentile ratings increased as they matured.

The Figure presents data<sup>5</sup> on populations approximately contemporary with American slaves. The abscissa of the Figure gives average height attained on the eve of adolescence as a percentage of modern standards, whereas the ordinate shows adult stature of the same population relative to modern standards. The heights were estimated from raw data using Preece-Baines Model 1. The first quadrant (NE) depicts populations that were well-off as children and young adults, whereas the third quadrant (SW) shows cases of deprivation in childhood followed by little catch-up growth. Because children and adults in most populations tend to attain similar cen-

tiles of modern height standards, one would expect to observe most populations in the first and third quadrants. The American slaves stand out in quadrant 2 (NW) as a case of extensive deprivation in childhood followed by substantial catch-up growth.

## Possible Explanations

Ingestion of toxic substances by the mother or young child may cause growth retardation in children. Could excessive alcohol or tobacco consumption have contributed to the small stature of young slaves? These products were available on many southern plantations, but the amounts consumed were probably modest in most cases, as intoxication and frequent breaks for smoking were incompatible with the considerable labor demands of slave agriculture. Moreover, heavy consumption of alcohol or tobacco is inconsistent with the substantial catch-up growth observed among slaves.

The generally high level of mortality and seasonal patterns of deaths tabulated from the records of large southern plantations, along with evidence from feeding patterns and work routines, suggest that nutritional deprivation stunted growth in early childhood.<sup>6</sup> Infant mortality probably occurred at a rate of about 350/1,000, and losses for the age group 1 to 4 were about 201/1,000. Overall, slave losses before age 5 were roughly double those of whites during the period 1830 to 1860. Perhaps two-thirds of the infant deaths involved neonates, and of these approximately three-fourths occurred in the 6 months of February through April and September through November. Stillbirths were concentrated in late autumn and, to a lesser extent, in the period of February through April.

Before the fifth month, pregnant slaves had little or no relief from the arduous work of the preparation and planting season of late winter and early spring and of the harvest of late summer and autumn. In addition, malaria and other fevers

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## Catch-up Growth

continued from page 5

were common during the "sickly season" of late summer and early autumn. Stillbirths and neonatal deaths often are secondary to deprivation at or near conception, and the neonatal death rate increases with deprivation during the third trimester.<sup>7</sup> This pattern of evidence points to seasonal nutritional deprivation of the fetus, brought on by hard work and, to a lesser extent, by disease.

Poor diet and infections also affected the health of postneonatal slave children. The evidence on breastfeeding practices is scanty, but it suggests that slave infants received breast milk for 9 months to 1 year, whereas white babies continued breastfeeding for more than 1 year. Within 3 months after delivery, the amounts of cotton picked by slave mothers returned to normal levels, and this suggests that one or more daytime breastfeedings may have been eliminated. The substitute diet featured starchy paps and gruels that were often contaminated or served with contaminated utensils. According

to owners' records, diarrhea was a common cause of death in slave infants.

Children who survived the hazardous period of infancy subsisted largely on hominy and fat, and their causes of death frequently included diseases aggravated by poor nutrition. In addition, aggregation of children on large plantations probably promoted the spread of communicable diseases, such as whooping cough, measles, and pneumonia.

By ages 8 to 12, work began to place a claim on the slaves' diets, and, other things being equal, the additional physical effort would have retarded growth. Yet it was at these ages that slaves realized some catch-up growth—an indicator that workers were rewarded with good diets, compared with children and other nonworkers. Those in the adult labor force usually received rations of pork (~0.5 lb/day) and other foods, principally corn. In addition, some slaves supplemented their rations with fish, game, poultry, garden vegetables, and other foods. Experienced workers also may have

benefited from practice or repetition that reduced their energy expenditure for particular tasks and left more nutrition for growth.

## Concluding Remarks

The growth profiles of American slaves suggest that humans have a remarkable capacity for catch-up growth. This finding should be qualified by recognizing that the harsh environment may have eliminated many children who adapted poorly to deprivation. Those who survived to adolescence may have been relatively efficient at utilizing a given level of nutrition for growth.

On the other hand, the chronic nutritional deprivation of young slaves may have stunted mental and emotional development in ways that adversely affected the economic progress of blacks after their emancipation. Most societies that attained near-modern nutritional standards for adults also had children who were relatively well-off. American slaves provide an exception that raises questions about the goals and incentives of resource allocation in free societies, as compared to slave societies. The unusual pattern of food allocation by age may have been achieved partly at the expense of the slave family; for example, workers ate breakfast and lunch in the fields and were probably fed after the young children in the evening, and this left little time for parents to spend with their children on a regular basis.

In summary, data collected for economic purposes may lend itself to analysis and evaluation of scientific parameters.

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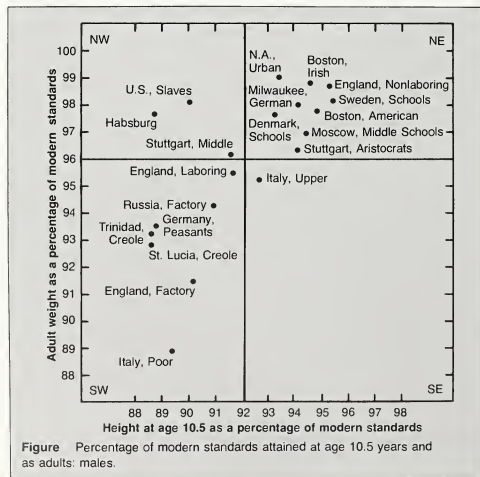


Figure 1. Percentage of modern standards attained at age 10.5 years and as adults: males.

## Height and Height Velocity

*Growth and growth-velocity curves have recently generated considerable interest. Because Dr. James M. Tanner, a member of our Editorial Board, has made significant contributions in this area, we present the following letter and reply. We feel that Growth, Genetics, and Hormones is an appropriate forum for the exchange of ideas on this critical issue.*

I greeted the Tanner and Davies article<sup>1</sup> enthusiastically. I anticipated that the new longitudinal height-velocity standards would provide definitive data about the normal range of height velocity. I had thought from the earlier work of Tanner and his associates that the lower percentiles on previous height-velocity charts were misleadingly wide because they were based on cross-sectional data and included children whose tempo of puberty was inordinately early or late.

Unfortunately, the Tanner and Davies charts do not solve this problem. For example, examine their longitudinal height-velocity chart for American boys between 8 and 9 years of age: height velocity is 3.75 cm/year at the

third percentile and about 3.75-4.0 cm/year for the fifth percentile. Are we then to consider that a linear growth rate of 4.0 cm/year is normal at this age? When one turns attention to the companion longitudinal data for height attained by American boys, one finds that boys in the fifth percentile for late maturers (that is, in the 0.125th percentile) grow from 117 cm at 8 years of age to 121.6 cm at 9 years, a velocity of 4.6 cm/year. Thus, a "normal" growth rate of 4.0 cm/year would cause a child to deviate from the normal growth channel.

These longitudinal standards do not get around the problem that Dr. Tanner had described with cross-sectional standards. Percentiles for velocity necessary to maintain height-channel position ("height-channel velocity") would seem more important in the diagnosis of growth disorders than the percentiles for height velocity since, as Dr. Tanner has taught us, prepubertal children grow to maintain their height-channel position rather than their height-velocity centile position. I would conclude that a height velocity below 4.6 cm between 8 and 9 years of age is subnormal. Why are there these discrep-

ancies? If short-term growth velocities had been used, that would explain the matter because they oscillate about the whole-year channel. However, whole-year velocities were apparently estimated by Tanner and Davies. Their appendix describes the construction of the 50th centile curves for height attained and peak height velocity but not of the outer limits. One cannot calculate growth velocities from the National Center for Health Statistics' curves because all their surveys are cross-sectional (T.A. Drizd, personal communication), so we badly need a more explicit explanation of the Tanner and Davies modeling procedure from which the centiles for height velocity were constructed.

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## Reply from Drs. Tanner and Healy

Dr. Rosenfield's letter regarding an article one of us coauthored<sup>1</sup> gets us into very deep water. Let us first clear away some misunderstandings. Dr. Rosenfield writes, "I had thought. . . that the lower centiles on previous height-velocity charts were misleadingly wide because they were based on cross-sectional data and included children whose tempo of puberty was inordinately early or late." To this we respond:

1. No velocity centiles were or can be based on cross-sectional data; such a thing is impossible.<sup>2</sup> Dr. Rosenfield presumably means *tempo-conditional data*,

which is longitudinal data plotted simply against chronologic age, with variations in tempo leading to early and late puberty being ignored.

2. Such tempo-unconditional velocity centiles do indeed give unpleasantly wide centiles from approximately 8.5 years in boys and 6.5 years in girls in America.<sup>3</sup> If the level of maturity is unknown, the information provided by velocities at these ages is relatively slight.

3. No published velocity standards, whether tempo-conditional or -unconditional, have included

"inordinately early or late" maturers so far as we know (if inordinately means that children more than, for example, 2 SD away from the mean for maturity are included in proportions greater than those occurring in the healthy population).

4. Prior to 8.5 years (for convenience, we shall talk about boys only from now on) there is only a small difference between tempo-conditional and tempo-unconditional centiles for velocity. Thus, between 8 and 9 years of age, the 50th centile velocity for 2 SD late maturers is only 0.2 cm/year less, and that for 2 SD



early maturers only 0.4 cm/year more, than the 5.4 cm/year appropriate to median maturers.

Dr. Rosenfield asks, "Are we to consider that a growth rate of 4.0 cm/year is normal" between 8 and 9 years of age? The answer to this question is straightforward. A velocity as small as this occurs at these ages in about 6% of all healthy boys, in a slightly larger percentage of late maturers, and in a slightly smaller percentage of early maturers. With the usual conventions, it must indeed be regarded as "normal." But, Dr. Rosenfield asks, what if the boy started at age 8 with an attained height at the fifth centile? A velocity of 4.0 cm/year would leave him, at age 9, at a position below his initial centile. Should he be regarded as abnormal?

This question is difficult in unexpected ways.<sup>4</sup> It involves the joint assessment of two pieces of information: the initial height and the velocity. In our case, both are near the conventional lower limit of "normality." However, between 2 years and the beginning of puberty, it is perhaps surprising that the correlation between distance attained and subsequent velocity is very small. This means that our judgment about the velocity as *such* is largely unaffected by its starting point: just about 6% of the subset of healthy boys who are at the fifth centile for height at age 8 will have a velocity as small as 4.0 cm/year over the subsequent year.

Looking at the question another way, the velocity required to keep a boy exactly on the fifth centile between ages 8 and 9 is 4.6 cm/year for a late maturer, and much the same (4.9 cm/year, in fact) for a median maturer. These velocities are around the 20th centile value, and a substantial proportion of healthy boys will have lower velocities. They will fall below their initial "channel" on the distance chart; this is bound to occur with some healthy children, just as some healthy children are bound to fall

below the 20th centile at any age. If Dr. Rosenfield were to conclude that a height velocity of 4.6 cm/year between 8 and 9 years of age was abnormal rather than merely "subnormal," then, based on current data, he would be aiming to treat some 20% of the healthy population.

The position is slightly more complicated during puberty, when tempo effects give rise to appreciable correlations between velocities and their starting values. Then the assessment of a velocity needs to take into account its starting value.<sup>5</sup> Regression height standards to make this possible have been published by Cameron,<sup>6</sup> although we are not aware that they have been used in practice. They do provide a practical version of tempo-conditional velocity standards over the pubertal age range where these are potentially useful. As an example, Cameron's figures show that the third centile velocity over ages 14.5 to 15 years is 2.6 cm/year for a median starting height, 2.0 cm/year for those starting 2 SD tall, and 3.1 cm/year for those starting 2 SD short.

Of course, a height at the fifth centile at age 8 years may be regarded as tentative evidence of abnormality and a subsequent yearly velocity of 4.0 cm/year may then be regarded as confirmatory. To assess this, we now need to have the probability in healthy boys of *both* a low starting height *and* a low velocity; thanks to the absence of correlation, this can be calculated as  $0.05^2 = 0.0025$ , a highly unlikely occurrence in healthy boys as a whole. Notice, though, that the probability of a height and a velocity both below the 15th centiles (two not very unlikely events in themselves) is only 0.02. Notice also that if we label as abnormal any boy with a height *or* a velocity below the third centile, we shall wrongly label not 3% but nearly 6% of the healthy population. This might suggest that the "normal" limit should be set at, for example, the 1.7th centile

to get back to an overall false-positive rate of 3%. This in turn would prevent us from detecting some truly abnormal children from their low starting height, and our false-negative rate would be increased. We have explored elsewhere<sup>7</sup> some possible ways of assessing two or more successive measurements in terms of the false-positive and false-negative rates of different tactics. We might, for example, declare as abnormal those boys with a starting height below the third centile, or those with both starting height and subsequent velocity below the 10th centile.

Finally, we answer the query in Dr. Rosenfield's last sentence. Briefly, to obtain velocity centiles, Tanner and Davies<sup>1</sup> followed the methods of Tanner, Whitehouse, and Takaishi.<sup>3</sup> Instead of British data, however, we used data from the Harvard School of Public Health and the Berkeley Growth Study. Individuals were lined up on their peak height velocity ages, and the SD was calculated for the preceding year, the year before that, etc. This led to an estimate of the SD of velocity for chronologic age for a given tempo cohort. Prior to puberty, the SD of whole-year velocities was estimated from the data straightforwardly. The junction of the prepubertal and pu-

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bortal SD lines was smoothed graphically (a minor adjustment). Centiles were then calculated, assuming (after test) a Gaussian distribution of velocities within tempo cohort.

We find that we encounter new theoretical problems each time we consider the best way to use velocity standards. We hope that others will add their views on this subject.

J.M. Tanner, M.D., D.Sc.  
M.J.R. Healy, M.D.

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## Letter to the Editor

I am writing in reference to your editorial comments (*Growth, Genetics, and Hormones*, Volume 4, Number 2) on our article, "Short-Term Testosterone Treatment at Bone Age 12-13 Years Does Not Reduce Adult Height in Boys with Constitutional Delay of Growth and Adolescence," by Zachmann M, Studer S, and Prader A (*Helv Paediatr Acta* 1987;42:21). You rightly pointed out the need for caution in interpreting mean data for groups when the individual results are not known.

Our article was a short version of a more detailed doctoral dissertation that was written by Dr. Studer under my guidance. In the abridged version, only mean values for groups were shown, to save space. In the original dissertation, however, there were three figures indicating each individual difference between predicted height (according to Bayley-Pinneau; Roche, Wainer, and Thissen; and Tanner et al) and the adult height actually reached. These figures showed that the differences are equally distributed among plus (ie, adult height greater than predicted) and minus (ie, adult height less than predicted), and that a few individual cases did not unduly influence the mean values. A copy of the dissertation is available to interested readers free of charge; keep in mind that it is written in German.

As far as the testosterone dose is concerned, it should be noted

that ours was a retrospective study and that some of the patients were receiving doses higher than what is today considered appropriate. Our present policy is to administer 50-100 mg of long-acting testosterone esters for a period of 6 months, when bone age has reached a value of 12.5 years or more. It is thus similar to the policy stated in your comment. In contrast, we do not treat younger boys with oxandrolone. In view of the recent characterization of one single androgen receptor, there seems to be no evidence that the action of oxandrolone or other synthetic androgens might be different in any way from that of testosterone

itself. Testosterone offers advantages of degradation (peripheral aromatization and glucuronide esterification), so it might be more physiologically appropriate to treat younger boys with extremely low doses of testosterone (eg, 5-10 mg of a long-acting preparation every 2 weeks). This dosage, however, will require further short-term metabolic and long-term treatment studies.

Milo Zachmann, M.D.  
Professor of Pediatric  
Endocrinology  
Department of Pediatrics  
University of Zürich  
Kinderspital, Zürich  
Switzerland

## Dr. Blizzard's reply

*The data in Dr. Zachmann's article (Helv Paediatr Acta 1987;42:21) and those in other recent reports indicate that low-dose depot testosterone (50-100 mg IM each month for 6 months), in boys with constitutional delay of growth and adolescence (CDGA) who have a bone age  $\geq$  12.5 years, generally does not decrease ultimate height. Dr. Zachmann adequately responded to my constructive questions regarding the data that could not be presented in the Helvetica article, and I am pleased to see this response.*

*In my editorial comment on Dr. Zachmann's article I suggested that oxandrolone might be preferable for young patients. Dr.*

*Zachmann legitimately questions whether oxandrolone has any advantages over testosterone at very low doses in boys who have CDGA and a bone age < 12.5 years. His argument that oxandrolone and testosterone should act similarly because only one androgen receptor has been identified is logical and very possibly correct. Confirmation of this argument, however, will require specific studies comparing the effects of testosterone at very low doses (5-10 mg every 2 weeks IM) with the usual doses of oxandrolone (0.1 mg/kg body weight every day) on nitrogen retention, growth acceleration, and virilization. Until such studies are accomplished, I am unprepared to say more than, "Possibly, Dr. Zachmann is correct."*

### **Slow Grows the Child: Psychosocial Aspects of Growth Delay**

Edited by Bryan Stabler, Ph.D.,  
and Louis B. Underwood, M.D.  
Published by Lawrence  
Erlbaum Associates, Hillsdale,  
New Jersey, 1986

*Slow Grows the Child* is a very readable and important book. It compiles for the first time information concerning the psychosocial aspects of short stature, a condition to which many physicians do not often pay close attention. It will also serve as a primary reference for those interested in performing the needed prospective longitudinal studies of cognition, achievement, psychosocial functioning, and other developmental and behavioral factors in short children.

This short volume (less than 200 pages) includes 14 papers that were presented at a 1984 symposium on the psychosocial aspects of growth delay held in Washington, D.C. A report based on that conference appeared in a previous issue of this publication (Vol. 1, No. 1). The book reviewed here is an excellent source for those who were unable to attend that symposium.

The value of the book is enhanced by the logical sequence of its chapters. In the first, Holmes et al describe the longitudinal evaluation of behavioral patterns in children with short stature and conclude that there is an age-related decline in the ability to adjust during early adolescence. In the next chapter, Richman et al discuss academic and emotional difficulties of children with constitutional delay of growth and describe how these children, who are often socially withdrawn and aloof, internalize emotional concerns. In the third chapter, Young-Hyman considers the social competence of children with growth hormone (GH) deficiency, constitutional delay of growth, and genetic short stature. The author concludes that short stature per se does not represent a handicapping condition, but the

age of onset and the perceived and real growth delay are critical factors affecting social competence.

The next series of chapters concerns children with GH deficiency and their academic and emotional functioning. Drs. Siegel and Hopwood report that children with hypopituitarism have overall average intelligence but show significant variability on cognitive testing and difficulty with visual-motor integration. Three chapters, by Dean et al, Clopper et al, and Mitchell et al, deal with the long-term psychosocial follow-up of children treated with GH. The results presented in these chapters, however, are often at variance with each other. For example, an 8% unemployment rate is described in one chapter while a 35% unemployment rate is described in another. Both of these chapters state that approximately 25% to 35% of the individuals queried are now living independently. Other findings are that only 58% of

GH-deficient adults have a driver's license, and 85% have never married. Although the majority (85%) of subjects in one study stated that short stature was not a serious problem, another study found that subjects had poor self-perception and 38% had sought psychological counseling.

Two chapters deal with cognitive and psychological studies in girls with Turner syndrome. As expected, visual-spatial problems were identified, but neuropsychological dysfunction appears to be even more prevalent. When one compares adults with Turner syndrome with adult women who have constitutional short stature, one finds that there are an equal number of major depressive episodes in both groups and no difference in the incidence or extent of psychological impairment.

A chapter by Drs. Wilson, Duncan, Dombusch, Ritter, and Rosenfeld looks at the effects of growth

### **Meet the Editorial Board**



Associate Editor  
William A. Horton, M.D.

Dr. Horton is Professor of Pediatrics and Medicine, Director of the Division of Medical Genetics, and Director of the Chondrodysplasia Laboratory at the University of Texas Health Science Center in Houston.

Dr. Horton received his bachelor's degree with honors in zoology from the University of Kansas and earned his medical degree in 1971 from the Univer-

sity of Kansas School of Medicine, where he received the Walter F. Sutton Award in Human Genetics and was elected to Alpha Omega Alpha. After completing his internship and residency at Kansas University Medical Center, he worked as a staff associate at the National Institutes of Health (NIH) and as a fellow in medical genetics under Dr. David L. Rimoian at Harbor-UCLA General Hospital, Los Angeles. Dr. Horton joined the faculty of the University of Texas in 1983.

Dr. Horton is a member of the Board of Directors and is Chairman of the Research Committee of the Human Growth Foundation. The author of more than 120 articles on the chondrodysplasias, he chaired the NIH Conference on the Biological Basis of Human Chondrodysplasias, held in September 1987. Dr. Horton is a fellow of the American College of Physicians and a member of the American Society of Human Genetics, the American Federation of Clinical Research, and the Society for Pediatric Research.

on intellectual function and describes an association between height and IQ scores. A retrospective study of children with emotionally based failure to thrive suggests that an early onset of chronic failure to thrive has a poor prognostic outcome; ultimately, these children exhibit poor attachment to their parents. A chapter on environment and intelligence suggests that manipulating the environment of children with psychosocial short stature can prevent permanent retardation and that significant intellectual catch-up growth can occur even if the child is not rescued until early adolescence. Finally, there is a chapter on the ambiva-

lence involved in parenting short-statured children.

Included in this volume is an address by Dr. Leonard P. Sawisch of the Michigan Department of Education, the keynote speaker at the symposium. Dr. Sawisch, who is 4' 4" tall, reviews the psychosocial aspects of short stature from his perspective. He describes his perception of himself as a short adult, and how short adults in general perceive the way society views them. The presentation is amusing and anecdotal but disappointing in terms of how we, as a society, have affected the lives of our children and others with handicaps.

Although there are limitations in

a number of the studies described in the book—some included small groups of subjects, some were performed retrospectively, some had diverse methods of evaluation, and some grouped together many discrete syndromes involving short stature—this book should nevertheless be on the required reading list of all who treat short children. Those interested in more information on psychosocial aspects of short stature are also referred to "The Disability of Short Stature," a recent paper by C.M. Law (*Arch Dis Child* 1988;62:855-859). Law carefully reviews many of the topics presented in depth in *Slow Grows the Child*.

William L. Clarke, M.D.

#### Abstracts From the Literature

### Decreased Height Velocity in Children and Adolescent Boys Before the Diagnosis of Crohn's Disease

To assess the prodromal growth patterns of Crohn's disease patients, sequential growth data and height and weight velocities were studied in 50 white children and prepubescent adolescents (31 boys, 19 girls) with Crohn's disease. Height and weight velocities of the patients were compared by calculating the SD score (z score). Inclusion criteria were at least four separate height recordings between the age of 4 years and the onset of symptoms attributable to Crohn's disease, premorbid height  $\geq$  fifth percentile, Tanner stage no greater than II, and absence of other chronic medical conditions. A decrease in height velocity was defined as a sustained decline of 25% from the premorbid height velocity or to the third percentile for height velocity.

Three distinct patterns of linear growth emerged in this patient population. Forty-six percent (23 patients) had a decrease in height velocity between 4 and 72 months (median, 12 months) before the onset of symptoms attributable to Crohn's disease. Forty-two percent (21 patients) had a decrease in height velocity after symptoms had developed but before Crohn's disease had been diagnosed.

Twelve percent (six patients) sustained a normal height velocity until Crohn's disease was diagnosed. There were no differences in the site of gastrointestinal involvement and symptoms between the patients who showed a decrease in height velocity and those who did not. Although the majority of patients with growth failure demonstrated poor weight gain, a subset (22%) had decreased linear growth while maintaining a normal weight velocity. Of the six patients with normal linear growth, five continued to maintain normal weight gain.

Kanof ME, Lake AM, Bayless TM. *Gastroenterology* 1988;95: 1523-1527.

**Editor's comment**—This interesting study reports on the pattern of growth of patients with Crohn's disease before presentation of symptoms and diagnosis. It illustrates a pattern of nutritional dwarfing in which cessation of body weight progression usually precedes height deceleration. It also shows that a body weight/height deficit is not always present in patients with Crohn's disease. Nutritional dwarfing has been reported

in patients who are overweight for their height; the best example, studied by Dietz and Hartung (*Am J Dis Child* 1985;139:704-708), is obese children given hypocaloric diets who fail to grow in height. Trowbridge and co-workers reported that this occurs in other malnourished populations as well (*Am J Clin Nutr* 1987;46:411-418). My colleagues and I have observed similar patterns of growth in children with atypical eating disorders leading to nutritional dwarfing who have no body weight/height deficits, but show a lack of weight progression (*Semin Adolesc Med* 1987;3:255-266). Nutritional alterations other than caloric or protein deficits may account for nutritional dwarfing without deceleration in weight velocity. For example, low blood zinc levels might precede both the onset of the symptoms and the diagnosis of inflammatory bowel disease (Lifshitz F, Nishi Y. In: Anast C, DeLuca H [eds]: *Pediatric Diseases Related to Calcium*. Elsevier, 1980). Unfortunately, this study did not address the possible etiology of the different growth patterns among these patients.

Fima Lifshitz, M.D.



## Birth Weight and Childhood Growth

Previous studies have recorded that low birth-weight (BWt) infants often exhibit rapid growth in infancy, but do not achieve the same weights and heights in childhood as do their counterparts with normal BWt. Furthermore, low BWt term infants do not grow as well as preterm low BWt infants after the first months of life, indicating the negative effect of intrauterine growth retardation (IUGR) on later growth.

In this report, the relationship between BWt and later childhood growth was studied in several thousand infants. The infants were divided into eight BWt categories, beginning with a BWt of 1,000 g and extending, in 500-g increments, to 5,000 g. Z scores of height for age (H/A), weight for age (W/A), and weight for height (W/H) were calculated. These z scores represent the distance of the observed value from the median of the age-specific and sex-specific reference curve, expressed in SD units. All children were observed from birth to 60 months of age. To evaluate the possible role of BWt in subsequent childhood obesity, the investigators calculated the percentage of children in each BWt category who had W/H z scores > 2.0.

The effect of low, intermediate, and high BWt on growth persisted during the 60 months, although the marked discrepancies in mean weight for the eight groups at birth (mean z score, -2 to +2) were less discrepant than those observed at 60 months of age (mean z score, -1 to +1). Each group continued in its own growth channel in relationship to the other groups, and, therefore, the infants born weighing between 1,000 and 1,500 g were the most weight-retarded at 60 months, whereas the infants with BWt values of 4,500 to 5,000 g were the most weight-accelerated.

The relationship between BWt and z scores for H/A were rela-

**Table.** Prevalence of abnormal growth by birth weight for children 36 to 41 months of age in the Tennessee Women, Infants, and Children Program, 1975 to 1985

| BWt category (g) | H/A<br>< -2.0 z<br>(%) | W/A<br>< -2.0 z<br>(%) | W/H<br>< -2.0 z<br>(%) | W/H<br>> 2.0 z<br>(%) |
|------------------|------------------------|------------------------|------------------------|-----------------------|
| 1,000-1,499      | 12.3                   | 19.8                   | 5.6                    | 1.0                   |
| 1,500-1,999      | 11.0                   | 10.4                   | 4.5                    | 1.0                   |
| 2,000-2,499      | 11.3                   | 10.6                   | 2.9                    | 1.9                   |
| 2,500-2,999      | 7.4                    | 5.9                    | 1.3                    | 2.1                   |
| 3,000-3,499      | 4.2                    | 2.9                    | 0.9                    | 2.4                   |
| 3,500-3,999      | 2.3                    | 0.9                    | 0.4                    | 3.7                   |
| 4,000-4,499      | 1.3                    | 0.5                    | 0.4                    | 5.0                   |
| 4,500-4,999      | 0.5                    | 0.0                    | 0.0                    | 8.7                   |

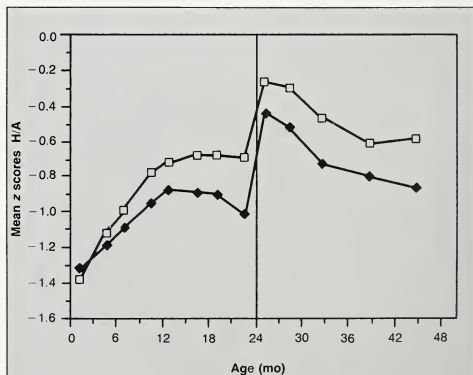
BWt = birth weight; H/A = height for age; W/A = weight for age; W/H = weight for height.

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tively constant after the 24th month. As BWt increased, the percentage of children who had a z score < 2.0 for height at 36 to 41 months declined considerably. However, 12.3% of the very low BWt infants continued to have a z score < 2.0, as compared with 2.3% of those with a BWt of 3,500

to 4,000 g and only 0.5% of those with a BWt of 4,500 to 5,000 g (Table). The W/A and W/H spread is also presented in the Table.

The authors compared the growth of preterm and term infants weighing 2,000 to 2,499 g at birth (Figure). H/A at 0 to 2 months was similar in both groups. Later, the



**Figure** Mean H/A z scores for intrauterine growth-retarded (◆) v premature (□) infants 2,000 to 2,499 g by 3-month age groupings for children < 36 months of age and 6-month groupings for children 36 to 47 months of age. Based on Tennessee-Linked Special Supplemental Food Program for Women, Infants, and Children and birth certificate records, 1975 to 1985.

Reproduced by permission of *Pediatrics*.

IUGR infants had lower z scores than did the premature infants with the same BWt. Median z scores in both premature infants and infants with IUGR remained considerably less than normal, however. Mean z scores for all parameters differed by 0.2 to -0.3 between the two groups, with the preterm infants being taller and heavier than the IUGR group.

High BWt appeared to be a risk factor for obesity (Table). The very high BWt group included 8.7% of the infants with z scores  $>-2.0$  (W/H).

The authors concluded that BWt

strongly predicts future growth in early childhood. Although low BWt infants exhibit significant weight gain in the first 12 months, they are likely to remain shorter and lighter in early childhood than children with higher BWt. Conversely, infants with higher BWt remain taller and heavier on average, and increased BWt is associated with a substantial increase in prevalence of childhood obesity. Finally, IUGR is a stronger risk factor than prematurity for short stature and low weight. Preterm children of the same BWt sustain less permanent growth impairment over the 60

months of observation than those who have IUGR, although both groups remain smaller than their normal BWt counterparts.

Binkin NJ, Yip R, Fleshood L, et al. *Pediatrics* 1988;82:828.

**Editor's comment**—This report provides important data to assist in predicting the height and weight at 5 years of age in infants of various sizes. The authors are to be commended for a study that was both much needed and precisely conducted.

Robert M. Blizzard, M.D.

## Natural History of Williams' Syndrome: Physical Characteristics

Williams' syndrome is a relatively common, sporadic condition marked by short stature, developmental disability, a characteristic craniofacial appearance, characteristic behavior, frequent failure to thrive in the newborn period, typical cardiac lesion (supravalvular aortic stenosis), and, occasionally, hypercalcemia. Most pediatricians feel that they can recognize the syndrome because of the typical elfin facies. Before this excellent study was conducted, however, the natural history of Williams' syndrome through adulthood, its medical complications, and its progressive nature had not been defined. Morris and colleagues collected information from multiple sources in Utah and Kentucky and from the Williams' Syndrome National Association. Evaluations varied from extensive, on an outpatient basis over a 2-day period, to a format of questionnaires answered by the subjects' parents. A total of 109 subjects were included in the study.

The intelligence of the study subjects varied widely, from severe mental retardation to normal (IQ range, 20 to 106), but most subjects had relative verbal and expressive strengths. Auditory input was much better than visual

and motor integration. Distractibility and attention deficits were common, occurring in 84% of subjects. Other frequent problems were esotropia (50%) and hyperopia. Eighty-five percent exhibited a unique hypersensitivity (hyperexaggerated startle) to sudden, loud sounds.

Most individuals had hoarse voices, irrespective of documented hypercalcemia. Most had small, widely-spaced teeth with malocclusions and hypoplastic enamel.

Seventy-nine percent of all subjects had cardiac murmurs, but only 18% required cardiac surgery. Enuresis and constipation were frequent. Joint contractures were progressive. Initial joint laxity was followed by progressive limi-

tation. Toe-walking, stiff and awkward gait, and lordosis occurred regularly; sequelae related to hypercalcemia, such as renal, intracranial, and abdominal aortic calcifications, also occurred regularly.

Most adults with Williams' syndrome were not able to live independently and had multiple chronic medical problems.

Morris CA, Demsey SA, Leonard CO, et al. *J Pediatr* 1988;113:318-326.

**Editor's comment**—This is an extremely important paper—essential for any physician caring for an individual with Williams' syndrome.

Judith G. Hall, M.D.

## Malformation due to Presumed Spontaneous Mutations in Newborn Infants

An estimate of the frequency of new mutations and the mutation rate among congenital anomalies has been made through an ongoing study of newborns. Congenital anomalies among live-born and stillborn infants of at least 20 weeks' gestation were tabulated over a 10-year period at one institution. Of 69,277 infants, 1,549 (2.24%) had some type of congenital anomaly. Anomalies suggestive of single-gene disorders were found in 48 of 69,277 infants

(0.07%). Family studies suggested that 11 of these 48 single-gene disorders represented new mutations: 10 were autosomal dominant and one was X-linked. The reported mutation rate, 11 of 69,227 (0.00016), was lower than rates quoted in similar studies. There were no differences in the ages of the female or male parents of the infants with mutations when compared with controls. The spontaneous rate for achondroplasia was 1.4/100,000

continued on page 14

**Mutations in Newborn Infants***continued from page 13*

births; the Apert syndrome occurred with the same frequency. The rate was 0.7/100,000 births for the Adams-Oliver, Freeman Sheldon, Hold Oram, Osteogenesis Imperfecta II, and spondylo-epiphyseal dysplasia congenital syndromes. As in many other studies, skeletal and limb anomalies are most easily recognized in newborns and, therefore, useful in estimating new mutation rates.

The causes of the congenital anomalies could be established in approximately half the cases, thanks to careful evaluation and follow-up (Table).

These findings emphasize the possibility that malformations caused by a single mutant gene occur unexpectedly among many infants born to healthy parents. There was no family history in 10 of the 21 infants (48%) with autosomal dominant disorders, in 5 of the 10 infants (50%) with auto-

**Table.** Causes of congenital abnormalities

|                           | No. of infants<br>(n = 1,549) | Percentage of infants |
|---------------------------|-------------------------------|-----------------------|
| Genetic                   |                               |                       |
| Chromosomal abnormalities | 157                           | 10.1                  |
| Single mutant gene        | 48                            | 3.1                   |
| Familial single gene      | 225                           | 14.5                  |
| Multifactorial            | 356                           | 23.0                  |
| Teratogen                 | 49                            | 3.2                   |
| Uterine factor            | 39                            | 2.5                   |
| Twinning                  | 6                             | 0.4                   |
| Unknown                   | 669                           | 43.2                  |

somal recessive disorders, and in 1 of the 5 infants (20%) with X-linked disorders. The authors found it useful to address this issue in genetic counseling, because parents often assume that the absence of a history of an affected family member rules out the possibility of a genetic cause.

Nelson K, Holmes LB. *N Engl J Med* 1989;320:19-23.

**Editor's comment**—This study is important, for both establishment

of baseline data and as an ongoing monitor of possible increasing rates. It is of interest that the mutation rates seem to be lower than those frequently quoted in the past. The authors' term "malformations" is something of a misnomer, because many of the anomalies described are deformations or disruptions. The generic term congenital anomaly is preferred, unless a true malformation (ie, failure of normal formation) has occurred.

Judith G. Hall, M.D.

## Physiological Growth Hormone Secretion During Recovery from Psychosocial Dwarfism: A Case Report

Stanhope and colleagues reported on 18-hour growth hormone (GH) profiles, sampled every 15 minutes from 1300 hours to 0800 hours, in a 6-year, 4-month-old boy with psychosocial dwarfism. On admission to the hospital, this boy had a height SD score of  $-3.3$  and an inadequate GH response to insulin hypoglycemia (maximum,  $1.8 \mu\text{g/L}$ ). Three GH profiles were performed: on admission, after 6 days, and at 18 days. During the initial profile, peak GH was greater during the day than overnight. After admission, there was a progressive increase in GH secretion, with maximum GH peaks occurring during early sleep. The increase in GH secretion was achieved by an increase in pulse amplitude without alteration of pulse frequency. Peak GH secretion during sleep rose from  $8.2 \mu\text{g/L}$  on admission to  $17.5$

$\mu\text{g/L}$  on day 6, and to  $25.0 \mu\text{g/L}$  on day 18. The pattern of GH pulsatility and peak GH achieved at the onset of sleep was consistent with data previously collected in normal children.

Stanhope R, Adlard P, Hamill G, et al. *Clin Endocrinol* 1988;28:335-339.

**Editor's comment**—Pharmacologic tests of growth hormone (GH)

secretion have demonstrated that the GH deficiency in psychosocial dwarfism is reversible. The present report demonstrates the pattern of that reversibility and corroborates the findings of earlier studies with pharmacologic stimuli. Although this is only a single case, the findings are very important. It would be interesting to study GH release in response to growth hormone-releasing hormone in these children.

William L. Clarke, M.D.

### In Future Issues

The Morbid and Functional Anatomy of the Human Chromosome Map in Endocrine Disorders and Hormonal Genes by Victor A. McKusick, M.D.

Assessment and Management of the Psychological Aspects of Short Stature

by Heino Meyer-Bahlburg, Ph.D.

Gonadotropin and Steroid Concentrations in the Fetus and Newborn by Claude Migeon, M.D.

Growth in Late Adolescence by Alex Roche, M.D.

## Oestrogen Treatment of Constitutionally Tall Girls with 0.1 mg/day Ethinyl Oestradiol

Thirty-five constitutionally tall girls (mean age, 12.5 years) were treated with ethinyl estradiol, in a dosage of only 0.1 mg/day. Their ultimate heights were compared with those of 23 untreated girls of similar initial ages, bone ages (BAs), and predicted adult heights, and with those of five girls treated with ethinyl estradiol, 0.3 mg/day. Treatment lasted more than 2 years for the girls whose BA was > 12.5. Final heights were defined

as heights measured at the end of a year during which growth was < 0.7 cm.

The Bayley-Pinneau predictions underestimated by an average of 0.7 cm the heights of controls whose BA was < 12.5 years, and overestimated by 0.6 cm the heights of controls with a BA > 12.5 years. Making allowance for these discrepancies, the investigators calculated that the reductions in predicted adult height achieved by estrogen treatment averaged 7.4 cm in girls with BA values > 12.5 years. The authors concluded that the higher dosage of estrogen offers little, if any ad-

vantage over the dosage of 0.1 mg/day.

Bartsch O, Weschke B, Weber B. *Eur J Pediatr* 1988;147:59.

**Editor's comment**—The authors admit that it is difficult to compare their results with those in the literature because of methodological differences in determining BA and predicting height. Nonetheless, it certainly seems that the lower, and hence more desirable, dosage of estrogen produces much the same results as the higher one. The authors' review of results in the literature is also a valuable one.

James M. Tanner, M.D., D.Sc.

## Changes in Serum Insulin Concentration During Puberty and Their Relationship to Growth Hormone

Hindmarsh and colleagues investigated the relationship between insulin concentration and growth hormone (GH) secretion during puberty in a cohort of 40 tall or short children. Oral glucose tolerance tests were performed in 34 children during puberty and in six adolescents who had reached their final height. The pubertal children were growing at a normal velocity for their stage of development and had no family history of insulin-dependent diabetes. Following glucose ingestion, blood samples were drawn at time 0, and at 30-minute intervals for 3 hours. In addition, 24-hour GH profiles were determined in 16 tall girls (four prepubertal, six in early puberty at breast stages II and III, and six in late puberty, at breast stages IV and V). Twenty-four-hour GH (sampled every 20 minutes) and insulin profiles were obtained in 13 of the 40 children (five short prepubertal, four tall prepubertal, and four tall pubertal). GH pulses were identified, and incremental areas under the glucose and insulin curves were calculated.

The 34 children and six young adults demonstrated no significant increase in fasting blood glucose or in incremental area under the glucose curve. In

both tall and short pubertal children there was a significant increase in fasting insulin concentration during puberty; this increase was related to pubertal status rather than stature. The incremental area under the insulin curve increased almost twofold. Age played no part in these changes. Among the six young adults, the glucose and insulin parameters resembled those of prepubertal children. Among individuals in whom GH secretion was studied, increases in fasting insulin were seen at breast stages II and III, and declines were seen at breast stages IV and V. The changes in GH pulse amplitude were coincident with the changes in fasting insulin concentration. There was a threefold increase in the mean sum of GH pulse amplitudes between prepubertal children and girls at breast stages II and III.

The authors concluded that they have demonstrated a threefold increase in serum insulin concentration during puberty and that this increase is coincident with the rise in GH associated with breast development stages II and III. They suggested that during pubertal growth in children with diabetes, the standard dose of insulin should

be doubled and possibly tripled to maintain good metabolic control and maximize pubertal growth. As further evidence that the changes in insulin secretion are due to GH, they cited the increase in fasting insulin concentrations in 14 prepubertal children during the first year of exogenous GH therapy.

Hindmarsh P, Di Silvio L, Pringle PJ, et al. *Clin Endocrinol (Oxf)* 1988;28:381-388.

**Editor's comment**—Amiel and colleagues (N Engl J Med 1986;315:215) have previously demonstrated impaired insulin action in pubertal children with diabetes and in their nondiabetic siblings. As Hindmarsh et al point out, the comparison with siblings of children with diabetes may not be appropriate, and clamp studies such as those performed by Amiel et al may not be as physiologically meaningful as the oral glucose loads given in this most recent study. Although this investigation studied children at a variety of prepubertal and pubertal phases, including differences of stature, its findings are significant and suggest one of the reasons for poor glucose control in adolescents with diabetes. Longitudinal studies are needed to corroborate these findings.

William L. Clarke, M.D.



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## MEETING CALENDAR

**July 9-12** Clinical Genetics Conference on Clinical Applications of Molecular Genetics. Lafayette Hotel, 1 Avenue de Lafayette, Boston, MA. Contact: Sue Greene, March of Dimes, 1275 Mamaronck Avenue, White Plains, NY 10605 (914-997-4524)

**September 23-25** 30th Annual Meeting of the American College of Nutrition. Omni International Hotel, Norfolk, Virginia. Contact: Kay Balun, Administrative Assistant, American College of Nutrition, 345 Central Avenue, Suite 207, Scarsdale, NY 10543 (914-723-4247)

**October 11-14** 41st Annual Postgraduate Assembly of The Endocrine Society. Fairmont Hotel, New Orleans, Louisiana. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**October 29-November 3** Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology (scientific sessions, October 30-November 1). Jerusalem Hilton, Jerusalem, Israel. Contact: Zvi Laron, M.D., Beilinson Medical Center, Petah Tikva 49 100 Israel

**November 12-15** The Annual Meeting of The American Society of Human Genetics, Convention Center, Baltimore, Maryland. Contact: Jean Francese, American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

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# GROWTH

## Genetics & Hormones

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## Mapping the Genes for Hormones and Growth Factors and the Mutations Causing Disorders of Growth

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In 1968, a Johns Hopkins Ph.D. candidate named Roger Donahue found linkage between the Duffy blood group gene and a particular heteromorphism of chromosome 1.<sup>1</sup> His report marked the first "mapping" of a specific gene to a specific autosomal location. Already by that time, 68 genes had been assigned with some confidence to the X chromosome, mostly via observations on the typical pedigree patterns of "sex-linked" inheritance. These 68 established X-linked traits, along with other, less certain linkages, were cataloged in the second edition of my book, *Mendelian Inheritance in Man*,<sup>2</sup> published in 1968.

In the remarkable decades since Donahue's report, specific genes have been assigned to specific chromosomes and chromosome regions at an ever-accelerating pace. The totals are now more than 150 genes assigned to the X chromosome and more than 1,300 assigned to specific autosomes. At least some regional information is available for more than half of the genes on the X chro-

mosome and for more than 80% of the autosomal loci—ie, not only the chromosome for these genes is known but also, with fair precision, where on the chromosome each resides.

### Methods for Mapping

The rapid advances in this field can be credited to the development of new methods, specifically, the commingling of four methodologic streams: family linkage studies, chromosome studies, somatic-cell hybridization studies, and molecular genetic studies. The latter two methods in particular have been largely responsible for the accelerated pace of discovery in recent years (Figure 1).

The early 1970s saw the introduction of somatic-cell hybridization, which correlates chromosome studies with the segregation of human cellular characteristics in subclones from rodent-human cell hybrids. As indicated in Table 1, somatic-cell hybridization in all of its variations has been responsible for the largest number of assignments of genes to autosomes.

Beginning about 1980, molecular genetic methods entered the methodologic mix. Southern blot analysis of DNA from somatic hybrid cells permitted the mapping of human genes, even though they are not expressed in the cultured

cells. Recombinant DNA restriction enzyme technology provided DNA markers (restriction fragment length polymorphisms, or RFLPs, pronounced "riflips") for use in family studies. Molecular genetics also provided the methods for in situ chromosomal hybridization, a combination of molecular genetic and chromosomal studies.

It is of considerable interest that in situ hybridization has risen to second place in the production of autosomal assignments (Table 1), because the methods for reliable in situ hybridization for mapping of single-copy genes did not become available until 1981. The first gene assigned a chromosomal location by in situ hybridization was that encoding the kappa light chain of immunoglobulin, mapped by Ferguson-Smith and colleagues in 1981. Also in that year, Harper and colleagues<sup>3</sup> used in situ hybridization to confirm the assignment of the insulin gene to chromosome 11 and to narrow the localization to the tip of the short arm of that chromosome.

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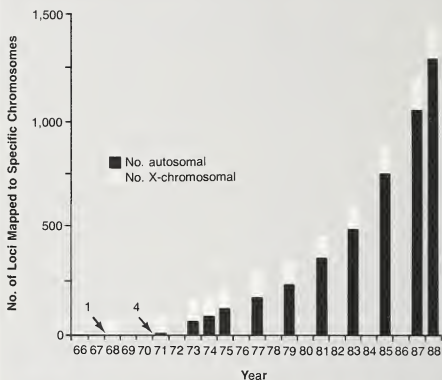
Today, as soon as a gene is cloned it is standard procedure to determine the chromosome that carries it by hybridization of the gene probe to DNA from a panel of rodent-human somatic-cell hybrids, and then to corroborate the chromosomal assignment and regionalize it by *in situ* hybridization. In the case of a gene that has not been cloned, other methods may be required. If the normal ("wild-type") gene is expressed biochemically, immunologically, or in other ways at the cellular level, it may be possible to map it by somatic-cell hybridization. But in the case of many hereditary diseases, the basic biochemical defect is unknown and, therefore, so is the nature of the gene. In such instances, mapping the disease gene requires family linkage studies using DNA or other markers. The Huntington's disease gene, assigned to chromosome 4 in 1983, was the first in an exciting succession of disease genes that have been mapped via family linkage methods.

Chromosomal banding methods have contributed as well, initially by providing a means for the unique identification of each chromosome. This was a boon to somatic-cell hybridization, as it permitted confident differentiation of the mouse and human chromosomes in the hybrid cells. More recently, high-resolution cytogenetics, *i.e.*, banding methods applied to the extended chromosomes of prophase or early prometaphase, have been used to detect small deletions and other abnormalities that serve as clues to the location of the genetic mutations responsible for several disorders, including various cancers. Such techniques have also figured in the localization of some wild-type genes.

### The Mapping of Genes for Hormones and Growth Factors

Genes for hormones and growth factors have figured disproportionately in the mapping process, most likely because much information was already available concerning the molecular structure of

**Figure 1** Growth in the field of gene mapping. The numbers relate exclusively to expressed genes.



these hormones and the enzymatic mechanisms by which they are synthesized. Figure 2 and Table 2 present the chromosomal loci of specific genes of endocrinologic interest. The genes for growth factor and growth factor receptors are listed in Table 3. In-nate mutation of these genes may well turn out to be the cause of some hamartomatous conditions and other congenital disorders.

Although a discussion of proto-oncogenes, which now number 60

or more, is beyond the scope of this presentation, it is noteworthy that many of them are growth factors or growth factor receptors. An example of the former is *SIS* = platelet-derived growth factor,  $\beta$  subunit; of the latter, *FMS* = receptor for colony-stimulating factor-1 (CSF-1R). Furthermore, many oncogenes seem to function in a paracrine or autocrine manner to determine tumor histophenotype, as exemplified by neovascularization. All oncogenes serve a function

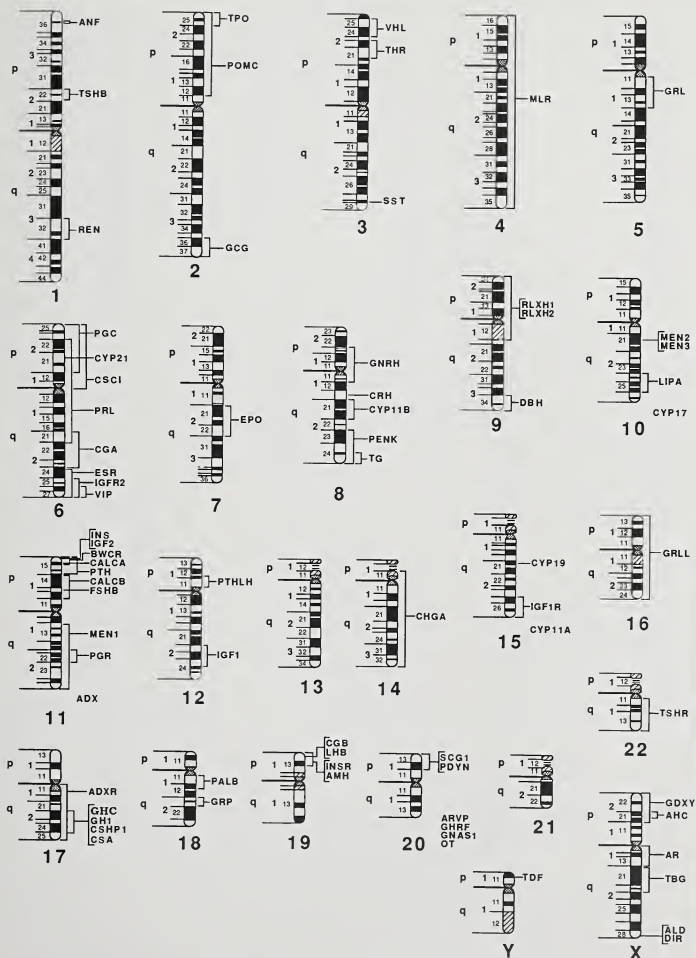
*text continued on page 5*

**Table 1.** Number of autosomal loci mapped by several methods (February 1, 1989)

| Method                             | No. of Loci Mapped |
|------------------------------------|--------------------|
| Somatic-cell hybridization         | 889                |
| In situ hybridization              | 444                |
| Family linkage studies             | 382                |
| Dosage effect                      | 132                |
| Chromosome aberrations             | 99                 |
| Restriction enzyme fine analysis   | 109                |
| Homology of syntenic               | 76                 |
| Radiation-induced gene segregation | 18                 |
| Others                             | 128                |
| <b>Total*</b>                      | <b>2,277</b>       |

\*The total exceeds the value of approximately 1,300 for autosomal gene loci mapped to date because many have been mapped by more than one method.

**Figure 2** Maps of selected genes, particularly those of endocrinologic interest. The symbols are defined in Table 2.





**Figure 3** The "morbidity map": location of mutations causing endocrinologic and growth disorders. (Boxes around the names of two or more disorders indicate that they are allelic, ie, caused by different mutations in one and the same gene.)

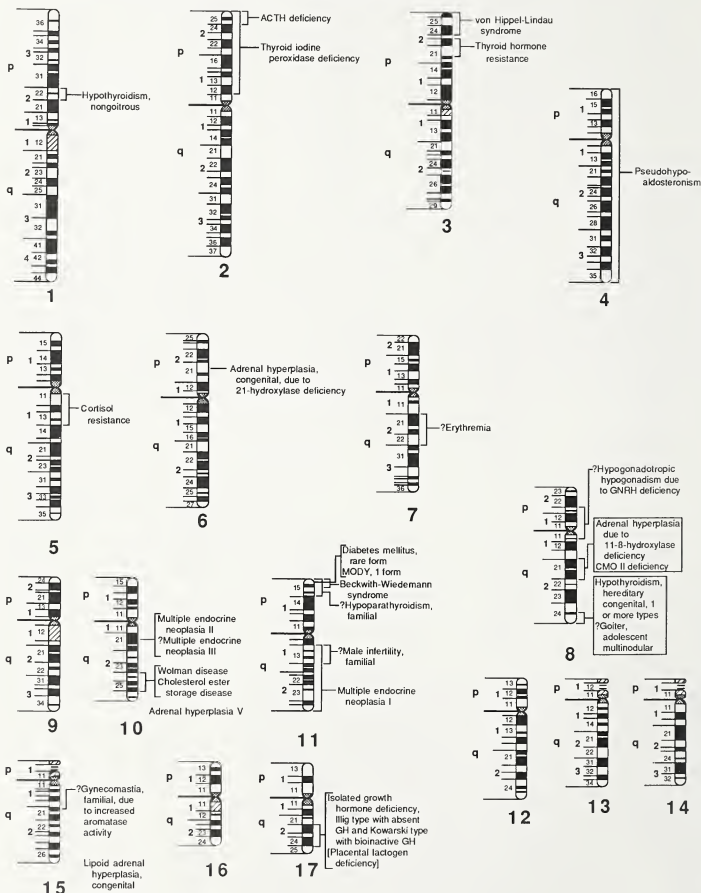
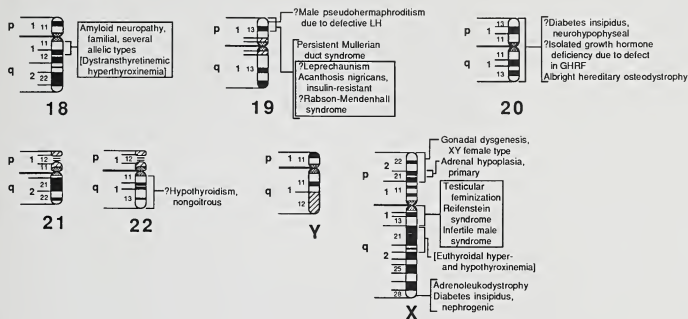


Figure 3 continued



damental role in normal cellular and tissue economy; the tumor-producing role is an aberration of the mutant form of the oncogene. In most instances, however, the normal functions are still unknown. The gene is not necessarily altered qualitatively when it produces cancer: amplification of a normal gene may also initiate a malignant process.

### The Morbid Anatomy of the Human Genome

The arrangement of genes on our chromosomes is as much a part of our anatomy as our organ systems or our extremities. This anatomic metaphor (apart from the usual cartographic one) allows the human genome to be viewed in terms of its comparative anatomy and evolution, eg. its functional, developmental, applied, and morbid anatomy.<sup>6</sup> The chromosomal sites of the mutations responsible for many genetic disorders have now been determined by mapping either the wild-type gene or the disease phenotype or both. With this information, a morbid anatomy of the human genome has been constructed, and that which applies to the endocrinopathies and selected growth disorders is schematized in Figure 3.

Before concluding that a mutation is located at a particular site of a wild-type gene, one must be certain that the mutation involves that structural gene. Demonstration of a change in the DNA of the gene, such as deletion or nucleotide substitution, is incontrovertible evidence, provided that the nucleotide change is not a polymorphism. This was the type of evidence presented by Phillips and colleagues<sup>5</sup> for isolated growth hormone (GH) deficiency caused by the absence of the gene on chromosome 17.

Francomano and colleagues<sup>6</sup> used another method, known as the candidate-gene-linkage approach, to show that one form of Stickler syndrome is caused by mutation in the gene for cartilage (type II) collagen; no crossover was observed between the disease phenotype and RFLPs of the type II collagen gene. Another excellent example involves the entity responsible for generalized thyroid hormone resistance: tight linkage with the ERBA2 oncogene, which appears to be identical to the gene for thyroid hormone receptor (THR) on chromosome 3, was demonstrated by Usala et al in 1988.<sup>7</sup>

Examples of endocrinopathies

in which the disease phenotype itself has been mapped include two forms of multiple endocrine neoplasia, MEN-1 and MEN-2, which are determined by mutations of the genes on chromosomes 11 and 10, respectively.

*text continued on page 9*

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Table 2. Gene loci of endocrinologic and related interest are listed here by the chromosome to which they have been mapped. Status: C = mapping confirmed; P = mapping provisional; L = mapping "in limbo" (tentative). MIM # = entry number in *Mendelian Inheritance in Man*<sup>2</sup> and its on-line version (OMIM). Method: A = in situ hybridization; C = chromosome-mediated gene transfer; Ch = chromosomal changes, visible; D = deletion mapping; EW = exclusion mapping; F = family linkage (Fc, with chromosomal heteromorphism or rearrangement; Fd, with DNA marker); H = homology mapping; HS = hybridization in solution; LD = linkage disequilibrium; M = microcell-mediated gene transfer; OT = ovarian teratoma; R = Goss-Harris method of radiation-induced gene segregation and modification of Cox and Myers ("zap mapping"); RE = restriction endonuclease methods (REa, combined with somatic-cell hybridization; REb, combined with chromosome sorting; REN, neighbor analysis in large segments); S = somatic-cell hybridization; V = viral change. The numbers in parentheses in column 8 refer to the means by which the mutation was positioned: by mapping the wild-type gene (1), by mapping the disease phenotype (2), or by both approaches.

| Location     | Symbol                  | Status | Title  | MIM # | Method      | Comments  | Disorder  | Mouse       |
|--------------|-------------------------|--------|--|-------|-------------|---|---|-------------|
| 1p36.2       | ANP, ANF, PND           | C      | Atrial natriuretic peptide; pronatriodiatin  | 10878 | REa, A, H   | centromeric to NGFB   | Hypothyroidism, nongoitrous (1)   | 4(Pud)      |
| 1p22         | TSHB                    | C      | Thyroid stimulating hormone, beta subunit  | 18854 | REa, RE, Fd |   |   | 3(Tabb)     |
| 1q32         | REN                     | C      | Renin  | 17982 | REa, A, D   | q32.3-q42.3 excluded by D <sub>1</sub> ; q42 = conflicting assignment |   | 1(Ren-1)    |
| 2pter-p12    | TPO, TPX                | C      | Thyroid peroxidase   | 27450 | REa         |   | Thyroid iodine peroxidase deficiency (1)  |             |
| 2p25         | POMC                    | C      | Proopiomelanocortin  | 17683 | REa         | ?close to ACP1  | ACTH deficiency (1)   | 12(Pomc-1)  |
| 2q36-q37     | GCG                     | C      | Glucagon   | 13803 | REa, A      |   | von Hippel-Lindau syndrome (1)  | 2(Cag)      |
| 3p25-p24     | VHL                     | P      | VHL of Hippel-Lindau syndrome  | 19330 | Fd          | linked to RAF1  |   |             |
| 3p22-p21.33  | THIR, THRB, THRI, ERBA2 | C      | Thyroid hormone receptor, beta (ERBA2)   | 19016 | REa         |   | Thyroid hormone resistance, 27430, 18857 (1)  |             |
| 3q28         | SST                     | C      | Somatostatin   | 18245 | REa         |   | Pseudohypoadosteronism (1)  | 16(Smat)    |
| Chr4         | MLR, MCR, MR            | P      | Mineralocorticoid receptor   | 26435 | REa, M      |   | Cortisol resistance (1)   | 18(Grl-1)   |
| 5q11-q13     | GRL                     | C      | Glucocorticoid receptor, lymphocyte  | 13804 | S, REa      |   |   |             |
| 6pter-p21.1  | PGC                     | P      | Progesterone receptor  | 16974 | REa         |   |   |             |
| 6p22.2-q21.3 | PKL                     | C      | Prolactin  | 17676 | REa, D      | ?between 6cen and GLO1  |   |             |
| 6p21.3       | CYP21, CA21H, CAH1      | C      | Congenital adrenal hyperplasia due to 21-hydroxylase deficiency; P450C21                 | 20191 | F, RE       | linked to C2, C4, BF, 2 loci, A and B; only B active                  | Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency (3)                             | 17(P450-21) |
| 6p           | CSCI                    | L      | Corticosterone side-chain isomerase  | 12255 | H           | linked to MHC   |   |             |
| 6q21.1-q23   | CGA                     | P      | Chorionic gonadotropin, alpha chain  | 11885 | REa, A      | shared with LH, FSH, TSH  |   | 4(Tsha)     |
| 6q24-q27     | ESR, ER                 | C      | Estrogen receptor  | 13343 | REa, A      |   |   |             |
| 6q25-q27     | IGF2R, MPRI             | P      | Insulin-like growth factor-2 receptor (mannose-6-phosphate receptor, cation-independent) | 14728 | REa, A      |   |   |             |
| 6q26-q27     | VIP                     | C      | Vasoactive intestinal peptide  | 19232 | REa, A, REb |   |   |             |
| 7q21-q22     | EPO                     | C      | Erythropoietin   | 13317 | REa, A, REb |   |   | 5(Epo)      |
| 8p21-q11.2   | GNRH, LHRH              | P      | Luteinizing hormone releasing hormone (gonadotropin releasing hormone)                   | 15276 | REa, A      | close to COL1A2; no recombination                                     | ?Erythremia (1)<br>?Hypogonadotropic hypogonadism due to GNRH deficiency, 22720 (1)               |             |
| 8q13         | CRH                     | P      | Corticotropin releasing hormone  | 12256 | REa, A      |   | Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency (1); CMO II deficiency (1) |             |
| 8q21         | CYP11B, P450C11         | P      | 11-beta-hydroxylase; corticosteroid methyl oxidase II (CMO II)                           | 20201 | REa, A, Ch  | multifunctional enzyme  |   |             |

Table 2. continued

| Location   | Symbol                                    | Status           | Title  | MIM #                            | Method                         | Comments  | Disorder   | Mouse            |
|--|---|------------------|--|----------------------------------|--------------------------------|---|--|------------------|
| 8q23-q24<br>8q24.2-q24.3                         | PENK<br>TG                                | P<br>C           | Proenkephalin<br>Thyroglobulin   | 13133<br>18845                   | REa, A<br>A, REa,<br>REb       | distal to MYC   | 1 hypothyroidism, hereditary<br>congenital, 1 or more types (1);<br>?Goiter, adolescent multinodular,<br>13880 (1) | 715(Tg)          |
| 9pter-q12<br>9pter-q12<br>9q34                   | RLXIII, RLNI<br>RLXII, RLNI<br>DBII       | P<br>P<br>P      | Relaxin, H1<br>Relaxin, H2<br>Dopamine-beta-hydroxylase  | 17973<br>17974<br>22336          | REa<br>REa<br>F                | tightly linked to ABO   |  |                  |
| 10q21.1  | MEN2                                      | C                | Multiple endocrine neoplasia, type II  | 17140                            | Fd                             | 19cM from D1065 at<br>10q21.1   | Multiple endocrine neoplasia II (2)  |                  |
| 10q21.1  | MEN3, MEN2B<br>LIPA                       | L<br>P           | Multiple endocrine neoplasia, type III (or IIIb)<br>Lysosomal acid lipase-A  | 16230<br>27800                   | Fd<br>S, H                     | ?allelic to MEN2<br>?close to GOT   | ?Multiple endocrine neoplasia III(2)<br>Wohman disease (1); Cholester-<br>ester storage disease (1)                | 19(Lip-1)        |
| Chr.10<br>1pter-p15.4                            | CYP17, P450C17<br>BWCR, BWS,<br>WBS       | P<br>C<br>C      | Steroid 17 $\alpha$ -hydroxylase / 17 $\beta$ hydrox-<br>yase<br>Beckwith-Wiedemann syndrome   | 20211<br>13065                   | REa<br>Ch                      | at least 2 genes<br>partial trisomy   | Adrenal hyperplasia V (1)<br>Beckwith-Wiedemann syndrome (2)   |                  |
| 11p15.5  | INS                                       | C                | Insulin  | 17673                            | IIS, A,<br>REb, Fd,<br>D       | 5'-JNS-12.6kb-IGF2--<br>3'-cen-11BHC-<br>10cM-JNS-2cM-<br>HIRAS1-3cM-THI<br>separate gene for<br>variant, 14741 | Diabetes mellitus, rare form (1);<br>MODY, 12585 (3)   | 6(Ms-1); 7(Ms-2) |
| 11p15.5  | IGF2                                      | C                | Insulin-like growth factor II, or somatomedin A  | 14747                            | REa, A, RE                     |   |  |                  |
| 11p15.4  | CALCA, CALCI                              | C                | Calcitonin/calcitonin gene related peptide, alpha<br>polypeptide   | 11413                            | REa, A,<br>REb, D,<br>Fd       |   |  | 7(Calc)          |
| 11p15  | PTII                                      | C                | Parathyroid hormone  | 16845                            | REa, REb,<br>A, Fd             |   | ?hypoparathyroidism, familial (1)  | 7(Pth)           |
| 11p14.2-p12                                      | CALCB, CALC2                              | C                | Calcitonin gene related peptide beta   | 11416                            | REb, D                         |   |  |                  |
| 11p13  | ESIB                                      | C                | Follicle stimulating hormone, beta polypeptide   | 13653                            | D, REa                         | disal to AN2  | ?Male infertility, familial (1)  | 2(Ushb)          |
| 11q13-qter<br>11q22                              | MEN1<br>PGR                               | C<br>C           | Multiple endocrine neoplasia 1<br>Progesterone receptor  | 13110<br>26408                   | Fd, D<br>REa, A,<br>REb        | linked to PYGM<br>11q13 = earlier<br>regionalization<br>pseudogene on 20  | Multiple endocrine neoplasia I (1)   |                  |
| Chr.11<br>12p12.1-p11.2<br>12q22-q24.1<br>Chr.14 | ADX<br>PTIILH<br>IGFI<br>CIIGA            | P<br>P<br>P<br>P | Adrenodoxin<br>Parathyroid hormone-like hormone<br>Insulin-like growth factor I, or somatomedin C<br>Chromogranin A (parathyroid secretory protein<br>1) | 10326<br>16847<br>14744<br>11891 | REa<br>REa, A<br>REa, A<br>REa |   |  |                  |
| 15q21.1  | CYP19, ARO                                | C                | Cytochrome P450 aromatization of androgen<br>(aromatase)   | 10791                            | REa, A                         |   | ?Glycomastasia, familial, due to<br>increased aromatase activity (1)   |                  |
| 15q25-q26<br>Chr.15                              | IGFIR<br>CYP11A,<br>P450SCC,<br>P450C11A1 | P<br>P           | Insulin-like growth factor-1 receptor<br>P450 side chain cleavage enzyme (20,22<br>desmolase)  | 14737<br>20171                   | REa, A<br>REa                  | ?relation to FES  | Lipoid adrenal hyperplasia,<br>congenital (1)  |                  |
| Chr.16<br>17cen-q25<br>17q22-q24                 | GRLL<br>ADXR<br>GHC                       | P<br>P<br>C      | Glucocorticoid receptor, lymphocyte-like<br>Adrenodoxin reductase<br>GROWTH HORMONE/PLACENTAL<br>LACTOGEN GENE CLUSTER                                   | 13806<br>10327<br>13925          | REb<br>REa<br>REa, A, C        |   |  |                  |



Table 2. continued

| Location      | Symbol            | Status | Title  | MIM #     | Method         | Comments   | Disorder   | Mouse   |
|---------------|-------------------|--------|--|-----------|----------------|--|--|---------|
| 17q22-q24     | GHI, GIN          | C      | Growth hormone, normal                           | 13925     | REa, A         | 5'-GHI-CSHPI-CSH1-GH2-CSH2-3'                    | Isolated growth hormone deficiency, IIlg type with absent GH and Kowarski type with bioinactive GH (3) |         |
| 17q22-q24     | CSHPI, CSL        | C      | Chorionic somatomammotropin pseudogene           | see 15020 | REa, A         |  | [Placental lactogen deficiency] (1)  |         |
| 17q22-q24     | CSA, PL, CSH1     | C      | Chorionic somatomammotropin A                    | 15020     | REa, A         |  | Amlyoid neuropathy, familial, several allelic types (3);   |         |
| 18q11.2-q12.1 | PALB, TTR, TBPA   | C      | Thyroxine-binding prealbumin (transthyretin)     | 17630     | REa, A         |  | [Dystranshyretinemic hyperthyroxinemia](1)   |         |
| 18q21         | GRP               | C      | Gastrin releasing peptide                        | 13726     | REa, A         | mammalian equivalent of bombesin                 | Persistent Mullerian duct syndrome   |         |
| 19p13.3-p13.2 | AMH, MIF          | P      | Anit-Mullerian hormone                           | 26155     | REa, A         |  | (1)  |         |
| 19p13.3-p13.2 | INSR              | C      | Insulin receptor                                 | 14767     | REa, A, REb    | 1 gene for alpha and beta subunits               | ?Leptocanthism (1); ?Acanthosis nigricans, insulin-resistant (1); ?Kabsen-Mendenhall syndrome (2)      | 8(Inst) |
| 19q13.32      | CGB               | C      | CHORIONIC GONADOTROPIN, BETA CHAIN               | 11886     | REa, H, A      | at least 5 genes                                 |  |         |
| 19q13.32      | LHB               | C      | Luteinizing hormone, beta chain                  | 15278     | RE             | beta chains of FSH, TSH on 11p, 1p, respectively | ?Male pseudohermaphroditism due to defective LH (1)  | 7(Lhb)  |
| 20pter-p12    | SCG1, CHGB        | P      | Chromogranin B (secretogranin B)                 | 11892     | REa, A         |  | ?Diabetes insipidus, neurohypophyseal, 12570 (1)   |         |
| 20pter-p12    | PDYN              | P      | Prodynorphin                                     | 13134     | REa, A         |  | ?Isolated growth hormone deficiency due to defect in GHRF (1)  |         |
| Chr20         | ARVP, VP          | P      | Arginine vasopressin-neurexysin II               | 19234     | REa, RE        |  | Albright hereditary osteodystrophy (1)   | 2(Gs-a) |
| Chr20         | GHRF              | C      | Growth hormone releasing factor; somatotropin    | 13919     | REa, REb       |  |  |         |
| Chr20         | GNAS1, GNAS, GPSA | P      | G-protein, stimulatory, alpha subunit (Gs-alpha) | 13932     | REa, H         |  |  |         |
| Chr20         | OT                | P      | Oxytocin-neurexysin I                            | 16705     | RE             | separated from VP by 12kb                        |  |         |
| 22q11-q13     | TSUR              | P      | Thyroid stimulating hormone receptor             | 18846     | REa            |  | ?Hypothyroidism, nongonitrous (1)  |         |
| Xp22-p21      | GDX1, TDFX        | P      | Gonadal dysgenesis, XY female type               | 30610     | F, Ch          |  | Gonadal dysgenesis, XY female type (2)   |         |
| Xp21.3-p21.2  | AIC, AIX          | C      | Primary adrenal hypoplasia                       | 30020     | D, Fd          | distal to GK                                     | Adrenal hypoplasia, primary (2)  |         |
| Xcen-q13      | AR, DHTR, TFM     | C      | Testicular feminization (androgen receptor)      | 31370     | S, Fd, REa, ?A |  | Testicular feminization (1); Reifenstein syndrome (1); Infertile male syndrome (1)                     | X(Tfm)  |
| Xq21-q22      | TBG               | P      | Thyroxine-binding globulin                       | 31420     | REa, A         |  | [Euthyroidal hyper- and hypothyroxinemia] (1)  |         |
| Xq28          | ALD               | C      | Adrenoleukodystrophy                             | 30010     | F, Fd, D       | cone pigment gene deleted in some ALD males      | Adrenoleukodystrophy (2)   |         |
| Xq28          | DIR, DII          | C      | Nephrogenic diabetes insipidus                   | 30480     | Fd             |  | Diabetes insipidus, nephrogenic (2)  |         |

**Table 3.** Genes for growth factors and growth factor receptors. (For definition of "status" and "MIM #," see Table 2.)

| <i>Location</i> | <i>Symbol</i>    | <i>Status</i> | <i>Title</i>  | <i>MIM#</i> |
|-----------------|------------------|---------------|---|-------------|
| 1p22            | NGFB             | C             | Nerve growth factor, beta   | 16203       |
| 2p13            | TGFA             | C             | Transforming (or tumor) growth factor, alpha type   | 19017       |
| 4q25-q27        | EGF              | C             | Epidermal growth factor   | 13153       |
| 4q26-q27        | IL2, TCGF        | C             | T-cell growth factor (interleukin-2)  | 14768       |
| 5q23-q32        | CSF2, GMCSF      | C             | Granulocyte-macrophage colony-stimulating factor  | 13896       |
| 5q31-q32        | PDGFR            | P             | Platelet-derived growth factor receptor   | 17341       |
| 5q31.3-q33.2    | ECGF             | C             | Endothelial cell growth factor  | 13122       |
| 5q33.1          | CSF1, MCSF       | P             | Macrophage colony-stimulating factor  | 12042       |
| 5q33.2-q33.3    | CSF1R, FMS       | C             | Oncogene FMS (McDonough feline sarcoma)   | 16477       |
| 6p23-q12        | INSL             | P             | Insulin-like DNA sequence   | 14749       |
| 6q25-q27        | IGF2R, MPRI      | P             | Insulin-like growth factor-2 receptor<br>(mannose-6-phosphate receptor, cation-independent) | 14728       |
| 7p13-p11        | EGFR             | C             | Epidermal growth factor receptor  | 13155       |
| 7p21            | IFNB2, IL6, BSF2 | C             | Interferon, beta-2 (hepatocyte-stimulating factor; interleukin-6)                           | 14762       |
| 7p22-q21        | PDGFA            | C             | Platelet-derived growth factor, A chain   | 17343       |
| 10p15-p14       | IL2R, TAK        | C             | Interleukin-2 receptor; T-cell growth factor receptor                                       | 14773       |
| 12q22-q24.1     | IGF1             | C             | Insulin-like growth factor 1, or somatomedin C  | 14744       |
| 15q25-q26       | IGF1R            | P             | Insulin-like growth factor 1 receptor   | 14737       |
| 17q21           | CSF3, GCSF       | C             | Granulocyte colony-stimulating factor-3   | 13897       |
| 17q21-q22       | NGFR             | C             | Nerve growth factor receptor  | 16201       |
| 19q13.1-q13.3   | TGFB             | P             | Transforming (or tumor) growth factor, beta form  | 19018       |
| 22q12.3-q13.1   | SIS, PDGFB       | C             | Oncogene SIS (platelet-derived growth factor, B chain)                                      | 19004       |
| Chr.4           | FGFB             | P             | Fibroblast growth factor, basic   | 13492       |
| Chr.5           | GFGA             | P             | Fibroblast growth factor, acidic  | 13491       |
| Chr.7           | IBP1             | P             | Insulin-like growth factor, low molecular weight  | 14673       |
| Chr.?           | BCGF             |               | B-cell growth factor  | 10954       |
| Chr.?           | CSF2R            |               | Granulocyte-macrophage colony-stimulating factor receptor                                   | 13898       |
| Chr.?           | FGF5             |               | Oncogene fibroblast growth factor-5   | 16519       |
| Chr.?           | NGFA             |               | Nerve growth factor, alpha polypeptide  | 16202       |
| Chr.?           | NGFG             |               | Nerve growth factor, gamma polypeptide  | 16204       |

Pheochromocytoma occurs in neurofibromatosis, which maps to proximal 17q, and in von Hippel-Lindau syndrome, which maps to 3p. These disorders all were mapped by linkage to RFLP markers.

Primary adrenal hypoplasia is encoded by a mutation located on Xp21 and another adrenal disorder, adrenoleukodystrophy, by a mutation on the Xq28 band. Nephrogenic diabetes also is determined by a mutation on the Xq28 band. Other interesting entities in this category include X-linked hypoparathyroidism, encoded at Xq26-q27; DiGeorge syndrome with hypoparathyroidism, at the proximal part of 22q; and the Beckwith-Wiedemann syndrome, in which cellular overgrowth includes nesidioblastosis of the pancreas with hypoglycemia, at the tip of chromosome 11p.

### The Applied Anatomy of the Human Genome

Mapping information has been particularly useful in the case of disorders for which the basic biochemical defect is not known. Great interest has been generated by the announcements of chromosomal mapping of the genes for Huntington's disease, cystic fibrosis, adenomatous polyposis of the colon, polycystic kidney disease, von Recklinghausen neurofibromatosis, Alzheimer's disease, Duchenne muscular dystrophy, and many others. Prior to mapping, no diagnostic means or rational therapy could be devised for any of these conditions. Once a marker closely situated to the locus of a mutant gene is known, however, genetic diagnosis can be done by the linkage principle, which involves prenatal and preclinical sampling and carrier de-

tection within a given family.

Furthermore, with neighboring or, better still, flanking markers, one can hope to walk in on the segment of DNA that contains the mutant gene and thereby identify both the gene and the precise nature of the change. This is so-called reverse genetics. Knowing the nature of the mutated gene opens the possibility of reconstructing the pathogenetic steps between gene and phenotype and devising therapeutic measures (short of gene replacement or repair) for ameliorating the effects of the disorder.

Defining the intragenic lesion is to chromosome mapping what microscopic anatomy is to gross anatomy. With every Mendelian disorder there must be a lesion in the DNA, and, increasingly, we will have the capability of going directly to the DNA—obtained from

circulating leukocytes, for example — to determine whether the lesion characteristic of a specific disorder is present. Indeed, this procedure might be called a biopsy of the human genome. John Phillips and colleagues<sup>5</sup> provided an early illustration of this approach when they demonstrated deletion of the GH gene on chromosome 17 in cases of isolated GH deficiency of the Illig type (type 1A). In other cases of isolated GH deficiency, they could exclude mutation in the structural gene by the linkage principle: affected sibs had inherited different chromosomes (17) from the parents, as indicated by RFLP markers, and no rearrangement or other struc-

tural abnormality of the GH gene could be identified by Southern blot analysis.

## Conclusions

Gene mapping is a technique that has expanded diagnostic and therapeutic potential immensely in the past decade. If adequate resources are made available, the human genome will be rapidly identified in its entirety. The implications are of great pertinence not only to geneticists but also to endocrinologists, nutritionists, and pediatricians. The reader is encouraged to follow closely this rapidly developing field, from which future genetics and endocrinology will evolve.

## To the Editor:

The article by A.D. Rogol, "Anabolic Steroid Hormones for Athletes: Efficacy or Fantasy?" (*Growth, Genetics, and Hormones*, December 1988; Vol. 4, No. 4), emphasizes the toxicity of these substances while failing to credit the known facts about their pronounced anabolic properties. It was a half century ago that Charles Kochakian and John R. Murlin first demonstrated the nitrogen-retaining properties of anabolic steroids<sup>1</sup>; later work showed increased muscle mass in experimental animals. Dr. Rogol correctly states that testosterone is principally responsible for the sex difference in muscle mass and strength that evolves during puberty.

What is generally not appreciated is the dose-dependent effect of anabolic steroids.<sup>2,3</sup> The small doses (total < 500 mg) used by some investigators produce very small amounts of nitrogen retention and increments in lean weight, so one would not expect significant increases in muscle strength. At total doses greater than 2,000 mg, there is a progressive increase in lean weight, as estimated by potassium 40 counting and total body nitrogen, and a fall in body fat. Under these circumstances the effect on lean body mass is far greater than has been reported from physical exercise and/or train-

ing alone. Moreover, Alén et al<sup>4</sup> have shown that large doses of anabolic steroids do indeed augment muscle strength and individual muscle fiber area, and to a much larger degree than does training alone. This is why athletes take steroids.<sup>5</sup>

Dr. Rogol supplies no evidence in support of his claims that anabolic steroids cause edema and that weight is quickly lost when they are discontinued. In our experience (R.C. Griggs, unpublished data) with several adult volunteers given anabolic steroids, it took 1 month or more for body weight and lean body mass to return to baseline after discontinuation of the drug.

Whatever our feelings about the use of anabolic steroids by athletes, we must admit that steroids truly are *anabolic*. We cannot dis-

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suaude people from taking them by downplaying the effects that are only too evident to the athletes themselves.

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5. Wade N. *Science* 1972;176: 1399-1403.

## In Future Issues

**Inflammatory Bowel Disease and Growth Retardation**  
by Richard Grand, M.D.

**Growth Hormone and IGF-1: Independent and Dependent Actions**  
by Olle Isaakson, M.D.

**Growth in Late Adolescence**  
by Alex Roche, M.D.

**Testicular Differentiating Factor: The Gene and its Clinical Importance**  
by Barbara Mc Gillivray, M.D.

**Mechanisms Responsible for Normal Bone Growth**  
by William Horton, M.D.

**Paracrine Aspects of Bone Metabolism**  
by David Baylink, M.D.

## Dr. Rogol's Reply

I thank Dr. Forbes for his letter regarding my article in *Growth, Genetics, and Hormones*. Dr. Forbes correctly points to the strong dose dependency (above a threshold level) of increase in lean body mass in most controlled studies. He refers to the work of Alén and co-workers, who have shown that "large" doses of androgenic steroid hormones augment muscle size as determined by morphometric analysis. However, there are many other studies that are equivocal or poorly executed. Although I agree that large doses of androgenic steroid hormones most likely augment lean body mass, there is great controversy over the magnitude of the effect and its precise mechanism.

My comment concerning the

possibility that edema is partially responsible for weight gain following the use of androgenic steroid hormones is indeed anecdotal, but marked weight loss within 24 hours of a weight-lifting competition (enabling the lifter to compete in a lower body-weight class) probably can be ascribed mainly to fluid loss. Dr. Forbes, in his own work<sup>1</sup> concerning the effect of testosterone on muscle mass, states this possibility: "The increase in body weight [following testosterone enanthate, 3 mg/kg intramuscularly each week for 12 weeks] could reflect a gain in water, but the increase in creatinine excretion, serum creatinine, and total body potassium suggests an increase in muscle mass [italics added]."

The controversy over the anabolic effects of androgenic ste-

roid hormones persists. It is likely, in my opinion, that large doses are in fact anabolic and increase muscle strength and athletic performance in some sports. It has not, however, been proved beyond a reasonable doubt that this is so. Athletes, being supreme pragmatists, will use steroids if they perceive that these compounds are helpful, even if they do not know the mechanism of action—which may be anticatabolic just as easily as anabolic.

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## Reference

1. Griggs RC et al. *J Appl Physiol* 1989;66:498-503.

## To the Editor:

I was delighted to read the much-needed review article entitled "Lipodystrophy" by William L. Clarke (*Growth, Genetics, and Hormones*, September 1988; Vol. 4, No. 3). I would like to add our experience with an additional, albeit rare, form of lipodystrophy to those reviewed by Dr. Clarke. An autosomal dominantly inherited form of partial lipodystrophy that spares the head and neck was first described by Dunnigan<sup>1</sup> and again by Kobberling.<sup>2</sup> In the literature, this entity hence has been referred to as Kobberling-Dunnigan syndrome or, alternatively, face-sparing lipodystrophy. To date, six families with the syndrome have been reported in the literature. We have encountered this syndrome in two additional kindreds, one of which encompasses three generations and includes 30 affected individuals. Like those with the other forms of lipodystrophy that were reviewed, the patients we have observed have insulin-resistant diabetes, acanthosis nigricans, elevated triglyceride levels, and hepatomegaly. Although Kobberling-

Dunnigan syndrome is rare, it may be distinguished from the sporadic, acquired type of lipodystrophy by its dominant inheritance. Whenever a face-sparing distribution of lipodystrophy is observed, a detailed family history should be obtained to rule out this genetic form.

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## Dr. Clarke's Reply

We thank Dr. McKeon for her important letter. We would agree that the Kobberling-Dunnigan syndrome probably is another form of inherited lipodystrophy. The paucity of case reports and information on this rare disorder led us to exclude it from our review. It is important that physicians accurately report their experiences with the lipodystrophic syndromes in the literature, so that a better understanding of these syndromes may be attained.

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## Acromegaly in an Infant

In discussing this case of a 21-month-old girl with excessive levels of growth hormone (GH; 135 ng/mL), prolactin (Prl; 370 ng/mL), and insulin-like growth factor-1 (IGF-1; 1,540 ng/mL), whose height was 97.6 cm (+4.4 SD) and whose head circumference was 55 cm (+5.5 SD), the authors briefly reviewed 22 cases of acromegaly reported in childhood. The majority had rapid linear growth, coarse facial features, and enlarged hands and feet; these are symptoms comparable to the findings that are discussed in the article. Interestingly, the 21-month-old girl had rapid head growth that preceded the significant rapid body growth. The authors postulate that the macrocephaly occurred because of rapid brain growth.

After a macroadenoma was removed from the suprasellar area, the GH and IGF-1 levels fell into the low normal range for preadolescent children. With pharmacologic testing, GH concentrations did not increase beyond 4 ng/mL, and Prl levels remained significantly elevated. GH-producing cells, but no Prl-producing cells, were observed under the microscope, utiliz-

ing immunologic techniques. The integrated GH concentration remained relatively stable overnight ( $\sim 2$  ng/mL), and peak GH concentrations did not exceed 3 ng/mL. The authors profess perplexity because this patient continued to grow at 6 cm/y over the next 2 years. Three possible explanations are offered: (1) hyperinsulinemia, which they subsequently exclude on the basis of insulin levels found during performance of a glucose tolerance test; (2) the continuing secretion of low levels of GH overnight, also discounted because the integrated GH value reportedly is lower than values obtained in control subjects; and (3) the elevated Prl levels, which contributed to the normal IGF-1 levels and the sustained growth. Several references are cited to support the final hypothesis.

In reviewing the literature, the authors note that hyperprolactinemia occurred in 12 of 15 pediatric cases. In seven cases where the tumor was examined by immunohistochemical techniques, both Prl and GH were present. In contrast, only GH was found in the case under discussion. The authors postulate, therefore, that dis-

ruption of the inhibitory centers and/or tracts accounted for the hyperprolactinemia in this patient.

Blumberg DL, Sklar CA, David R, et al. *Pediatrics* 1989;83: 998-1002.

**Editor's comment**—This article provides stimulating reading for pediatric endocrinologists. It updates the count of children reported with acromegaly and the immune histochemical findings in the pituitary of these children. It also raises again the question regarding the capability of Prl to increase IGF-1 levels. The postoperative Prl levels in this girl were 30 to 120 ng/mL. Previously, Clemmons et al (*J Clin Endocrinol Metab* 1981;52:731) reported Prl levels greater than 100 ng/mL associated with normal adult IGF-1 levels in 20 GH-deficient patients. Very possibly, we as clinicians do not pay adequate attention to the role that increased Prl levels may play in producing normal IGF-1 levels in GH-deficient patients. All "suspect" GH-deficient patients whose IGF-1 is not in the GH-deficient range should be screened for high Prl.

Robert M. Blizzard, M.D.

## Verification of the Fetal Valproate Syndrome Phenotype

Valproic acid (VPA) is a relatively new anticonvulsant that was approved for use in the United States in 1978. Its main indication is for the treatment of absence seizures, although it has been used, often in combination with other anticonvulsants, to treat a variety of other seizure disorders.

In 1984, DiLiberti et al described a consistent facial phenotype in seven children who were exposed to VPA in utero. The facial abnormalities included epicanthic folds, flat nasal bridge, small upturned nose, long upper lip, shallow philtrum, thin lower lip, and downturned mouth. The authors also

suggested an association of this phenotype with other anomalies, such as low birth weight, psychomotor delay, congenital heart defects, neural tube defects, hypospadias, strabismus, and nystagmus.

In the current study, 19 children who were exposed to VPA in utero were carefully examined. No consistent alterations of pre- or postnatal growth deficiency were found with exposure to VPA alone. Postnatal growth deficiency and microcephaly were present, however, in two thirds of the children exposed to VPA in combination with other anticonvulsants. Developmental delay or neurologic abnormality was found in 71% of those exposed to VPA alone and in 90% of those exposed to VPA and other anticonvulsants. Craniofac-

cial anomalies, which can be seen in children exposed to other anticonvulsants in utero, were also found in infants whose mothers received VPA. The anomalies included midface hypoplasia; short nose with a broad and/or flat bridge; epicanthic folds; minor abnormalities of the ear, philtrum, or lip; and micrognathia. Prominent metopic ridge and outer orbital ridge deficiency or bifrontal narrowing, and certain major anomalies such as tracheomalacia, talipes equinovarus, and lumbosacral meningomyelocele, seem to be peculiar to infants with VPA exposure.

Ardinger HH, Atkin JF, Blackston RD, et al. *Am J Med Genet* 1988; 29:171.

**Editor's comment**—The authors confirm the presence of a distinct fetal valproate syndrome that shares many features with the other syndromes secondary to prenatal exposure to anticonvulsants, ie, developmental delay, craniofacial abnormalities, congenital heart defects, urogenital anomalies, and limb

anomalies. In contrast to the fetal diphenylhydantoin, trimethadione, and phenobarbital/primidone syndromes, neither prenatal nor postnatal growth deficiency is present in the fetal VPA syndrome. Indeed, only those children who were exposed to VPA and another anticonvulsant in utero had growth deficiency. Thus, each of the

commonly used anticonvulsants appears to produce a distinct fetal malformation syndrome phenotype. It is most important, as the authors have done in this study, to isolate the specific effects of each of the anticonvulsants since so many women requiring treatment for seizures take more than one.

David L. Rimoin, M.D., Ph.D.

## Development of Human Palmar and Digital Flexion Creases

Normal flexion creases of the fingers and palm reflect normal movement and development of the upper limbs during embryonic and fetal life. A study by Stevens and colleagues of the flexion creases in the human fetus has established the time during fetal life when these creases first appear. A hand malformation or specific insult that occurs before the time of crease development may cause secondary alterations in crease patterns of the hand. The presence of creases may also be used to date the age of an abortion.

The authors measured the development of hand creases by examining the hands of 100 human fetuses that had been obtained by random selection after therapeutic abortion. At 7 weeks' gestation, the fingers are separated but the hand is smooth and glove like, without pads, creases, or distinguishing features between the dorsal and volar surfaces. At 8 weeks, the distal interphalangeal and metacarpophalangeal creases are faintly visible. Digital and interdigital pads begin to be seen, and the thumb begins to rotate to another plane. At 9 weeks, the proximal interphalangeal and thenar creases become visible, and the nail beds begin to form. At 10 weeks the digital creases are well formed. The interdigital pads begin to regress, and a depression is seen in the center of the digital pads. Thickenings appear in the palm, and the nails are well de-

## Transient Growth Deceleration in Normal Short Children: A Potential Source of Bias in Growth Studies

A child whose growth fluctuates from one 6-month period to the next is more likely to be diagnosed as growth hormone (GH) deficient at the end of the slow growth period than at the end of the rapid growth period. Under these circumstances there is a strong selection bias, and one would expect a regression toward the mean velocity during the 6 months after diagnosis, whether or not the child was treated. To test this hypothesis, the authors followed 21 short children who had 6-month growth velocities  $< 4$  cm/y but whose response to GH stimulation testing was  $> 5$  ng/mL and showed that they were not GH deficient. All but three of the children had increases in velocity  $> 0.5$  cm/y during the subsequent 6 months of observation. These increases were significant in both the prepubertal and pubertal groups: the mean growth rates increased from 3.4 to 5.1 cm/y and from 3.4 to 6.3 cm/y,

respectively. In growth studies in which children are used as their own controls, this effect must be taken into account.

Polychronakos C, Abu-Srair H, Guyda HJ. *Eur J Pediatr* 1988; 147:582.

**Editor's comment**—"Regression toward the mean" is an important aspect of any longitudinal study in which the initial value is not based on a random selection from the population. It stems from unreliability of measurement, as well as from seasonal and other fluctuations. In any growth study in which a treatment is given after a control period, the most likely velocity in the absence of treatment must be estimated. Bias is introduced, however, if the most likely velocity is estimated simply as a continuation of the velocity in the control period.

James M. Tanner, M.D., D.Sc.

fined. At 11 weeks, the distal palmar creases appear. At 12 weeks, the proximal palmar creases are seen and the digital pads are still present, but the interdigital pads are gone. By 13 weeks, all palmar and digital creases are well defined, digital pads are regressing, and the thumb is opposable. The digital pads disappear by 15 weeks.

This work establishes the normal pattern and development of digital and palmar creases.

Stevens CA, Carey JC, Shah M, et al. *J Pediatr* 1988; 113:128-132.

**Editor's comment**—Creases of the hand are examined in all children. With specific dating of the development of these creases, we can much more accurately time insults and abnormalities. This study makes a real contribution to defining normal development.

Judith G. Hall, M.D.

## Atlantoaxial Instability in Down Syndrome

At least 10% of individuals with Down syndrome have atlantoaxial instability, as measured by an atlantodens interval of  $>5.0$  mm. Because of the risk of spinal cord damage, Special Olympics, Inc., and the American Academy of Pediatrics have recommended evaluation, including cervical roentgenography, for all individuals with Down syndrome who wish to take part in sports.

Davidson recently reviewed the evidence that individuals with Down syndrome are at increased risk for unexpected spinal cord injury when taking part in sports. He found that in almost all published cases of spinal cord injury in Down syndrome patients there had been signs or symptoms of cervical subluxation for weeks or months prior to the injury. He points out in his review that the incidence and real

risk of cervical subluxation, the results of longitudinal studies in cases of Down syndrome with atlantodens intervals  $>5.0$  mm, and the exact measurements that should lead to concern at a particular age have not been defined. Thus, exclusion from sports may be inappropriate unless the individual has had a roentgenographic series or studies of cervical spine flexion and extension. He does agree that sports that lead to

## New Concepts of the Growth Spurt of Puberty

The authors emphasize that *final height attained is independent of the timing and intensity of the growth spurt*. Thus, those who enter puberty late have a relatively smaller growth velocity (GV) during the adolescent growth spurt, and they reach a final height identical to what it would have been had they entered puberty earlier and experienced a greater GV for a shorter period. A previous article by these investigators reported that short normal children and children with central precocious puberty (CPP) who had puberty arrested with a gonadotropin-releasing hormone agonist-analog (GnRHa) did not have an improved height prognosis.

The authors attribute the earlier onset of puberty in girls, compared with boys, to differences in luteinizing hormone (LH) pulsatility. Girls require lower doses of luteinizing hormone-releasing hormone (LHRH) to cause the release of LH than do boys, and LHRH agonist-analogs (LHRHa) block the release of LH more readily in girls than in boys. These observations may also help to explain why CPP is more common in girls than in boys and why the converse is true of constitutional growth delay.

The timing of the growth spurt is related to an increase in growth hormone (GH) production, particularly to the amplitude of the GH peaks, according to the authors. GV and GH secretion peaked when the testicular volume reached 12 mL in boys; in girls, this correlation was noted before any increase in serum estradiol or uterine size was detected by ultrasonography. The evidence suggests that changes in GH secretion are modulated by factors other than sex steroids, and the recent demonstration that inhibin can affect the GH response to growth hormone-releasing hormone (GHRH) (*J Endocrinol* 1988;116:301) may be relevant.

In discussing the effect of GnRH on growth in CPP, the authors state that GnRHa do not increase ultimate height. This phenomenon relates to the decreased GH production that occurs with the use of GnRHa. The researchers postulate that GH given concomitantly with GnRHa may play a beneficial role and that delay in the timing of puberty alone will probably not improve final height prognosis.

Stanhope R, Preece MA, Grant BD, et al. *Acta Paediatr Scand* 1988;347(suppl):30.

**Editor's comment**—*The studies reported and the concepts proposed are well worth reading in detail. The growth spurt of adolescence undoubtedly is attributable to increased GH production, as reported by Martha et al at the Society for Pediatric Research meetings in May 1989. Boys in stages 3 and 4 of sexual development have GH levels two to three times those found during stages 1 and 2 of puberty and after epiphyseal fusion occurs. There is a strong correlation among GV, GH production, and insulin-like growth factor-1 generation, as demonstrated in those studies. As for the lack of effectiveness of GnRHa in enhancing the ultimate height of girls with CPP, I disagree. Table 2 in the Boepple and Crowley article (Growth, Genetics, and Hormones March 1989; Vol. 5, No. 1) clearly indicates that the appropriate use of GnRHa prevents the loss of height that occurs in patients with untreated CPP. Among such children, those with a bone age greater than 13 years have an average predicted adult height of  $-3.7$  SD. Patients with treated CPP whose bone ages at the time of treatment are less than 10 years remain at essentially the same mean score ( $-1.1$  for bone age) after 3 years of GnRHa treatment.*

Robert M. Blizzard, M.D.

hyperextension or radical flexion of, or direct pressure on, the neck or spine (eg, tumbling or trampolining) may place the patient in jeopardy. It is possible that car accidents, rheumatoid disease, and general anesthesia also may increase the risk of spinal cord injury in individuals with Down syndrome. Interestingly, the number of injuries leading to symptoms of subluxation appears to be increasing in girls and women with

Down syndrome.

Davidson RG. *Pediatrics* 1988; 81:857-865.

Pueschel SM. *Pediatrics* 1988; 81:879-880.

Atlantoaxial instability in Down syndrome. Editorial. *Lancet* 1989; 1:24.

**Editor's comment**—What should we recommend to the families of

individuals with Down syndrome concerning sports participation? Clearly, better information is needed to define the risks and make appropriate recommendations for studies and follow-up. The crucial issue is, Are we going to do unnecessary testing and unnecessarily restrict activities in the name of safety?

Judith G. Hall, M.D.

## Identification of the Molecular Defect in a Family With Spondyloepiphyseal Dysplasia

The problem of gene defects is of intense interest to geneticists and molecular biologists. The spondyloepiphyseal dysplasias (SED) are a heterogeneous group of inherited disorders characterized by disproportionately short stature and pleiotropic involvement of the skeletal and ocular systems. Recent investigations suggest an association between some forms of SED and a defect in type II collagen. In this study coarse scanning by Southern blot hybridization of the *COL2A1* gene, which encodes type II collagen, identified an abnormal restriction pattern in the DNA of one of the affected members of a relatively large family with SED. Analysis of selected genomic fragments localized the molecular defect, all affected family members carried the same heterozygous single-exon deletion.

The proband was a 3.5-year-old girl, apparently normal at birth, who had a history of ear infections, slowed growth, and genu valgum. She was short and had lordosis, mild kyphosis, and rhizomelic shortening of the extremities. Epiphyseal centers were affected with no metaphyseal involvement. The father,

four paternal aunts, and two nieces had kyphoscoliosis, retinal detachment, myopia, genu valgum, cervical instability, and dwarfism. Analysis of the proband's DNA yielded a novel 3.3-kb *EcoRI* fragment that was not seen in the control samples. This segment of *COL2A1* contains exons 45 to 52, which code for the C-terminal propeptide and the last 123 amino acid residues of the triple-helical domain. Amplification of *COL2A1* exons 47 to 52 from genomic DNA of unaffected family members produced a single 3.2-kb fragment. Analogous amplifications of DNA from affected family members produced the normal 3.2-kb fragment and a deleted 2.8-kb fragment. This finding established the segregation of the deleted *COL2A1* allele with the abnormal SED phenotype. The deletion accounts for the elimination of the whole of exon 48, including 36 amino acids of the type II triple-helical domain. The authors conclude that the *COL2A1* deletion is responsible for this type of dwarfism.

Lee B, Vissing H, Ramirez F, et al. *Science* 1989;244:978.

**Editor's comment**—Chondrodysplasias are a highly heterogeneous group of disorders that includes endochondral ossifica-

tion and simple abnormal skeletal growth. These entities are believed to result from mutations affecting either the structural integrity of cartilage matrix components or the regulatory pathways of chondrogenesis. *COL2A1* has been linked to the Stickler syndrome by genetic analysis, and, as noted by the authors, biochemical analysis of small cartilage samples from chondrodysplastic individuals has recently suggested that some of these conditions, such as the spondyloepiphyseal dysplasias, Kniest dysplasia, and type II achondrogenesis-hypochondrogenesis, may be associated with type II collagen defects. The association seems to be confirmed for this particular family with SED.

Fibroblasts from patients with osteogenesis imperfecta have a type I collagen defect, and defects in type III collagen have been demonstrated in patients with Ehlers-Danlos syndrome. In these disorders, structural mutations in the type I and type III collagen subunits are believed to decrease the rate of helical assembly and expose greater regions of unassembled chains to overmodification. The location of a defect within the helical domain may affect directly the degree of collagen modification.

Robert M. Blizzard, M.D.



## MEETING CALENDAR

**November 12-15** The 40th Annual Meeting of The American Society of Human Genetics, Convention Center, Baltimore, Maryland. Contact: Jean Francese, American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

**January 10-13, 1990** 37th Postgraduate Course, American Diabetes Association. Hyatt Regency Grand Cypress, Orlando, Florida. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22314 (800-232-3472)

**February 4-8, 1990** 17th Annual Seminar in Pediatric Nephrology: Current Concepts in Diagnosis and Management. Diplomat Resort and Country Club, Hollywood, Florida. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101 (305-549-6726)

**February 6-9, 1990** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, California. Contact: David K. Stevenson, M.D., Department of Pediatrics, Room S222, Stanford University School of Medicine, Stanford, CA 94305 (415-723-5711)

**April 28-May 3, 1990** Spring Session, American Academy of Pediatrics. Seattle, Washington. Contact: Department of Education, American Academy of Pediatrics, P.O. Box 927, Elk Grove Village, IL 60007 (800-322-9016)

**May 7-11, 1990** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Hilton Hotel, Anaheim, California. Contact: Debbie Agnostelis, Executive Director, Society for Pediatric Research, 2650 Yale Boulevard S.E., Suite 104, Albuquerque, NM 87106 (505-764-9099)

**May 9, 1990** Diabetes Symposium, Lawson Wilkins Pediatric Endocrine Society. Hilton Hotel, Anaheim, California. Contact: Gilbert August, M.D., Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue N.W., Washington, DC 20010 (202-745-2121)

**May 11, 1990** Annual Scientific Session, Lawson Wilkins Pediatric Endocrine Society. Hilton Hotel, Anaheim, California. Contact: Gilbert August, M.D., Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue N.W., Washington, DC 20010 (202-745-2121)

**June 8-11, 1990** 30th Meeting of the Teratology Society. Empress Hotel and Convention Center, Vancouver, British Columbia, Canada. Contact: Alexandria Ventura, Administrative Assistant, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1841)

**June 20-23, 1990** 72nd Annual Meeting of The Endocrine Society. Atlanta Convention Center, Atlanta, Georgia. Contact: Scott Hunt, Executive Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**July 9-11, 1990** Annual March of Dimes Clinical Genetics Conference: Gastrointestinal Disorders. Westin Hotel, Detroit, Michigan. Contact: Orlando J. Miller, M.D., Wayne State University, 2316 Scott Hall, 540 East Canfield Avenue, Detroit, MI 48201 (313-577-5323)

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# GROWTH

## Genetics & Hormones

Vol. 5 No. 4

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## Testicular Differentiating Factor: Current Concepts

Barbara C. McGillivray, M.D.  
*Associate Professor*  
*University of British Columbia*  
*Co-director, Prenatal Diagnosis*  
*Program*  
*Grace Hospital*  
*Vancouver, British Columbia*  
*Canada*

Sex determination in humans is a complicated process and one that is still not fully understood. Recently, a region on the short arm of the Y chromosome was sequenced and was postulated to be responsible for determining the testis. This finding was made possible in part by analyzing DNA from both XX and XY individuals having sex reversal. Several questions remain regarding the function of the identified sequence; these involve the interaction between similar sequences on the X chromosome and the identity of the next gene or genes involved in the process of male sexual differentiation.

Generally, all individuals having a Y chromosome will develop a testis regardless of the number of X chromosomes, and individuals lacking a Y chromosome will develop an ovary (Table). However, a variety of clinical situations appear to contradict this general rule. XX males with no obvious Y chromosome develop a normal testis, as do XO males. XY females, often with no obvious chromosomal abnormality, develop ovaries, although these may be streaks. The true hermaphrodite with an XX chromosomal complement may

still develop a variety of gonadal structures, including a testis or an ovotestis.

### X-Y Interchange in XX Males

XX males appear to have the chromosomes of a normal female, with no obvious Y chromosomal material. The incidence of this condition is approximately 1 in 20,000 males. In 1966, Ferguson-Smith<sup>1</sup> suggested that XX males may be the result of an interchange between the X and the Y chromosomes, which would allow transfer of Y chromosomal material (presumably including the testis-determining region) to the X chromosome. This interchange would have to occur during recombination at meiosis, and so the transfer would be between the paternal X and Y. Page and de la Chapelle<sup>2</sup> demonstrated that most XX males had one maternally derived and one paternally derived X chromosome. Andersson et al.,<sup>3</sup> using the technique of in situ hybridization, were able to show that probes detecting Y chromosome short arm sequences clustered on the distal portion of one of the X chromosome short arms. The most terminal band of the X chromosome showed the greatest concentration of the probe, and, significantly, the autosomes were not labeled. Again, these findings suggested that Y DNA was transferred to the short arm of the X chromosome at male meiosis. If the Y chromosome normally contained a testis determining function, then the material translocated

to the X chromosome in the XX males should contain the testicular differentiating factor (TDF) gene or genes.

An X-Y interchange of this kind was thought possible because the X and Y chromosomes, at the distal short arm, share a region that regularly combines in male meiosis (the pseudoautosomal region). If recombination extends beyond the pseudoautosomal region, strictly Y-linked sequences could be transferred to the X chromosome. The mechanism might be similar to the well-described X-Y interchange in XXr mice. Several pseudoautosomal loci were mapped, and the TDF region was thought to be very close to the junction of the pseudoautosomal region on the Y chromosome. In fact, the majority of XX males were shown to have paternal pseudoautosomal loci and to have lost specific DNA sequences from the paternally derived distal X chromosome. However, not all XX males had such findings,<sup>4</sup> and Y chromosome mosaicism, or a mutation involving the X or an autosomal chromosome, was also thought possible.

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## Deletion-Mapping the Y Chromosome

In 1984, Magenis et al<sup>5</sup> demonstrated that a female infant presenting with features of Turner's syndrome had an XY karyotype and gonadoblastomas. High resolution chromosomal studies revealed a deletion of a portion of the Y short arm, with no evidence of mosaicism. This finding provided additional evidence that a gene or genes responsible for male determination was found on the distal half of the Y short arm, as deletion of this small area allowed sex reversal.


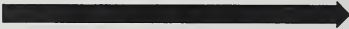
Using DNA from 27 individuals<sup>6</sup> (most of them either XX males or XY females with detectable deletions), a deletion map of the Y chromosome was constructed. Before this was possible, cloned Y chromosomal DNA sequences were isolated with the use of DNA libraries or with human-hamster hybrid techniques. The latter technique involved the introduction of a single human chromosome (in this case, the Y chromosome) into hamster chromosomes, thus allowing easier identification of sequences specific for the human Y chromosome.

In this study,<sup>6</sup> the presence or absence of 23 restriction fragments was analyzed. The size and placement of the sequences were ordered to produce a deletion map of the Y chromosome, spanning seven identified intervals. Intervals 1 to 3 were on the short arm of the Y chromosome, interval 4 represented the centromere, and intervals 5 to 7 comprised the long arm of the Y chromosome (Figure 1). The results from the XX males having Y material placed the testis-determining function in interval 1. Interestingly, infertile but phenotypically XY males had deletions on the long arm of the Y chromosome.

## Closing In on the Gene

Further work on the XX males and the XY females with deletions allowed interval 1 to be broken down into subregions. Page et al<sup>7</sup> used an XX male with the smallest identified segment of Y DNA and a fe-

**Table.** Presence of Y chromosome generally determines testicular differentiation

|                                  |   |        |
|----------------------------------|---|--------|
| XY                               |   |        |
| XXY                              |  | TESTIS |
| XXY                              |   |        |
| XX                               |   |        |
| XO                               |  | OVARY  |
| XXX, etc.                        |   |        |
| Exceptions: XX males, XY females |   |        |

male with a tY:22 autosomal translocation (and a demonstrated lack of regions 1A2 and 1B) as a starting point to clone the putative testis-determining gene (Figure 2). The gene was cloned by conventional walking methods using the probe closest to the breakpoint of the deleted Y chromosome. In one direction, the investigators hit segments found in common with the XX male; in the other direction, segments in common with the other end of the translocation breakpoint were hit. The deletion thus identified was thought to consist of approximately 120 kilobases, while the entire 1A2 interval was thought to be 140 kilobases, or 1/500 of the Y chromosome.

As the sequenced area was believed likely to contain the testis-determining function, evolutionary conservation was then investigated. DNA from a variety of heterogametic animals was used to make a "Noah's Ark blot." A male-specific band was identified in all animals but chickens.

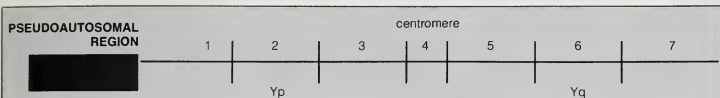
The identified sequence appeared to represent a long open reading frame and to encode a protein most like transcription fac-

tor IIIA from the frog. This transcription factor was known to be a DNA binding protein. The Y encoded protein had a repetitive array of structural elements with zinc fingers. The "fingers" formed a tetrahedral shape around the zinc and were thought possibly to project into the grooves of DNA. Page postulated that the protein had a transcriptional regulatory function and that it was neither a hormone nor a cell surface protein.

Importantly, a similar sequence was found on the short arm of the X chromosome in an area normally inactivated. This raised the question of the function of the X-linked testis-determining region (TDX). Four models were proposed. First, the TDX region was unrelated to the Y-linked testis-determining region (TDY) or to a distinct function. Second, the two regions could cooperate in forming a heterodimer. Third, the X and Y functions could be competitive, perhaps for a common binding site. The fourth and most appealing theory has TDX and TDY being interchangeable, with dosage being of crucial importance. The TDX from one of the two X

## Erratum

In the article on occult celiac disease by Asaria Ashkenazi, M.D. (*Growth, Genetics, and Hormones* Volume 5, Number 2, pp 1 to 4), an editing error altered the meaning of one of the author's sentences. The sentence should read: "In recent studies, we demonstrated the toxic effect of purified gliadin-derived peptides on intestinal mucosa cultures of CD patients ingesting a normal gluten-containing diet."



**Figure 1** Y chromosome deletion-mapping intervals.

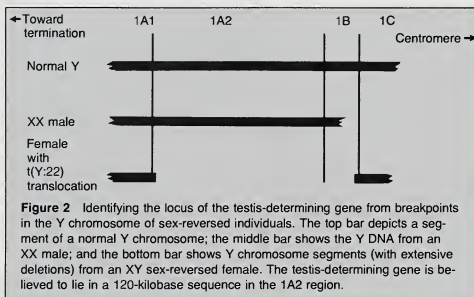
chromosomes of a normal female would normally show inactivation, while the TDX and TDY in a normal male would not show inactivation. In the XX male, the TDY from the X-Y interchange would escape inactivation and allow male development.

### New Findings, New Questions

These results were indeed exciting, and they helped explain the clinical findings with sex-reversed individuals. Subsequent work,<sup>8</sup> however, has raised still more questions while providing a few answers. As the Y-encoded protein was not yet proven to be the primary sex determinant signal, its terminology was changed from TDY to zinc finger Y (ZFY). The X-linked gene was correspondingly zinc finger X (ZFX). Interval 1A2 of the Y chromosome was found to contain four restriction fragments of highly conserved sequences, one of which encoded the zinc finger protein. Preliminary evidence suggested that at least two of the other three segments might also be exons of the same gene. These four conserved segments of 1A2 also hybridized to similar segments on the X chromosome. The X-chromosomal counterparts were cloned and compared to the segments from the Y chromosome and were found to be quite similar. Both had a carboxyl-terminal exon encoding the tandem array of zinc fingers with two cysteines and two histidines.

Surprisingly, the transcription of ZFX did not appear to be subject to X inactivation. The level of ZFX's transcription increased with the number of X chromosomes. Other studies demonstrated that the ZFX gene was transcribed whether it was on the "active" or "inactive" X chromosome.

The similar sequential structures



**Figure 2** Identifying the locus of the testis-determining gene from breakpoints in the Y chromosome of sex-reversed individuals. The top bar depicts a segment of a normal Y chromosome; the middle bar shows the Y DNA from an XX male; and the bottom bar shows Y chromosome segments (with extensive deletions) from an XY sex-reversed female. The testis-determining gene is believed to lie in a 120-kilobase sequence in the 1A2 region.

of the ZFX and ZFY genes suggest derivation from a common ancestral gene. A possible explanation is that the ancestral gene was initially on a pair of autosomes and may be highly conserved because of evolutionary selective pressure.

The sequence similarity of the zinc finger proteins of ZFX and ZFY also suggests a common nucleic acid binding sequence. While the two gene products could regulate transcription of the same downstream gene, the minor variations between the two genes might also significantly affect binding specificity or affinity.

Another question involves the overall function of ZFY and ZFX. Cultured cells from a variety of human tissues show transcription of the genes and possibly suggest other functions in addition to sex differentiation.

A recent and exciting development has been the demonstration of a Y-linked and closely related X-linked gene transcribing a regulatory protein. The interaction of the two genes is not yet clear, but these could be differentially expressed during embryonic development because of as yet unde-

scribed regulatory mechanisms. Further studies of the expression of these genes in embryos, or analysis of transgenic mice, must be performed to document the sex-determining role of ZFY.

Finally, the role of ZFY in conditions such as Y-negative XX males or XX true hermaphrodites is also unclear.

The importance of clinical observations to the promising developments described here cannot be understated. Continuing collaboration between clinician and basic researcher have over time led to delineation of the crucial steps to testis formation.

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# The Final Phase of Growth in Stature

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## Editor's Note

*The dilemma of prognosticating how much growth may yet occur after certain milestones of growth are met often frustrates the clinician. A second dilemma of when to stop growth hormone, anavar, estrogen, or other treatment for growth follows from the first. In this article, Dr. Roche addresses these questions and provides some answers. For example, growth in stature ceases in males and females at median ages of  $21.2 \pm 2.5$  years and  $17.3 \pm 2.5$  years, respectively. Children often grow an extra centimeter or slightly more after their growth velocity has decreased to  $<1.0$  cm during the previous year; the later the occurrence of peak height velocity, the later menarche occurs, and subsequent growth in stature after late menarche is less than that in girls with earlier menarche. The reader will find that the abstract entitled, "New Concepts of the Growth Spurt of Puberty," which appeared in the previous issue of Growth, Genetics, and Hormones (Volume 5, Number 3) will supplement the reading of this article.*

Robert M. Blizzard, M.D.

Knowledge of the "normal" range of growth after particular ages and pubescent events may assist the clinical management of older children with unusual statures. Serial studies are needed to determine the distributions of these growth increments. Partly because the protocols for almost all growth studies cease at 18 years, and partly because marked attrition of the sample population after 17 years is common, there are few reported data concerning the final phase of growth in stature for nor-

mal children and almost none for children with pathologic conditions.

The following account summarizes some of the findings from the Fels Longitudinal Study in 1972<sup>1</sup> and presents further analyses of the larger database that is now available from this study. In 1972, data were analyzed for 192 participants aged 28 years or more at their most recent examinations. Data are now available for 520 participants, with serial stature measurements extending to at least 20 years. Many of the Fels participants are siblings or offspring of older participants, and the inclusion of related individuals theoretically could introduce bias. However, the differences between sex-specific analyses in which all the available data were included and those in which only one participant per family was included, are inconsequential. The participants involved in the present analyses were born between 1929 and 1968, but there are no secular trends in these data. Although there was no strict sampling design, distribution statistics for Fels statures are similar to those from national U.S. surveys.

At the time of enrollment, the families of the participants lived in southwestern Ohio. These families were generally of middle socioeconomic status, with distributions of education and occupation that matched those from national samples, except that the lowest group was slightly underrepresented among those born after 1939. All the children were white and healthy.

Stature was measured with a wooden stadiometer until 1971, when a Holtain stadiometer was introduced. Both instruments were calibrated monthly, and the procedures used to measure stature match current recommendations.<sup>2</sup> All measurements were made by two anthropometrists working independently who repeated their measurements when they differed by more than 1.0 cm. The mean

inter-observer difference for stature measurements from 12 to 18 years was 0.16 cm (SD 0.15 cm).

## Cessation of Growth in Stature

It is difficult to determine when growth in stature ceases. In earlier analyses,<sup>1</sup> a pair of mathematical functions was fitted to the serial data for individuals; the second function was a horizontal line to represent the lack of change during early adulthood. The age at which the fit was maximal for the two functions combined was accepted as the age at which growth in stature ceased. The median ages were 21.2 years for males and 17.3 years for females, with ranges of about 5 years from the 10th to the 90th percentiles.<sup>1</sup> This sex difference in the timing of cessation is greater than that for peak height velocity (PHV) and presumably results from sex-associated differences in growth patterns during the final phase of growth.

Some authors<sup>3</sup> consider adult stature to be reached when an annual growth increment is less than 1.0 cm, but the median increases in stature after such an increment exceed 1 cm in each sex.<sup>1</sup> The data in Table 1 and other findings<sup>4</sup> show that the total median for growth in stature, after an annual increment  $<1.0$  cm, exceeds 1 cm for most age groups. These data demonstrate also that the total growth after an annual increment  $<1.0$  cm was negatively associated with the age at which the low increment occurred. The total increment after four successive 6-month increments, each less than 0.5 cm, is only a few millimeters.<sup>1</sup>

Other investigators contend that adult stature has been reached when maturation of the hand-wrist is complete. After this occurs, however, total increments of 1.6 cm for males and 2.3 cm for females have been reported.<sup>5</sup> In addition, after maturation is complete in both the tibia and femur, the median increases in stature are

1.5 cm for males and 1.0 cm for females.<sup>1</sup> The final phase of growth in stature probably reflects elongation in the vertebral column.

### Increments after Peak Height Velocity (PHV) and Menarche

The distributions of the total increments after PHV are slightly larger for boys than for girls, with large differences between the 10th and 90th percentile levels in each sex (Table 2). These sex-associated differences are particularly large for the first year after PHV. The annual increments decrease rapidly after PHV, with almost as much growth in the first year as in the second to fifth years combined. The data for annual increments after PHV do not add up to the total increments, because the groups for the annual increments differ across intervals. It is noteworthy that at least 10% of the boys and of the girls had increments greater than 1.0 cm even from 4 to 5 years after PHV.

The median total stature increment after menarche was 7.4 cm with a 10th to 90th percentile range from 4.3 to 10.6 cm. These findings are in agreement with reports of growth from menarche to the end of school attendance or to 18.25 years.<sup>6,7</sup> The median increments during the first year after menarche exceeded the sum of the median annual increments over the next 4 years.

### Relationships to the Timing of PHV and Menarche

The later the occurrence of PHV and menarche, the lesser the

**Table 1.** Total growth in stature (cm) after an annual increment of less than 1.0 cm

| Age (years) at increment<br><1.0 cm/year | Percentiles |     |     |       |     |     |
|--|-------------|-----|-----|-------|-----|-----|
|  | Boys        |     |     | Girls |     |     |
|  | 10          | 50  | 90  | 10    | 50  | 90  |
| 13-14                                    | —           | —   | —   | 0.7   | 1.8 | 3.3 |
| 14-15                                    | —           | —   | —   | 0.4   | 1.4 | 2.6 |
| 15-16                                    | 0.4         | 1.2 | 1.9 | 0.2   | 1.3 | 2.2 |
| 16-17                                    | 0.4         | 1.1 | 2.4 | 0     | 1.1 | 2.4 |
| 17-18                                    | -0.2        | 0.4 | 1.6 | -0.4  | 0.0 | 1.6 |

growth in stature after these events<sup>1,4,8,9</sup> (Figure). After PHV, there was considerably more growth in boys than in girls for groups matched in age at PHV, but the ranges from the 5th to the 95th percentiles and the slopes of the regressions were almost identical in each sex. The increments in boys from 14 to 17 years are also negatively related to stature at 14 years.<sup>10</sup>

### Clinical Applications

The preceding data may serve as guides to the potential for growth in stature, depending on the patient's maturational status. This information therefore may assist decisions about the initiation or cessation of therapy. Such decisions will be influenced as well by the presence and nature of any pathological condition and by the attitude of the patient and the patient's family.

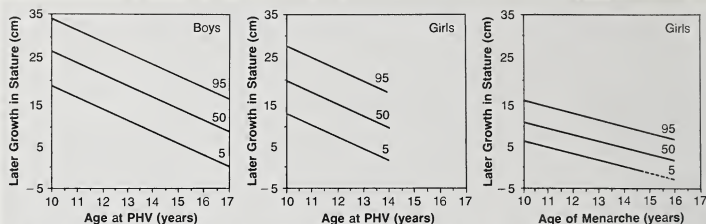
More complex procedures utilize regression equations to pre-

dict adult stature from childhood variables.<sup>3,11,12</sup> These require assessments of skeletal age and, therefore, cannot be used after maturation of the hand-wrist is complete. Prediction methods based on regression are likely to be misleading when applied to children with chronic diseases that affect growth, with large over-predictions more likely than under-predictions. The Bayley-Pinneau method<sup>13</sup> is the best current procedure for predicting the adult statures of children with diseases; but even with this method, the prediction errors are large.<sup>12</sup> The present data should be applicable to healthy children, including those with statures or maturational levels unusual for their chronological ages.

In making decisions regarding the cessation of growth-promoting therapy, clinicians may utilize the distributions of age at which growth in stature ceases.<sup>1</sup> This approach is limited in value, how-

**Table 2.** Growth in stature (cm) after peak height velocity (PHV) and menarche

| Age Intervals          | PHV - Boys |      |      | PHV - Girls |      |      | Menarche |     |      |
|------------------------|------------|------|------|-------------|------|------|----------|-----|------|
|                        | 10         | 50   | 90   | 10          | 50   | 90   | 10       | 50  | 90   |
| Total                  | 11.6       | 17.8 | 23.7 | 10.8        | 15.8 | 22.3 | 4.3      | 7.4 | 10.6 |
| 0 to 1.0 years later   | 5.5        | 8.0  | 9.9  | 5.0         | 6.9  | 8.5  | 1.9      | 3.9 | 6.0  |
| 1.0 to 2.0 years later | 2.9        | 4.7  | 6.8  | 2.0         | 4.3  | 6.1  | 0.6      | 1.5 | 3.1  |
| 2.0 to 3.0 years later | 1.1        | 2.2  | 3.5  | 0.7         | 1.6  | 3.5  | 0.2      | 0.9 | 1.7  |
| 3.0 to 4.0 years later | 0.4        | 1.0  | 2.0  | 0.3         | 0.9  | 1.9  | 0.0      | 0.5 | 1.1  |
| 4.0 to 5.0 years later | -0.1       | 0.6  | 1.5  | 0.0         | 0.6  | 1.1  | -0.3     | 0.3 | 0.8  |



**Figure** Selected percentiles for the remaining growth in stature (cm) after peak height velocity (PHV) and menarche in relation to the ages at which these events occur. The interrupted portion of the 5th percentile for growth in stature after menarche represents values that are less than zero. These are unacceptable biologically but are found in actual data due to measurement errors.

ever, because growth is very slow prior to cessation, and a decision made on this basis would commonly prolong therapy beyond the age at which it is effective. The present data for expected growth after particular maturational events may offer a more useful alternative for basing clinical judgments. In the absence of an effective stature prediction method for children with diseases, it is recommended that surveillance and treatment continue until about 3 years after PHV or 2 years after menarche. The later these events occur, the shorter the following period during which treatment or surveillance should be continued.

### Research Applications

Another major potential application of these data is in study design. If the planned statistical analyses relate to differences in stature increments between experimental and control groups, the present data could assist a power analysis of the sample sizes required. They could also indicate the necessary duration of the study relative to age and maturity levels. If the study is to extend until growth in stature is complete in 90% of the group, data collection must continue until about age 23.5 years in males and 21.1 years in females.<sup>1</sup>

### Conclusion

The data discussed here describe

late growth in normal children. Presumably, late growth occurs as well in children with chronic diseases, although the magnitude of such changes is likely to be different. Application of these data therefore would require adjustment for the altered rate of growth produced by many diseases. There is, however, insufficient evidence on which to base these adjustments, and further studies are required to provide such support.

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## Letter from the Editor

This is the 20th issue of *Growth, Genetics, and Hormones*, completing five years of publication. The goals of the Editorial Board have been to assimilate the interests of geneticists, endocrinologists, nutritionists, and pediatricians in respect to the factors that affect growth in children, and to publish articles and abstracts in accordance with these interests. The Editorial Board has received much satisfaction in pursuing its endeavors and expresses its appreciation to Genentech Corporation for the educational grant that has made this publication possible. The Board hopes to continue its efforts in the future; please advise us how we can better accomplish our goals.

For the Editorial Board,  
Robert M. Blizzard, M.D.

## Special Report:

### 7th International Symposium on Growth and Growth Disorders

April 21-22, 1989, Rome, Italy

Robert M. Blizzard, M.D.  
Chairman  
*Growth, Genetics, and Hormones*

One entire session of this symposium dealt with adult patients with growth hormone deficiency (GHD). Prof. Bjork of Sweden queried 65 adult patients with GHD who were treated with growth hormone (GH) in the past. Twenty-three responded to the questionnaire. There were 47 controls. The conclusion of this inquiry was that patients with GHD were "worse off" than controls with respect to their quality of life. These patients reported that they felt isolated socially, were less active and mobile, had sleep disturbances, and felt less adequate emotionally than the controls. The data suggested that a significant proportion of patients with GHD have psychological damage from their chronic disease. Several individuals from the audience suggested that the control group should not have consisted of normal individuals, but individuals with other types of chronic illness or patients with short stature who were not growth hormone deficient. The various discussants agreed that the GHD patients were handicapped, but there was no way to know how they compared to individuals with other handicaps.

Drs. O.M. Rutherford and M. Preece of London reported

changes in skeletal muscle after discontinuing GH in GHD patients. The study was established because a significant decrease in the cross section of the thigh, as determined by CT scan, was noticed in one patient with GHD who had stopped GH treatment upon becoming an adult. In this study 7 of 8 patients who stopped GH because they were late adolescents or adults experienced decreases in muscle strength and size of the quadriceps. Biopsy demonstrated that the fiber area of the quadriceps was decreased. Interestingly, only one of the subjects was found to have a decrease in triceps area, and no change in strength was found in this muscle.

Drs. H. Whitehead and D. Had-den of Belfast looked at muscle fiber size in 13 GHD patients 19 to 52 years of age. Seven had been treated with GH, but not for at least 6 months before the study was undertaken. In these patients, GH was adequately replaced with hydrocortisone, thyroxine, and sex steroids. The study was of a double-blind crossover design, in which subjects received GH, 0.5 U/kg/wk divided into daily injections, for 6 months. Eleven of the 13 completed the study. The data from biopsies indicated that 6 months of treatment had no effect on the size or the type of muscle fibers. The authors emphasized that this

was a small population.

Prof. Sonksen of London then discussed the effects of 6 months of GH treatment on body composition in adults with GHD. Twelve subjects received placebo and 12 received GH. Their average age was  $30 \pm 3$  years. Studies included body composition (assessing total body potassium), a CT scan of the thigh, and measurement of various chemical parameters. The results indicated that the lean body mass increased, but fat mass decreased significantly. The fat area in the thigh did not change, but the skin fold thickness decreased by 25%; this decrease was most marked in the abdominal area.

Dr. G. McGauley of London assessed the quality of life before and after GH treatment in adults with GHD. Seventeen GHD patients who received GH were compared to 17 who did not. The former perceived that they incurred less illness and had a better quality of life. The actual measurements of differences were minimal, however.

Prof. J.M. Connor of Glasgow presented an excellent discussion of the molecular genetics of Turner's syndrome. "We must consider DNA analysis as well as chromosome analysis in patients with Turner's syndrome," he stated. Connor reported an interesting observation that in all 14 cases tested, the X chromosome came from the mother. Connor



emphasized that DNA analysis can pick up translocations and that Y material can occur in individuals with XO/XX syndrome. In such individuals gonadectomy is indicated. Y determinants should be looked for in all XO/XX Turner's patients, and this can best be done by DNA probes. Connor stated that 10% of XO/XX girls have unexpected chromosome material. He also noted that 2% of all pregnancies start with 45 XO chromosome karyotype and that the majority of these abort spontaneously.

Prof. R. Rappaport of Paris discussed the theories of growth retardation in patients with Turner's syndrome. The endocrine hypothesis—i.e., GHD—was considered first. He carefully reviewed the data from the literature, which he agreed is confusing. He emphasized that complete GHD is rare and explained that the differences in data from various clinics may be related to the fact that GH production is not the same at various stages of preadolescent life, and various investigators have reported on patients of varying ages. Values of integrated GH concentrations in patients with Turner's syndrome must be compared with those of normal preadolescents, as GH production increases in puberty. Rappaport believes that GHD is *not* significant in patients with Turner's syndrome. Furthermore, patients with Turner's syndrome do not respond as well to GH therapy as do GHD patients, and this mitigates against the possible diagnosis of GHD. He then considered the possibility of a chromosomal defect and stated that the genes on the short arm of X and Y are believed to be genes accounting for stature. Absence of these genes will produce short stature. Rappaport also talked about the likelihood of these patients having a primary skeletal defect. He presented histological data collected several years ago by Dr. Sciencu regarding the growth plate cartilage of patients with Turner's

syndrome. Clusters of chondrocytes were found in such patients, and there was abnormal organization of cartilage. In the discussion session that followed, Prof. Bierich of West Germany reported testing 36 patients with Turner's syndrome for integrated concentrations of GH. Several had decreased GH production compared to normals.

Prof. A. Ferrandez of Spain and Dr. R. Rosenfeld of Stanford (USA) then discussed studies of GH therapy in patients with Turner's syndrome. Ferrandez reported that patients receiving GH, GH plus anavar, or GH plus estrogen in small doses had an increase in the cortical thickness of bone and no change in bone age. There also were increases in bone density and in growth. Skin folds decreased in all

groups. Dr. Rosenfeld reported on 4 years of collaborative experience with the Genentech DNA recombinant growth hormone. The summation was that patients with Turner's syndrome receiving GH plus anavar, or even GH alone, are now exceeding the heights projected at the time they began GH therapy.

This conference focused on adult patients with GHD and on patients with Turner's syndrome. Our understanding of Turner's syndrome is now at the point where such patients possibly should have the opportunity to receive GH as a therapeutic agent. However, we will need more information concerning GH production in normal adults before we can conclude that GH is also good therapy for adults with GHD.

## Meet the Editorial Board



Associate Editor  
Jean-Claude Job, M.D.

Dr. Job is Professor and Chairman of Pediatrics at the Hôpital Saint-Vincent de Paul in Paris. He also heads the pediatric clinic, in which he organized a division of endocrinology.

A leading investigator of biosynthetic human growth hor-

mones in France, Dr. Job developed a research laboratory on human growth under the auspices of the French national institute of health, INSERM, and has served as research director since 1978. He organized the French national committee for hGH (France-Hypophyse), which he served first as secretary and, since 1983, as president. He also established a working group on hGH for the European Society of Pediatric Endocrinology.

Dr. Job earned his medical degree from the University of Paris in 1952. He has published more than 450 papers in French and international medical journals and is the editor of a textbook on pediatric endocrinology.

Dr. Job is a member of the European Society for Pediatric Endocrinology, the Société Française de Pédiatrie, and the Société Française d'Endocrinologie. He is also a corresponding member of the Lawson Wilkins Pediatric Society.

## Special Report

### The American Diabetes Association Scientific Meeting

June 3-6, 1989, Detroit, Michigan

William L. Clarke, M.D.

Associate Editor

*Growth, Genetics, and Hormones*

Several of the presentations at this meeting may be of interest to readers of *Growth, Genetics, and Hormones*. Geneticists, in particular, will be interested in the symposium on "New Developments in Immunology and Genetics of Insulin-Dependent Diabetes Mellitus [IDDM]," which included a talk by John I. Bell (Oxford, England) entitled, "Genetics of Insulin-Dependent Diabetes Mellitus—Has a Susceptibility Gene Been Found?" Dr. Bell described the epidemiologic and laboratory studies that have led to the identification of the association between the amino acid at position 57 of the human leukocyte antigen-DQ (HLA-DQ) beta chain and IDDM susceptibility in whites. The hypothesis is that aspartic acid (Asp) at position 57 protects against IDDM.

Dorman and Trucco (*Diabetes* 1989;38[2]:34A) reported on the contribution of the HLA-DQ phenotype to the incidence of IDDM in Allegheny County, Pennsylvania. Their previous studies demonstrated that the relative risk of developing IDDM for individuals homozygous for lack of Asp at position 57 of the DQ beta chain, compared to those with at least one DQ gene with Asp, was 107. The incidence of IDDM for non-Asp homozygotes was calculated to be 74 per 100,000, and for those with at least one Asp allele it was 0.69 per 100,000. The annual incidence attributable to the phenotype was thus 73.3 per 100,000. They then calculated the population attributable fraction to be 95%. The non-Asp/non-Asp phenotype is therefore a major determinant of the incidence of IDDM in Allegheny County, Pennsylvania. Trucco et al (*Diabetes* 1989;38[2]:19A) described a relatively simple and quick test that within 24 hours

can detect the presence or absence of Asp-57 without using either allele-specific oligonucleotide probes or radioactive probes. Ikegami et al (*Diabetes* 1989;38[2]:19A) analyzed HLA-DQ beta chain sequences in Japanese patients and determined that the DQ beta characteristics in Japanese IDDM patients are different from those in white populations, and that the DQ-alpha and/or DR sequence also may affect susceptibility.

Presentations regarding the relationship of growth hormone and diabetic retinopathy also were of interest. Rymaszewski et al (*Diabetes* 1989;38[2]:30A) studied the response of retinal capillary endothelial cells of humans in vitro to human growth hormone (hGH) stimulation. They determined, using long-term cultures of retinal endothelial cells from normal, postmortem human eyes, that exposure to hGH (200 ng/mL x 4 days) after the second passage in the presence of 10% horse serum, resulted in a  $55 \pm 9\%$  greater cell number versus controls. Tritiated thymidine incorporation was stimulated at hGH concentrations as low as 1.2 ng/mL. Thus, physiologic concentrations of hGH stimulated mitotic activity of highly purified human retinal capillary endothelial cells. These studies suggest a direct responsiveness of the retinal endothelium to hGH. Dills et al (*Diabetes* 1989;38[2]:5A) measured insulin-like growth factor I (IGF-I) serum levels in 876 subjects with diabetes diagnosed at 30 years of age or older. Proliferative retinopathy was found in 15.6% of the insulin-taking population (N = 488). After controlling for duration of diabetes, glycosylated hemoglobin, blood pressure, proteinuria, and age at diagnosis, higher levels of IGF-I were associated with an increased risk of proliferative retinopathy in those subjects taking insulin. The authors suggest that

high IGF-I levels may be a factor for the development of proliferative retinopathy. Grant et al (*Diabetes* 1989;38[2]:56A) measured vitreous concentrations of IGF-I and -II by radioimmunoassay in 40 subjects with retinopathy and 18 nondiabetic subjects. Seventy-two percent of the diabetic subjects had vitreous concentrations of IGF-I capable of inducing increases in chemotaxis of human retinal endothelial cells ( $>5.0$  ng/mL). IGF-II concentrations in the vitreous exhibited a distribution similar to IGF-I levels, and the concentrations of both correlated moderately with their serum concentrations.

Horber and Haymond (*Diabetes* 1989;38[2]:56A) studied the insulin resistance induced by hGH and prednisone in nondiabetic subjects. Glucose and leucine oxidation after an 18-hour fast, and during gut infusion of glucose and amino acids, was measured. Subjects were studied after 7 days of placebo, hGH 0.1 mg/kg/day, prednisone 0.8 mg/kg/day, or hGH plus prednisone. Fasting glucose was similar during the placebo and hGH administration, but was elevated during prednisone administration and during the combination of hGH and prednisone. Leucine oxidation was increased by prednisone but decreased by hGH administration and unchanged during combined treatment. By indirect calorimetry, glucose oxidation was similar in all groups. Insulin levels were higher during combined therapy than during placebo, hGH, or prednisone treatment. In summary, the insulin resistance of hGH and prednisone was demonstrated to be additive. The authors concluded that the insulin resistance of hGH and of prednisone may be caused by independent mechanisms. Prednisone decreased fat oxidation and increased leucine oxidation, whereas hGH treatment did the opposite. hGH and prednisone may reciprocally regulate oxidation of protein and fat, while decreasing the efficiency of glucose disposal.

## Variation in Lower Leg Growth With Alternate-Day Steroid Treatment

The growth of a boy with Crohn's disease was studied intensively over 4 weeks. Lower leg length was measured with a knemometer (mean of four measurements) every day (except for weekends). The standing height was measured weekly. The subject was 11.6 years old and had had the disease for 5 years. He was on a regimen of 1 g of sulfasalazine twice daily and 7.5 mg soluble prednisolone on alternate days (taken after the measurement session, which was 1 hour after arriving on the ward). In the fourth week the steroid was put

"out of phase" by 1 day, to see if the pattern of growth reversed.

A clear distribution of leg-length gains was shown. Despite some overlap, a highly significant difference was demonstrated between the means of the steroid days and those of the steroid abstinence days. Growth on the days of steroid ingestion was fractionally below zero, but the gain on the days the patient was off steroid averaged 0.5 mm ( $t = 3.6$ ). Soft tissue changes at the knee and heel were measured by ultrasound, but no consistent change was noted.

Wales JKH, Milner RDG. *Arch Dis Child* 1988;63:981-983.

**Editor's comment**—This report is important in showing exactly what the knemometer does best: measuring short-term growth changes in the context of physiologic or pharmacologic investigations of the growth plate, or of factors affecting it. It provides direct evidence of the expected inhibiting effect of high doses of steroid on growth, followed by catch-up as soon as the steroid is removed.

James Tanner, M.D.

## The Role of the Vitamin D Endocrine System in Health and Disease

Vitamin D is not only a vitamin, but also a hormone.  $1,25(\text{OH})_2\text{D}_3$  and  $1,25(\text{OH})_2\text{D}_2$  are the principal vitamin D mediators that regulate bone and mineral metabolism in humans. There are, however, other actions of  $1,25(\text{OH})_2\text{D}_3$ —and probably  $1,25(\text{OH})_2\text{D}_2$ —that have not been well recognized by practitioners. This article reviews current concepts in this field.

The circulating  $25(\text{OH})\text{D}_3$  level reflects the availability of vitamin D<sub>3</sub> and is thought to be the best indicator of vitamin D levels. Feedback mechanisms play pertinent roles, as they do in the other endocrine systems. For example,  $1,25(\text{OH})_2\text{D}_3$  decreases the level of  $25(\text{OH})\text{D}_3$ .  $1,25(\text{OH})_2\text{D}_3$  in excess also decreases its own level by shifting the synthesis of  $25(\text{OH})\text{D}_3$  to  $24,25(\text{OH})_2\text{D}_3$  instead of continuing to synthesize  $1,25(\text{OH})_2\text{D}_3$ .

Other factors regulating the synthesis of  $1,25(\text{OH})_2\text{D}_3$  include parathyroid hormone (PTH), which stimulates  $1\alpha$ -hydroxylase activity, as do low dietary phosphate and hypophosphatemia. Hyperphosphatemia, in contrast to hypophosphatemia, decreases  $1\alpha$ -hydroxylation. Several other hormones secondarily affect  $1,25(\text{OH})_2\text{D}_3$  levels. Estrogen, for

example, increases  $1,25(\text{OH})_2\text{D}_3$  because vitamin D binding protein is increased.

Synthesis of  $1,25(\text{OH})_2\text{D}_3$  occurs to some extent in organs other than the kidney, eg, in patients with sarcoidosis who are anephric. Ectopic synthesis also occurs during pregnancy, as placental and decidual cells produce the hormone.

Substantial evidence has accumulated that the mechanism of action of  $1,25(\text{OH})_2\text{D}_3$  is similar to that of other steroid hormones, in that the hormone-receptor complex is associated with DNA in the nucleus. Here it either initiates the synthesis of specific RNA encoding proteins or mediates a selective repression of gene transcription. The  $1,25(\text{OH})_2\text{D}_3$  receptor protein is expressed in almost every tissue examined so far.

With respect to calcium metabolism,  $1,25(\text{OH})_2\text{D}_3$ , in concert with PTH and calcitonin (CT), acts on bone, intestine, and kidney.  $1,25(\text{OH})_2\text{D}_3$  plays a role in the regulation of osteoblast function, although its effect on bone growth and mineralization is probably not mediated directly via osteoblasts. As for osteoclast activity, which contributes to bone resorption: administration of  $1,25(\text{OH})_2\text{D}_3$  in-

creases the number of osteoclasts found in rats.

In the parathyroid gland  $1,25(\text{OH})_2\text{D}_3$  decreases PTH release by increasing serum calcium and by a direct short-loop feedback message that inhibits the synthesis of PTH through an interaction with the prepro-PTH gene. In the intestine, it stimulates the influx of calcium and phosphorus from the lumen through the intestinal wall and into the plasma. For calcium, this is done by activating an increased production of calbindin-D in the intestinal wall which, in turn, enhances calcium absorption.

The authors also discuss at length the role of  $1,25(\text{OH})_2\text{D}_3$  in involutional osteoporosis, rickets, granulomatous diseases, cellular growth and differentiation, interaction with the hematopoietic system, effects on lymphocytes, and interaction with cancer cells.

Reichel H, Koeffler HP, Norman AW. *N Engl J Med* 1989;320:980.

**Editor's comment**—This is a lengthy and excellent review of the contributions of vitamin D to both normal and pathologic conditions. The data and concepts are up to date. Readers interested in the details of vitamin D metabolism are encouraged to use this article as a reference.

Robert M. Blizzard, M.D.

## Growth Failure: A Complication of Dietary Treatment of Hypercholesterolemia

A group of 40 children were advised to pursue a low-cholesterol and low-fat diet because of relative or unequivocal hypercholesterolemia. Few studies evaluating the benefits and risks of dietary recommendations to children with hypercholesterolemia have been reported. Thirty-two of the patients were considered to have normal growth, although some were seen relatively shortly after the diagnosis was made and treatment was initiated. The remaining 8 were considered to have growth failure associated with the dietary treatment. Three had growth inhibition primarily of height and 5 primarily of weight. The 8 patients were ingesting approximately 65% of the calories necessary for energy expenditure and approximately 40% of the dietary requirement for zinc. The 3 patients with growth inhibition of stature were obtaining only 20% of their energy expenditure through fat ingestion and consumed even less calories than the other 5 (<60% of the established energy requirement for their ideal weight, sex, and age).

The authors comment that these data demonstrate that the diagno-

sis and unsupervised dietary treatment of hypercholesterolemia in children may have adverse consequences. In this study a high proportion of patients who were advised to eat a low-fat, low-cholesterol diet because of hypercholesterolemia consumed diets inappropriate to sustain normal growth and weight gain and to initiate pubertal development. The diets consumed by those with growth failure were mainly inadequate in energy and zinc. The authors conclude that a reduction in fat intake to less than 30% of total energy may not be routinely warranted in children with hypercholesterolemia and that such restrictions should be reserved for those who fail to reduce their serum cholesterol levels when following a prudent diet. Sufficient dairy products, red meat, and eggs to meet nutritional standards should be included in the diet, and this can be done without increasing the fat and cholesterol intake beyond the aforementioned guidelines. These recommendations are in accord with those of the Committee on Nutrition of the American Academy of Pediatrics, who concluded in

their report that any restrictions on dietary patterns during the first 20 years of life should be viewed with caution. The authors strongly recommend assistance from a dietitian or nutritionist when planning diets for children with hypercholesterolemia.

Lifshitz F, Moses N. *Am J Dis Child* 1989;143:537-542.

**Editor's comment**—*I can only re-emphasize the comments made by Dr. Laurence Finberg, in an editorial entitled "Dietary Advice: Responsibility for Monitoring" that appeared in the American Journal of Diseases of Children. Dr. Finberg noted that the dietary requirements of children differ from those of adults in many respects, eg, children need more calories for energy and a variety of nutrients at higher levels to promote optimal growth. Finberg agreed that a prudent diet in the presence of hypercholesterolemia was indicated for the patients reported here. In the 20% of patients (8/40) with growth retardation, the failure lay in the monitoring of growth and in the provision of advice concerning the intake of all necessary nutrients.*

Robert M. Blizzard, M.D.

## Growth and Endocrine Disorders Secondary to Cranial Irradiation

Rappaport and Brauner present data from the literature and their own studies concerning cranial and spinal irradiation therapy and its effect on growth and pubertal development. Of a group of children given 2,400 rad as prophylactic irradiation for acute lymphoblastic leukemia, 56% had growth hormone (GH) deficiency with a peak GH response to arginine-insulin of <8 ng/mL. Complete GH deficiency (two consecutive GH peak responses <5 ng/mL) was observed in 30% of the same population. Eight children treated with

1,800 rad had normal GH responses at least 4 years after radiation. In addition, normal GH secretion was found in a group of children treated for retinoblastoma who received <2,000 rad. All children who received >4,500 rad for optic glioma had GH deficiency. Younger children were reported to be more vulnerable to the effects of radiation than older children or adults. In addition, the timing of the occurrence of GH deficiency was reported to be related to the radiation dose. GH deficiencies may appear during the first year after radiation in patients receiving more than 4,500 rad, and most of these children are GH deficient within 2 to 3 years. In the authors' experience, GH deficiency will almost always

appear within 5 years of radiation, and no affected child has resumed GH secretion. The authors also discussed the use of different stimulation tests, plasma insulin-like growth factor I values, and possible mechanisms for GH deficiency.

Pubertal development also was discussed. Five of 45 children treated with 2,500 to 5,000 rad before or during puberty showed complete gonadotropin deficiency at pubertal ages, while two children had partial gonadotropin deficiency. Diabetes insipidus has not been reported after cranial irradiation and hypothyroidism is infrequent.

Growth after cranial irradiation was dose dependent. Radiation doses in excess of 3,000 rad will



reduce final height in most children, whereas low-dose cranial irradiation (1,800 to 2,400 rad) produces variable responses. Spinal irradiation may have an effect on sitting height that is independent of GH deficiency and that results from decreased growth of the spine.

The final section of this report deals with GH therapy in cranially irradiated children. Although patients initially have catch-up growth, the authors' data (unpublished) confirm that prolonged GH therapy does not significantly improve the mean height SDs of

patients given cranial and/or spinal irradiation. The possible reasons for this include 1) a shorter duration of GH deficiency, 2) a less retarded bone age at the onset of GH therapy, 3) a lower initial (first year) growth velocity response, and 4) the presence of early puberty, which had accelerated bone age faster than the growth velocity. Despite these less than optimal responses, the authors state that it is essential to begin GH therapy as soon as growth velocity declines and radiation therapy has been concluded. They consider treating

any child with a height loss of 1 SD or more who has proven GH deficiency. The follow-up period after radiation must be 2 years or more.

Rappaport R, Brauner R. *Pediatr Res* 1989;25(6):561-567.

**Editor's comment**—This paper presents few new data, but it is a good review of the effects of cranial spinal irradiation on GH secretion and pubertal development. As such, it is quite comprehensive and deserving of close scrutiny.

William L. Clarke, M.D.

## Puberty in the Syndrome of Septo-optic Dysplasia

Hanna et al retrospectively evaluated pubertal development in 13 patients with septo-optic dysplasia. The patients were grouped according to the timing of puberty. Six of the 13 patients comprised group 1; they had clinical signs of puberty beginning earlier than anticipated (bone age <10.5 years in girls, <11.5 years in boys) and experienced rapid progression of puberty associated with bone ages advancing more rapidly than chronological age. Growth rates were normal-to-increased in this group, but because of the rapid advancement in bone age, these patients lost growth potential. Three of the 13 patients were classified in group 2, with puberty beginning at the expected time and the progression of puberty considered to be normal. The remaining four patients (group 3) were judged to be gonadotropin deficient by low serum levels of follicle-stimulating hormone and luteinizing hormone at bone ages of 12 years in the three girls and 13 years in the boy. Minimal signs of puberty were present at a mean chronological/bone age of 16.8/13.7 in the girls and 17/13 in the boy, and replacement therapy with sex steroids was instituted.

The authors comment that sexual precocity in girls with septo-optic dysplasia has been described previously. However, they note

that precocity affects boys as well as girls, is most often associated with isolated GH deficiency, and is independent of visual limitation.

Hanna CE, Mandel SH, LaFranchi SH. *Am J Dis Child* 1989;143:186-189.

**Editor's comment**—Although this report is a retrospective study, its contributions are important. Twelve of the 13 patients reported in this study received growth hormone therapy. However, only four of these (group 3) had multiple

hormonal deficiencies and significant pubertal delay or absence. The others either had normal progression of puberty or sexual precocity. Despite the high percentage of patients with abnormal puberty, it is important to note that not all patients with septo-optic dysplasia experience abnormalities of pubertal timing and progression. Thus, it is not possible at this time to make predictions concerning puberty in those children who do not have multiple hormonal deficiencies.

William L. Clarke, M.D.

## Birth Prevalence of Skeletal Dysplasias

The prevalence of skeletal dysplasias at birth has received relatively little attention, and the completeness of the available data has been viewed with concern. Two recently reported prospective, population-based studies shed light on this subject.

Stoll and colleagues examined birth records, roentgenographic reports, autopsy reports, follow-up pediatrician notes, and other available data from 11 maternity hospitals where all births were recorded from Strasbourg, France and the surrounding region, from 1979 to 1986. The data from fetuses delivered with a minimum age of 20 weeks and from pregnancies interrupted following prenatal diagnosis of a skeletal dysplasia were included; ascertainment was thought

to be complete. A skeletal dysplasia was diagnosed in 34 cases out of 105,374 births to give a prevalence rate of 32.2 per 100,000. The rates per 100,000 births for several of the more common disorders were: achondroplasia, 6.4; thanatophoric dysplasia, 2.8; achondrogenesis, 2.8; osteogenesis imperfecta, 6.4; osteopetrosis, 1.8; and multiple exostoses, 1.8. Roughly half of the patients had disorders that are usually lethal in the newborn period.

The second study, by Andersen, examined the birth prevalence of lethal bone dysplasias. Clinical and radiographic findings were analyzed from all births, including stillbirths, in the county of Fyn, Denmark, from 1970 to 1983. Twelve

## Limb Lengthening by Epiphyseal Distraction in Chondrodystrophic Bone: An Experimental Study in the Canine Femur

Distraction of the left distal femoral epiphysis was carried out in 18 chondrodystrophic dogs at 19 to 22 weeks, an age comparable to early adolescence in humans. The distraction rate was 0.5 mm/day. Epiphysiolysis occurred after 4 to 9 days, and the treatment was continued for 3 weeks. The average gain in length (measured on radiographs at cessation of treatment), compared to the contralateral control side, was  $1.4 \pm 0.3$  cm. At 2 weeks callus appeared in the gap of the lengthening zone. Osteogenic activity was most distinct at the metaphyseal side. Periosteal reaction along the diaphysis produced widening of the diaphyseal diameter. After removal of the pins, the distracted zone appeared as radiodense immature bone, which soon became mature. Closure of the distal epiphyses took place at about 8 months on the operated side and 9 months on the other.

Animals were killed 3 weeks

after cessation of distraction (group 1,  $n=5$ ), at 19 weeks (group 2,  $n=10$ ), or 71 weeks (group 3,  $n=3$ ). At postmortem, femoral lengthening in group 1 was confirmed at 1.2 cm (12.3% of metaphyseal length); in groups 2 and 3, observed after leg growth had ceased, the gain was 0.7 cm (6.1%). That is, there was a loss of residual growth potential in the distracted epiphysis. The torsional strength of the distracted femur ranged from 83% (group 1) to 98% (group 2) to 107% (group 3) of that of the contralateral control. Degenerative changes in the knee joint were observed in three animals in each group.

In conclusion, lengthening by epiphysial distraction of the distal femur of chondrodystrophic dogs resulted in reduction of residual growth in the involved growth plate. This finding is in accordance with what we previously have observed in analogous studies of animals with normal growth. The procedure obviously had an adverse effect on the growth plate. It is unlikely that the situation would be different in humans. The observed retardation effect on the traumatized growth plate is a sequela that, in general,

restricts the use of epiphysial distraction to the late adolescent period when residual growth is negligible. In successive bilateral lengthening of multiple bone segments by epiphysial distraction, a procedure that requires an early onset of lengthening, retardation of residual growth may cause significant reduction of gained length. The development of degenerative joint changes is a potential risk that probably does not legitimate epiphysial distraction as the method of choice in the femur.

Fjeld TO, Steen H. *J Orthop Res* 1989;7:184-191.

**Editor's comment**—With the increasing demand for leg-lengthening operations, basic studies such as this one are very much needed. Its findings are clear and unequivocal. At least in dogs (and also in goats, according to an earlier report), epiphysiolysis damages the growth potential of the split-off growth plate—a not unexpected finding. This seems to restrict the technique to late adolescence, when, unfortunately, less growth potential remains.

James M. Tanner, M.D.

lethal bone dysplasias were diagnosed out of 77,977 total births to give a prevalence of 15.4 per 100,000. Three cases of thanatophoric dysplasia (including one with cloverleaf skull) and five cases of achondrogenesis type II were identified, yielding respective prevalence values of 3.8 and 6.4 per 100,000 births, respectively.

Stoll C, Dott B, Roth M-P, et al. *Clin Genet* 1989;35:88-92.

Anderson PE. *Am J Med Genet* 1989;32:484-489.

**Editor's comment**—These reports provide relatively similar birth prevalence rates for skeletal dysplasias when one takes into account that the study by Stoll and colleagues examined both lethal and nonlethal conditions whereas

Andersen looked only at lethal disorders. Together the data suggest that the prevalence of all skeletal dysplasias is slightly greater than 30 per 100,000 births, of which approximately half are lethal in the perinatal period. This figure translates into a rate of around 1 per 3,000 births. This rate, which is actually an underestimate as many disorders are not evident at birth, is much higher than is generally ap-

preciated. For comparison, approximate prevalence rates for several well-known genetic conditions are as follows: Down syndrome, 1 per 700 births; cystic fibrosis, 1 per 1,600 births; muscular dystrophy, 1 per 3,500 male births; hemophilia, 1 per 5,000 male births; phenylketonuria, 1 per 15,000 births; and albinism, 1 per 40,000 births.

William A. Horton, M.D.

### In Future Issues

Inflammatory Bowel Disease and Growth Retardation by Richard Grand, M.D.

IGF-Binding Proteins: Their Physiological and Clinical Importance by Michael Ranke, M.D.

Genomic Imprinting by Judith G. Hall, M.D.

How Bones Grow by William A. Horton, M.D.

Paracrine Aspects of Bone Metabolism by David Baylink, M.D.

## Surgically Curable Hypophosphatemic Rickets

All patients with apparent hypophosphatemic rickets (HR) do not have an inherited defect. A small but significant proportion have a tumor, which results in a very similar clinical picture. The history of one such patient and a review of the literature comprise the contents of this article.

An 8-year-old boy with rickets had swollen wrists for 6 months and knee pain for 30 months. His height had continued between the 25th and 50th percentiles. The only physical findings were tenderness and swelling of the wrists and right knee and genu valgum.

The findings were consistent with the proposed diagnosis of HR. Roentgenography confirmed the diagnosis. Demineralization of the pelvis, an occurrence seen in severe rickets, was present. A large lytic 6 x 2.5 cm lesion with sclerotic borders was noted at mid-femur on the right.

Following treatment with calcitriol (1.5 µg/day) and NeutraPhos, a phosphorus replacement

supplement (0.5 g three times daily), for 4 months the rickets improved. After discontinuing treatment for 2 weeks the lytic lesion was surgically removed. Within 15 days postoperation, serum phosphorus rose to normal levels. The patient was cured as evaluated by chemical analysis of serum and urine. The histopathology of the tumor was consistent with a diagnosis of hemangiopericytoma.

Only six other cases of HR associated with bone tumors in children have been reported in the literature. The tumors were classified as fibrous dysplasia, fibroma, osteoblastoma-like variants, and non-osteous soft tissue tumors. In adults, HR occurs with connective tissue tumors located in soft tissues that have morphologic features of hemangiopericytoma.

The authors conclude that the tumor produced a phosphaturic substance that impaired phosphate resorption by kidney tubule cells, although production of a substance inhibiting vitamin D metabo-

lism has been implicated in other cases. In this patient, an associated amino aciduria was of diagnostic import in distinguishing genetic from tumor-caused etiology. Most importantly, the authors urge that the possibility of a tumor be considered in sporadic cases of HR.

Hanukoglu A, Chalew SA, Sun CJ, et al. *Clin Pediatr* 1989;28:321-325.

**Editor's comment**—*The diagnosis of tumor could readily be missed in sporadic cases of HR. The presence of amino aciduria is found in vitamin D deficiency and vitamin D dependency rickets but not in HR unless a tumor is present. Although most physicians probably do not check for amino aciduria, the presence of this substance should be evaluated in all sporadic cases. If found, screening for tumor should follow. The absence of amino aciduria may not absolutely exclude the possibility of tumor, but it makes it much less likely.*

Robert M. Blizzard, M.D.

## Management of Idiopathic GH-Deficient Patients During Puberty

At the Fifth International Symposium Regarding Growth and Growth Disorders, Berlin, April 1988, Price et al presented data from their clinic and from the literature regarding the growth of patients with growth hormone deficiency (GHD) during spontaneous or induced puberty. Boys with idiopathic GHD had a significantly later onset of puberty (15.0 to 15.9 years) than normal boys (11.5 to 12.0 years). The peak height velocity (PHV) occurred at 16.0 to 16.4 years, compared with 14 years in normal boys. The bone ages were comparable (13.5 to 14.0 years) at the time of PHV in the two groups. The PHV was less

in the GHD group and the total gain after G2 sex development was 17.0 to 22.8 cm (means of four groups of GHD patients studied at different centers) vs 27.4 cm in normals and 18.0 cm in boys with constitutional delayed growth (CDG). The loss in final height SD score ( $-2$  to  $-2.5$ ) may reflect pretreatment loss rather than a failure of adequate treatment during puberty, because the total gain after G2 sex development was comparable to that of boys with CDG. The GH treatment was unsophisticated by modern methods, with fixed doses independent of body size given two to three times per week. Data regarding girls were very limited and, therefore, are not reported here.

With respect to treatment, the authors did not encourage an increased dose of GH during puberty

because significantly increased growth velocity occurs spontaneously in boys with isolated GHD and because of the greater cost of larger doses. They urged, however, that daily rather than intermittent doses be used. They also stated the need for further information before dose schedules for pubertal patients can be firmly recommended.

Manipulation of puberty was recommended in patients who have GHD and either gonadotropin deficiency or sexual precocity. In the former, the authors recommended strongly that physicians consider the induction of puberty at 14 to 15 years of age in boys and 13 to 14 years of age in girls. They also suggested that this approach be considered in patients with isolated GHD if puberty has not developed spontaneously. Their ar-

guments are based on the psychological need of adolescents to develop at these chronologic ages and the disproportionate stature that develops if treatment is prolonged. Six boys with luteinizing hormone deficiency who were not treated with testosterone until late had an SD score for mean leg length/sitting height of 1.4, compared with 0.6 in isolated GHD. Low doses of testosterone (25 mg twice monthly) are advised in boys to counteract the shorter pubertal duration of 2.7 vs 4.2 years observed when 100 mg was given monthly. Estrogen, 2 to 5  $\mu\text{g}/\text{d}$ , was recommended for girls. The authors suggested that delay of puberty with gonadotropin-releasing

hormone analogs should be considered in patients with sexual precocity, but they readily admit that data are not available to evaluate the effectiveness of delaying epiphyseal fusion in order to increase height.

Price DA, Shaleta SM, Clayton PE. *Acta Paediatr Scand* 1988;347 (suppl):44-51.

**Editor's comment**—*The fact that testosterone, endogenous or exogenous, stimulates growth in GH-deficient patients, and that Laron dwarfs have an adolescent growth spurt, support the concept that the growth spurt at adolescence is derived, at least in part, from a direct action of testosterone on the*

*growth plate. The second, and possibly more influential, action is via the increased GH secretion that occurs under the influence of testosterone. Whether additional GH should be given to GH-deficient patients while they are passing through adolescence is still debatable. Each case should be individualized, and the decision to treat should be made on the basis of current height, bone age, mid-parental height, and cost. Some very short GH-deficient patients certainly should be given the opportunity to grow maximally while passing through adolescence and should be considered for additional GH treatment.*

Robert M. Blizzard, M.D.

## Urea Synthesis, Nitrogen Balance, and Glucose Turnover in Growth-Hormone-Deficient Children Before and After Growth Hormone Administration

Dahms et al studied urea synthesis and glucose turnover using a primed constant infusion of  $^{15}\text{N}_2$ -labeled urea and a constant infusion of  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  in 10 prepubertal, growth hormone (GH)-deficient children prior to and after 6 days of human GH (hGH) therapy. The patients were admitted following diagnosis of GH deficiency, which was established by failure to respond to at least three stimulation tests. The first 6 days of hospitalization constituted a control period, during which a liquid diet that provided 9% of energy as high biologic-value protein was given. On day 6, tracer infusion studies were performed following an overnight fast. hGH (NPA) was then administered (0.1 U/kg/day, intramuscularly) between 10:00 P.M. and 11:00 P.M. and the studies were repeated on day 12.

The patients' height and energy intake did not vary during the pro-

tol. However, plasma urea nitrogen decreased significantly by the second day of hGH therapy. Urea synthesis also decreased significantly after 6 days of hGH therapy. Nitrogen excretion, determined by total stool and urinary nitrogen, was decreased, and this was accounted for by decreased urea excretion. The decrease in urea excretion was the result of decreased urea synthesis. Plasma glucose increased in eight of the 10 patients during hGH therapy, but there was no significant change in the rate of glucose turnover. There was no correlation between subsequent growth velocity while the patients were on hGH and the quantitative decrease of urea nitrogen during the acute administration of hGH.

Dahms WT, Owens RP, Kalhan SC, et al. *Metabolism* 1989; 38(3):197-203.

**Editor's comment**—*The authors state that previous studies have looked at the effect of GH on nitrogen balance using classic nitrogen balance studies. The present studies, however, demonstrate that the mechanism of the decrease in nitrogen secretion induced by GH is a decrease in urea synthesis. The authors further suggest that the*

*most likely explanation for the decreased urea synthesis is the decreased production of ureagenic substrates by peripheral tissue, and state that the observed decreases in the plasma concentrations of the amino acids after hGH administration support this hypothesis. These carefully performed studies help to explain the changes that occur during GH administration. Unfortunately there was no correlation between the 6-month growth rate and the change in urea synthesis or blood urea nitrogen during the 7-day treatment.*

William L. Clarke, M.D.

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## MEETING CALENDAR

**January 24, 1990** Endocrinology in Pediatric Practice, a one-day symposium. Garden City Hotel, Garden City, Long Island, New York. Contact: Denise DiSisto, Division of Continuing Education, Long Island Jewish Medical Center, New Hyde Park, NY 11042 (718-470-8650)

**February 6-9, 1990** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, California. Contact: David K. Stevenson, M.D., Department of Pediatrics, Room S222, Stanford University School of Medicine, Stanford, CA 94305 (415-723-5711)

**May 7-11, 1990** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Hilton Hotel, Anaheim, California. Contact: Debbie Anagnosopoulis, Society for Pediatric Research, 2650 Yale Boulevard S.E., Suite 104, Albuquerque, NM 87106 (505-764-9099)

**May 9, 1990** Diabetes Symposium, Lawson Wilkins Pediatric Endocrine Society. Hilton Hotel, Anaheim, California. Contact: Dr. Gilbert August, Children's Hospital, 111 Michigan Avenue N.W., Washington, DC 20010 (202-745-2121)

**May 11, 1990** Annual Scientific Session Lawson Wilkins Pediatric Endocrine Society. Hilton Hotel, Anaheim, California. Contact: Dr. Gilbert August, Department of Endocrinology and Metabolism, Children's Hospital, 111 Michigan Avenue N.W., Washington, DC 20010 (202-745-2121)

**June 8-11, 1990** 30th Meeting of the Teratology Society. Empress Hotel and Convention Center, Vancouver, British Columbia. Contact: Ms. Alexandria Ventura, Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1841)

**June 16-19, 1990** 50th Annual Meeting and Scientific Sessions, American Diabetes Association. Georgia World Congress Center, Atlanta, Georgia. Contact: American Diabetes Association, 1660 Duke Street, Alexandria, VA 22314 (800-232-3472)

**June 20-23, 1990** 72nd Annual Meeting of The Endocrine Society. Convention Center, Atlanta, Georgia. Contact: Ann Singer, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**July 9-11, 1990** Annual March of Dimes National Genetics Conference: Gastrointestinal Disorders. Westin Hotel, Detroit, Michigan. Contact: Orlando J. Miller, MD, Wayne State University, 2316 Scott Hall, 540 East Canfield Avenue, Detroit, MI 48201 (313-577-5323)

**July 18-21, 1990** 59th Annual Meeting of the Genetics Society of America (co-hosted with the Genetics Society of Canada). San Francisco Hilton, San Francisco, California. Contact: Jean Francese, The Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825).

**October 12-14, 1990** 31st Annual Meeting of the American College of Nutrition. Ramada Classic, Albuquerque, New Mexico. Contact: Kay Balun, American College of Nutrition, 345 Central Avenue, Suite 207, Scarsdale, NY 10543 (914-723-4247)

**October 16-20, 1990** Annual Meeting of The American Society for Human Genetics. Convention Center, Cincinnati, Ohio. Contact: Notten Boom, Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20814 (301-530-7010)

**October 28-November 1, 1990** 42nd Annual Postgraduate Assembly of The Endocrine Society. Sheraton Waikiki, Honolulu, Hawaii. Contact: Ann Singer, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

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# GROWTH

## Genetics & Hormones

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## Growth, Thyroid Function, and Sexual Maturation in Down Syndrome

Siegfried M. Pueschel, M.D., Ph.D.  
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During the past three decades advances in the biomedical sciences have brought about more effective approaches in the care of children with Down syndrome. This article focuses on new information relating to longitudinal growth, thyroid disorders, and sexual development in these individuals.

### Longitudinal Growth

Previous studies suggest that stature and growth rate are reduced at most ages from birth to adolescence in persons with Down syndrome.<sup>1,2</sup> We have evaluated heights and weights from birth to 18 years in children with this syndrome.<sup>3,4</sup> Compared with growth data from the National Center for Health Statistics, children of either sex with Down syndrome were significantly smaller at all age intervals. The mean stature for girls with Down syndrome was reduced from the normal mean by 1.5 to 2.5 SD until 12 years of age, and by more than 3 SD from 12 to 17 years, according to different series. Mean stature for boys was reduced by 2 to 3 SD until 13 years and by 2 to 4 SD thereafter.<sup>4</sup>

Centile charts for 1-month- to 18-year-old children with Down syndrome were constructed from these data. Figures 1 and 2 portray these data for boys and girls, respectively, from 2 to 18 years. For

all centiles of children with Down syndrome, stature was less than the equivalent centiles for the National Center for Health Statistics data over the entire age range. The growth charts give smoothed values for five centiles for stature for each sex and two age intervals, from 1 to 36 months and from 2 to 18 years. The centiles for stature reflect the expected smaller size and slower growth rate of children with Down syndrome. It was observed that deficiencies in growth velocity occur at varying times in children with Down syndrome and are of widely different magnitude, particularly in infancy. Thus, compared with normal children, we emphasize that a child with Down syndrome may at various times appear in very different centile levels on these charts.

These studies also revealed that boys were significantly longer and heavier than girls from 3 to 24 months and taller and heavier than girls again after 13 years of age. Differences in the intervening period were not significant. Differences in mean height for those children without congenital heart disease from those with moderate or severe congenital heart disease were approximately +2 cm in boys and +1.5 cm in girls until about 8 years. Growth rate was reduced approximately 20% during infancy in each sex, but only about 5% between 3 and 10 years in girls, and 10% between 3 and 12 years in boys. During the remainder of the growing period, reduc-

tion in growth rate was 27% for girls and 50% for boys. This indirectly supports the observation that the adolescent growth spurt in youngsters with Down syndrome is less marked than in normal children. Rarick and Seefeldt<sup>5</sup> observed that average growth velocity during adolescence was somewhat reduced in a population with Down syndrome, but peak height velocity was achieved at an age similar to that of the control group. These investigators also measured sitting height and found that the reduction in stature was largely due to a reduction in lower segment length throughout the period of the study (8 to 18 years).

Although we were unable to determine growth velocities in our cohort, "pseudovelocities" (defined as the mean value for an age interval subtracted from the mean of the subsequent age interval) were computed and compared with reference data from the Fels Research Institute. We observed that the growth pseudovelocities fell between the 10th and 25th centiles of normal girls from 2 to about 13 years, and between the 3rd and

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25th centiles for boys from 2 to 11 years. For the periods ending at 14 to 15 years for girls and at 12 to 13 years for boys, pseudovelocities for stature were between the 50th and 90th centiles.

Anneren and co-investigators<sup>6</sup> studied the growth and somatomedin responses to growth hormone (GH) in three girls and two boys with Down syndrome whose height was less than 3 SD below mean for age. All five children had normal GH responses to arginine-insulin and were treated with human growth hormone (hGH) for 6 months (0.5 U/kg/wk in three divided doses). During this time, growth velocity increased in all subjects, from a range of 2.3 to 2.8 cm per 6 months to 3.3 to 5.8 cm, or 50% to 200%. Serum concentrations of insulin-like growth factor I were low before therapy and increased during treatment. The authors concluded that children with Down syndrome respond to this dose of hGH with an increase in growth velocity, although this increase is not as much as that observed in otherwise normal children with GH deficiency.

### Thyroid Dysfunction

The most often observed thyroid abnormality in Down syndrome is hypothyroidism.<sup>7-9</sup> However, hyperthyroidism and thyroiditis without hypothyroidism have been reported.<sup>10-13</sup> Moreover, associations of hypothyroidism and diabetes mellitus,<sup>14</sup> hypothyroidism and precocious sexual development,<sup>15</sup> and autoimmune hypothyroidism and hypoparathyroidism<sup>16</sup> have also been observed in Down syndrome. Autoimmune disease in Down syndrome primarily affects the thyroid.

In a recent study we compared serum thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3), free triiodothyronine (FT3), triiodothyronine uptake (T3U), thyroid-stimulating hormone (TSH), and thyroxine-binding globulin (TBG) levels in 181 individuals with Down syndrome with those in 163 controls. We found a significant difference between the two groups for T4, T3, and TSH

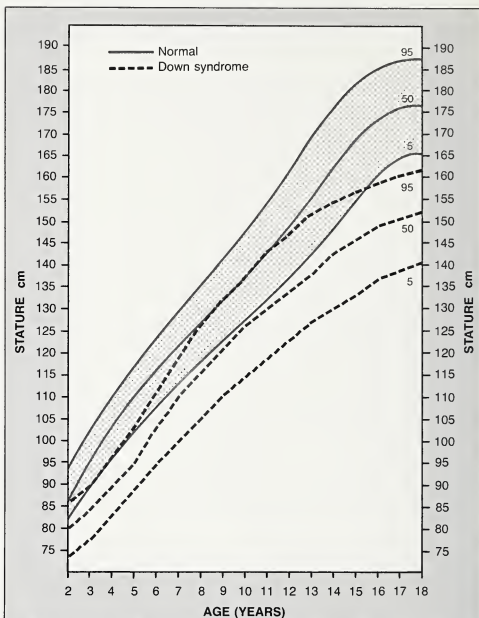


Figure 1 Growth chart for boys with Down syndrome from age 2 to 18 years.

levels: T4 was significantly lower and T3 and TSH levels were significantly higher in the Down syndrome population (Table 1). Of the 181 patients with Down syndrome, 29 (16%) showed evidence of hypothyroidism. Of these 29, 25 had elevated serum TSH and 11 had reduced serum T4 concentrations. We also observed a significantly low T4 in four patients in whom the TSH concentration was not raised. Only one patient had a significantly elevated T4 level.<sup>17</sup> When the individuals with Down syndrome were divided into 5-year age groups, there were statistically significant differences between most of the age groups in the different thyroid function categories. In particular, a gradual decline of the mean T4, FT4, T3, FT3,

and TBG values was observed with advancing age. As expected, thyroid microsomal autoantibody titers also were significantly inversely correlated with T4, FT4, T3, and FT3. Thyroglobulin autoantibody titers showed a significant inverse correlation with T4 and FT4 and a positive correlation with TSH.

In a previous study<sup>18</sup> we investigated the relationship between thyroid function and mental ability in persons with Down syndrome. Intellectual function in patients with both abnormally high TSH and very low T4 levels was significantly lower (mean IQ, 42) than in patients with Down syndrome with increased TSH values only (mean IQ, 54), or in patients with Down syndrome who had normal thyroid

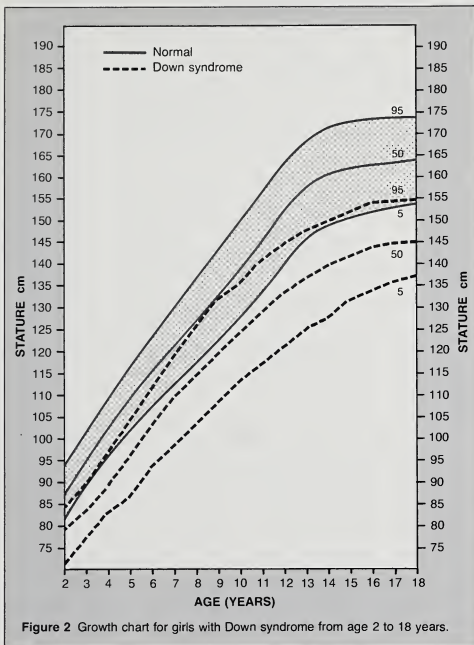


Figure 2 Growth chart for girls with Down syndrome from age 2 to 18 years.

function (mean IQ, 55). It is possible that a decline in IQ in persons with Down syndrome is related to hypothyroidism.<sup>19</sup>

Many features of hypothyroidism are similar to those seen in Down syndrome, which makes it difficult at times to diagnose hypothyroidism clinically in these patients. Because hypothyroidism may compromise normal central nervous system functioning and because clinical symptoms of hypothyroidism are sometimes interpreted as being part of the "Down syndrome gestalt," thyroid function studies, including thyroid antibodies, should be obtained in persons with Down syndrome at regular intervals. Particularly during adolescence and adulthood, annual screening for thyroid dys-

function is recommended. Early detection of thyroid hormone dysfunction and prompt hormone treatment, if hypothyroidism is present, may prevent further cognitive decline and enhance growth and development.

### Sexual Maturation

Because the few reports available on sexual maturation in Down syndrome individuals deal primarily with those who have been institutionalized, we investigated the development of primary and secondary sex characteristics and measured gonadotropin and testosterone levels in 45 male adolescents with Down syndrome reared at home.<sup>20</sup> Pubic hair development did not differ significantly from that of normal boys. As

is true for normal adolescent males, males with Down syndrome initially have darkening of the villosus hair at the base of the penis; hair growth is then observed at the inguinal regions, the mons pubis, and the adjacent portion of the lower abdominal wall, later extending to the umbilical area.

Genital size of these patients was contrasted with that of an age-appropriate normal population. Mean testicular volume and penile length and circumference were not significantly different from the normal population at any age.

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels attained in a single morning sample were increased with advancing age and with sexual maturation, as shown in Table 2. No significant differences were found in those hormone levels when compared with normative data. Other investigators, however, have reported that serum FSH and LH levels were significantly higher in males with Down syndrome when compared with controls.<sup>21-23</sup>

Because most of the subjects in these older studies were residents in state institutions and were much older than the individuals in our study, it is possible that the observed testicular failure, including germinal cell hypoplasia and decreased Leydig cell function, was

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**Table 1.** Thyroid function studies in patients with Down syndrome and controls, mean  $\pm$  SD

| Group         | N   | T4<br>$\mu$ g/dL | FT4<br>ng/dL  | T3<br>ng/dL      | FT3<br>pg/dL  | T3U<br>%       | TSH<br>$\mu$ IU/mL | TBG<br>$\mu$ g/dL |
|---------------|-----|------------------|---------------|------------------|---------------|----------------|--------------------|-------------------|
| Down syndrome | 181 | 8.0 $\pm$ 2.2    | 1.5 $\pm$ 0.4 | 145.5 $\pm$ 32.7 | 2.9 $\pm$ 0.8 | 31.3 $\pm$ 2.4 | 6.9 $\pm$ 14       | 27.7 $\pm$ 11.5   |
| Controls      | 163 | 8.7 $\pm$ 2.3    | 1.6 $\pm$ 0.4 | 128.2 $\pm$ 37.5 | 2.6 $\pm$ 1.1 | 31.2 $\pm$ 4.4 | 2.8 $\pm$ 7.8      | 29.4 $\pm$ 9.6    |
| P value       |     | 0.003            | 0.09          | 0.0001           | 0.08          | 0.84           | 0.0014             | 0.14              |

T4, serum thyroxine; FT4, free thyroxine; T3, triiodothyronine; FT3, free triiodothyronine; T3U, triiodothyronine uptake; TSH, thyroid-stimulating hormone; TBG, thyroxine-binding globulin.

**Table 2.** FSH, LH, and testosterone levels in male adolescents and young adults with Down syndrome according to Tanner Genital Stages\*

| Tanner Stage | FSH, mIU/mL    |          |              | LH, mIU/mL     |          |              | Testosterone, ng/mL |         |              |
|--------------|----------------|----------|--------------|----------------|----------|--------------|---------------------|---------|--------------|
|              | Mean $\pm$ SD  | Range    | Normal Range | Mean $\pm$ SD  | Range    | Normal Range | Mean $\pm$ SD       | Range   | Normal Range |
| 1 (n = 4)    | 3.2 $\pm$ 3.4  | 1.0-9.0  | 3-9          | 4.3 $\pm$ 1.7  | 2.0-6.3  | 4-12         | 0.4 $\pm$ 0.2       | 0.1-0.6 | 0.03-0.1     |
| 2 (n = 7)    | 2.2 $\pm$ 0.5  | 1.8-2.9  | 3-14         | 3.7 $\pm$ 0.5  | 3.2-4.4  | 6-11         | 0.9 $\pm$ 1.0       | 0.1-2.4 | 0.1-0.3      |
| 3 (n = 8)    | 7.0 $\pm$ 3.7  | 2.2-14.0 | 3-15         | 9.3 $\pm$ 2.9  | 5.7-14.9 | 6-16         | 3.4 $\pm$ 1.8       | 0.8-5.6 | 0.7-4.0      |
| 4 (n = 11)   | 10.5 $\pm$ 7.8 | 3.9-30.0 | 4-15         | 15.5 $\pm$ 9.0 | 4.3-33.0 | 7-19         | 5.7 $\pm$ 1.3       | 2.9-7.4 | 2.5-9.0      |
| 5 (n = 12)   | 8.6 $\pm$ 4.6  | 2.7-19.9 | 4-13         | 11.4 $\pm$ 5.0 | 5.3-21.6 | 6-23         | 4.7 $\pm$ 1.9       | 1.3-8.3 | 3.5-12.0     |

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

\*Tanner stages are according to a previous study.

responsible for their findings. These discrepancies between our data and others' should prompt further investigation.

Fertility by males with Down syndrome is exceedingly rare. A recent report is the first of a non-mosaic male with Down syndrome conceiving a child with normal karyotype.<sup>24</sup>

Young females with Down syndrome have menarche at a mean age of 12 years 6 months, an age not significantly different from the menarche of their sisters who did not have Down syndrome.<sup>24</sup> Of the 38 females with Down syndrome who had menstruated at least once, 29 reported regularly occurring menses. The nine females with irregular cycles included three who had menarche only recently, two who had spotted several times without an established pattern, and four who had very irregular patterns but a normal to heavy flow. The average length of the monthly cycle varied from 22 to 33 days, with average menstrual flow lasting about 4 days. Thus, most young women with Down syndrome living in the community have regular menstrual cycles with age of onset similar to that of the

normal female population.<sup>25</sup>

Our investigations of pituitary and ovarian hormones revealed that adolescent females with Down syndrome have concentrations of FSH, LH, and estradiol similar to those of a control population. The rise of FSH and LH during sexual maturation that is observed in individuals without Down syndrome also was observed in our population.

An investigation of follicular development in ovaries of females with Down syndrome was conducted by Hojager and co-workers.<sup>26</sup> They reported that all ovaries from patients with Down syndrome were abnormal, that 42% of their ovaries were quiescent with small, resting follicles and no follicular growth, and that the number, as well as the size, of the antral follicles differed from those in the normal ovary. In another study, Tricomi and co-investigators<sup>27</sup> examined the ovulatory patterns in vaginal smears of females with Down syndrome. They found that nearly 40% of these women had a definite pattern of ovulation, another 15% probably ovulated, and another 15% possibly ovulated. There was

no evidence of ovulation in the remaining 30% of females.

Pogue<sup>28</sup> reported in 1917 that women with Down syndrome are capable of reproduction. Since then, 30 pregnancies occurring in 26 women with Down syndrome have been reported.<sup>25</sup> Approximately half of these children were normal and half had Down syndrome. These reports suggest adequate ovarian function in at least some females with Down syndrome.

In summary, although some endocrine studies have been performed in individuals with Down syndrome, much remains to be done. The relationship between thyroid dysfunction and Down syndrome needs to be elucidated further. Although pubertal development appears normal, the overall potential for reproductive capability remains marginal; conception and childbearing in some females certainly does occur. Stature is usually reduced. Sophisticated studies of growth hormone, LH, and FSH pulsatility have not been reported; thus, little is known about the physiology of pituitary hormone secretion in these individuals.

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# Inflammatory Bowel Disease and Growth Retardation

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Severe impairment of linear growth is a well-known complication of chronic inflammatory bowel disease (IBD) in childhood. Growth failure is often the major complication for which treatment is sought by the chronic IBD patient, and restitution of normal growth often signals remission of disease. Of all the manifestations of IBD in childhood, none is as poorly understood or as resistant to therapy as chronic growth failure. However, reversal of growth failure can be achieved by purely nutritional means.<sup>1</sup>

## Growth Failure

Growth failure in children with IBD is a common and ominous complication (Figure 1). Impairment of linear growth, lack of weight gain,

retarded bone development, and delayed onset of sexual maturation are seen in 15% to 40% of patients with IBD under 21 years of age.

Growth failure may precede clinical illness, often by years. Furthermore, growth failure may occur when clinical disease is quiescent. Under these circumstances, it must be assumed that the chronic demands placed on the body by the presence of undiagnosed inflammatory disease accounts for alterations leading to poor growth. Growth failure is rarely if ever associated with endocrine abnormalities. Tests of hormonal function generally are normal. Recent reports have demonstrated that some chronic IBD children with growth failure have low serum somatomedin-C levels.<sup>2</sup> However, somatomedins are dependent on protein intake, and serum levels rise after repletion of protein nutriture. Thus, this potential mediator requires further study and should not be identified as the final common pathway for growth failure in IBD.

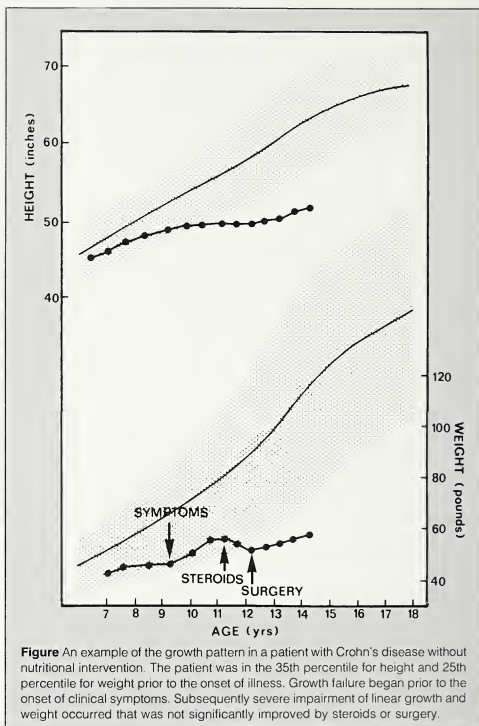
## Etiology of Malnutrition and Growth Failure

The etiology of malnutrition in patients with IBD is multifactorial and generally cannot be ascribed to a single event.<sup>1</sup> The major factors include inadequate dietary intake, excessive gastrointestinal losses, malabsorption, and increased nutritional requirements (Table 1). Inadequate dietary intakes in patients with IBD may occur as a result of the anorexia associated with chronic illness or recurrent bouts of inflammatory activity. Often children refuse to eat because of increased diarrhea or abdominal pain associated with the ingestion of food. Excessive losses of nutrients may originate from the gastrointestinal tract or through the kidneys. Large dosages of exogenous corticosteroids or the endogenous stress-induced response to acute inflammation may lead to increased urinary nutrient losses. Hematochezia, protein-losing enteropathy, and increased fecal losses of cellular constituents are consequences of chronic inflammation and damage to the in-

testinal mucosa. Bile salt-losing enteropathy and subsequent fat malabsorption result from ileal disease, resection, or fistulas.

Malabsorption is more common in patients with Crohn's disease, particularly individuals with small bowel involvement, and less common in ulcerative colitis. Hypoalbuminemia is found in at least 50% of patients due to under-nutrition and/or increased fecal protein loss. Approximately 16% of IBD patients will have abnormal xylose absorption tests, whereas 33% will have a moderate degree of steatorrhea and increased bile acid malabsorption in conjunction with mucosal injury and bacterial overgrowth. Lactose intolerance may also be present either due to the presence of small bowel Crohn's disease or to the genetic background of the patient. Hypocalcemia and hypomagnesemia, when present, are generally associated with enteric protein loss or steatorrhea. Vitamin D deficiency has been described in 25% of older patients evaluated for bone disease associated with Crohn's disease. Vitamin K deficiency, when it occurs, is usually a consequence of steatorrhea. Reductions in serum iron and folate levels are common, and in severe ileal disease or resection, vitamin B<sub>12</sub> deficiency is inevitable. Some children with Crohn's disease have reduced serum zinc levels, but the role of this trace element in conjunction with malnutrition and growth failure is unclear.

Increased nutritional requirements may result from increased inflammatory activity, fever, intestinal fistulas, or periods of rapid growth, particularly during adolescence. Inflammation leads to negative energy and nitrogen balances as a result of decreased dietary intake and increased metabolic activity. Additional nutrient requirements are also a consequence of the demands on growth in children. With a peak weight gain of 7 kg per 6-month interval during puberty and at an energy cost of up to 4.4 cal/g of tissue gained, an additional energy intake of 170 kcal/day may be



**Figure** An example of the growth pattern in a patient with Crohn's disease without nutritional intervention. The patient was in the 35th percentile for height and 25th percentile for weight prior to the onset of illness. Growth failure began prior to the onset of clinical symptoms. Subsequently severe impairment of linear growth and weight occurred that was not significantly improved by steroids or surgery.

needed during the adolescent growth spurt.

### Nutritional Assessment

Regular evaluations are necessary to assess the initial impact of nutritional failure on the child with IBD and growth failure, and to measure the success of therapy over time. Recommendations for nutritional assessment are shown in Table 2. It should be stressed that the use of this sequential assessment allows the clinician to maintain close surveillance not only over nutritional status but also over measurements of linear and ponderal

growth. Alterations in therapy must be made in order to achieve and maintain normal expected growth rates. Carefully maintained data regarding growth and nutrition are mainstays of treatment of children with growth failure and IBD.

### Treatment of Growth Failure

**Medical.** In the routine management of IBD, with or without growth failure, control of inflammatory activity is the first goal of medical treatment. Medications currently used for children with IBD are listed in Table 3, and discussed in detail elsewhere.<sup>1,3</sup> Sulfasalazine

**Table 1.** Etiology of malnutrition in IBD

| Inadequate intake                         | Excessive intestinal losses  |
|---|------------------------------|
| Anorexia                                  | Protein-losing enteropathy   |
| Altered taste                             | Hematochezia                 |
| Abdominal pain                            | Bile salt-losing enteropathy |
| Diarrhea                                  |                              |
| Early satiety                             |                              |
| Malabsorption                             | Increased requirements       |
| Protein                                   | Fever                        |
| Carbohydrate                              | Fistulas                     |
| (xylose, lactose)                         | Repletion of body stores     |
| Minerals (Ca, Mg, Fe, Zn)                 | Growth                       |
| Vitamins (folate, B <sub>12</sub> , D, K) |                              |
| Bacterial overgrowth                      |                              |
| Drug inhibition (folate)                  |                              |

**Table 2.** Evaluation of the nutritional status of children with IBD

| History  |
|--|
| Appetite, extracurricular activity   |
| Type and duration of IBD, frequency of relapse   |
| Severity and extent of current symptoms*   |
| Medications  |
| Three-day diet record  |
| Physical examination   |
| Height, weight, arm circumference, triceps skinfold measurements   |
| Loss of subcutaneous fat, muscle wasting, edema, pallor, skin rash, hepatomegaly                         |
| Laboratory tests   |
| CBC and differential, reticulocyte and platelet count, sedimentation rate, urinalysis                    |
| Stool guaiac, cultures for bacteria, smears for ova, parasites, and fat                                  |
| Serum total proteins, albumin, transferrin, retinol binding protein, orosomucoid, immunoglobulins        |
| Serum electrolytes, calcium, magnesium, phosphate, iron, zinc  |
| Serum folate, vitamins A, E, D, B <sub>12</sub>  |
| Special tests  |
| Xylose absorption, 72-hour fecal fat, fecal $\alpha$ -1-antitrypsin, lactose breath test, Schilling test |
| Radiology  |
| Upper GI series with small bowel follow-through  |
| Air-contrast barium enema  |
| Colonoscopy with biopsies  |

\*Crohn's Disease Activity Index (*Gastroenterology* 1976;70:439) or Lloyd Still Clinical Scoring System (*Dig Dis Sci* 1979;24:620) may be useful in the assessment.

**Table 3.** Commonly used drugs in treatment of IBD

| Drug                                | Daily Dose           | Comment   |
|-------------------------------------|----------------------|---|
| Sulfasalazine                       | 50 mg/kg             | May increase to 75 mg/kg or standard adult dose   |
| Steroids (prednisone, prednisolone) | 1-2 mg/kg            | Single AM dose when possible<br>Dose depends upon severity<br>Not to exceed standard adult dose |
| Azathioprine or 6-MP                | 2 mg/kg<br>1.5 mg/kg | Not to exceed standard adult dose   |
| Metronidazole                       | 15-20 mg/kg          | Not to exceed 1.0 g   |

is recommended for the treatment of mild acute attacks and maintenance of remission when the colon is involved. Some patients with small bowel Crohn's disease will also respond to sulfasalazine therapy, but less predictably.

In contrast, prednisone is more effective in treating moderate to severe activity of disease. Corticosteroids induce remissions, but do not prevent relapses, and may, in fact, increase overall morbidity when used as maintenance therapy. Therefore, corticosteroids are generally recommended in limited courses, using a single morning dose when the severity of the disease permits this form of therapy. Sometimes, twice daily or more frequent oral doses are necessary. Therapy should be maintained for 4 to 6 weeks, tapering to an alternate-day regimen by decreasing the dosage by 5 mg every other day at 5- to 7-day intervals. If necessary, prolonged alternate-day therapy may be maintained. In most cases, this regimen allows for a gradual decrease of medication without flare-up of disease. Low-dose, alternate-day steroid therapy is an acceptable form of long-term treatment.

Many patients with IBD demonstrate accelerated linear growth, despite high-dose steroid therapy, presumably because inflammatory activity is suppressed.<sup>3</sup> An improvement in appetite may account in part for the growth response, because increased dietary protein and energy intakes are associated with corticosteroid use. This may be particularly true when alternate-day steroid therapy is used for a prolonged period of time.

Other medications may be valuable in bringing disease activity under control. Azathioprine and 6-mercaptopurine may allow reduction in the dosage of steroids required, prolong remission, avoid surgery, and allow prolonged maintenance in patients who would not be candidates for other forms of therapy. Metronidazole is valuable for perianal disease, and this agent or vancomycin may be



helpful in those patients whose flare-up of disease activity is associated with *Clostridium difficile* overgrowth.<sup>3</sup>

**Surgical.** Surgical resection of disease has been considered as an alternative in the management of growth failure in patients with IBD, but the results of this approach have not supported its routine use for this purpose. In most studies, children with Crohn's ileocolitis have only limited response to removal of active disease, with only 14% to 28% of patients showing postoperative catch-up growth. Virtually all children who have had catch-up growth after surgery were prepubertal at the time of operation. In general, pubertal patients have shown no catch-up growth after surgery. At the present time, bowel resection should be reserved for those patients in whom there is another clear indication for surgery besides growth failure. In selected cases, where medical and nutritional therapy have failed to alter growth arrest, surgical treatment may be beneficial in prepubertal children.<sup>3</sup>

**Nutritional.** Even in the absence of nutritional failure or growth retardation, the indications (Table 4) and benefits of nutritional therapy in IBD have become apparent. In the nutritional management of children with growth failure and IBD, the major aims are to replace the nutrient losses that are associated with inflammatory processes, to correct body deficits, and to provide sufficient nutrients to promote energy and nitrogen balance for normal metabolic function. In children, additional nutrients must be provided to restore normal growth and to provide catch-up growth.<sup>1</sup>

Both enteral and parenteral routes are available for the treatment of nutritional disorders in IBD (Table 5). The easiest way to provide nutritional supplementation is to increase intake enterally, using standard table foods. No specific diet has been shown to alter the course of ulcerative colitis or Crohn's disease in patients who are in remission. There is also no clear evidence that the con-

sumption or avoidance of specific foods influences the severity of disease or the frequency of relapses, or induces remission. Accordingly, patients should be encouraged to eat an adequate, well-balanced diet and to avoid food fads. In children and adolescents, it is preferable to allow the intake of favorite foods and beverages rather than force a limited energy intake.

When disease is active, when specific foods exacerbate symptoms, or when laboratory tests suggest specific abnormalities such as steatorrhea or lactose intolerance, the diet should be appropriately modified. In the presence of postprandial pain, a low-residue diet, administered as frequent small meals, is often recommended. In children with watery diarrhea due to bile acid or hydroxy fatty acid excretion, a low-fat diet supplemented with medium chain triglycerides and the use of cholestyramine may be helpful in the control of symptoms. However, care must be taken to ensure that patients on low-fat diets are consuming adequate energy intakes.

**Table 4.** Indications for nutritional therapy in IBD

**Primary therapy for disease activity**

Newly diagnosed IBD  
Chronic disease unresponsive to medical management  
Short bowel syndrome  
Closure of fistulas  
Small bowel obstruction  
Ostomy care

**Supportive therapy for disease activity**

Inoperable diffuse disease  
Preoperative nutritional rehabilitation

**Drug-nutrient interactions**

Sulfasalazine (folic acid)

**Abnormalities of specific laboratory test**

Anemia (microcytic, macrocytic)  
Hypoproteinemia  
Fat malabsorption  
Lactose intolerance  
Serum mineral deficiencies (Fe, Ca, Mg, K)  
Serum vitamin deficiencies (folate, B<sub>12</sub>, A, D)  
Prolonged prothrombin time (vitamin K)  
Depressed alkaline phosphatase (Zn)

**Complications of IBD**

Malnutrition  
Growth failure

**Table 5.** Nutritional therapy of IBD

**Well-balanced, high-protein and -energy diet**

± Low residue  
± Lactose-free  
± Low fat; MCT and cholestyramine supplemented

**Enteral supplementation**

(140% to 150% of Recommended Daily Allowances for height age)  
Continuous intermittent nasogastric tube feeding  
Continuous or intermittent feeding gastrostomy

**Total parenteral nutrition**

(140% to 150% of Recommended Daily Allowances for height age)  
Peripheral  
Central

**Minerals and Vitamins**

**Therapeutic**

|  |   |
|--|---|
| Iron                                   |   |
| Ferrous sulfate (20% Fe)               | 6 mg elemental Fe/kg/day, divided in 3 oral doses   |
| Ferrous gluconate (11.5% Fe)           |   |
| Iron dextran (intramuscular) (Imferon) | Follow directions on package insert                 |
| Magnesium                              | 200-400 mg elemental Mg/day, IV                     |
| Zinc sulfate (22% Zn)                  | 50-100 mg elemental Zn/day, divided in 3 oral doses |
| Vitamin B <sub>12</sub>                | 1,000 mg at 3-month intervals, SC or IM             |
| Folate                                 | 1 mg daily  |
| Supplemental                           |   |
| Multivitamins with minerals (daily)    |   |

**Table 6.** Effect of nutritional supplementation on growth in adolescents with IBD

| Measurements*              | Control<br>(n = 5) | Crohn's Disease (n = 6) |                             |
|----------------------------|--------------------|-------------------------|-----------------------------|
|                            |                    | Before<br>Supplement    | After<br>Supplement         |
| Observation<br>period (mo) | 6.5 ± 0.2          | 10 ± 1.4                | 7 ± 0.8                     |
| Height gain†<br>(cm/mo)    | 0.38 ± 0.12        | 0.1 ± 0.08              | 0.5 ± 0.16 <sup>§</sup>     |
| Weight gain†<br>(kg/mo)    | 0.4 ± 0.17         | 0.21 ± 0.09             | 1.22 ± 0.25 <sup>§,  </sup> |

\*Values are mean ± SEM; data are derived from Motil et al, *J Pediatr*

1982;101:345-351

<sup>§</sup>P<0.05 vs before supplementation

†Expected linear rate for age, 0.47 cm/mo <sup>||</sup>P<0.01 vs control

\*Expected weight gain for age, 0.5 kg/mo

Multivitamins with minerals should be administered routinely to replace deficits in the diet. Oral iron and folic acid therapy should be provided when laboratory findings are consistent with a deficiency state. Parenteral administration of vitamin B<sub>12</sub> may be necessary in patients with extensive ileal resection. Despite an association between serum zinc levels and linear growth delay, very few patients with growth failure have low serum zinc levels. However, those who have this abnormality are generally treated with oral zinc supplements.

When the patient is unable to increase dietary protein and energy intakes with larger meals or palatable snacks, oral supplementation with a commercially available liquid formula should be attempted. Successful supplementation may be achieved with such formulas; however, many patients experience early satiety when taking these, and will not increase their total nutrient intake significantly. Under these circumstances nutritional supplementation can be accomplished by intragastric feedings or parenteral alimentation.

Nasogastric infusions, used either continuously or intermittently, have been effective in reversing metabolic imbalances and improving nutritional status, linear and ponderal growth rates, and the clinical well-being of patients with IBD.<sup>4-10</sup> If the patient does not tolerate this form of therapy, a gastrostomy may be performed for ei-

ther continuous or intermittent tube feedings in the same manner as in the nasogastric regimen. The gastrostomy tube is advantageous because it is cosmetically acceptable and easily cared for, and because large increases in the amount of formula can be administered in spite of the patient's lack of appetite. In our experience the only complication associated with intragastric tube feedings has been reversible diarrhea secondary to overly rapid administration of the nutritional supplement.

The amount of supplementation administered via the nasogastric or gastrostomy tube will vary, depending on the nutritional requirements and tolerance level of the individual. In our adolescent

patients, up to 1,500 mL of a commercial formula, administered nightly for 8 to 10 hours, is usually well tolerated. This volume of supplemental formula, in addition to usual meals and snacks, provides protein intake of 3 g/kg/day and energy intake of 95 kcal/kg/day. Results of supplementation are shown in Table 6. After 3 weeks of nutritional supplementation, a weight gain of as much as 4 kg may occur, nitrogen balance improves, and total body potassium increases significantly. After 7 months of nutritional supplementation, average height and weight velocities were at least five times greater than those observed during the 10 months prior to supplementation, and equaled or exceeded velocities of normal adolescents.

These observations demonstrate that the abnormalities in the nutritional status of adolescents with Crohn's disease, malnutrition, and growth failure are not related to intrinsic defects in their metabolic pathways, and that with appropriate nutritional supplementation, growth occurs. Moreover, in these patients, neither the presence of chronic inflammation nor the use of corticosteroids interfered with their rehabilitation.<sup>11,12</sup> We also have recommended that commercially prepared formulas

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be used as an adjunct rather than as the sole source of long-term nutritional intake in order to avoid potential nutrient imbalances.

When patients with IBD are unable to tolerate adequate amounts of enteral alimentation because of disease activity or diarrhea, parenteral alimentation may provide substantial benefits. Parenteral nutrition appropriately improves nutritional status as demonstrated by linear and ponderal growth rates, lean body mass deposition, and postoperative recovery.<sup>5</sup> Nutritional rehabilitation may also induce a clinical remission.<sup>1</sup> Home parenteral alimentation is available for those patients who require long-term nutritional support for active disease, short bowel syndrome, or growth failure.<sup>13</sup> In general, the nutritional recommendations are similar to those used for enteral nutrition support.<sup>1</sup> Patients may be monitored by their own hospital programs or by a com-

mercial nutritional maintenance company.

## Conclusions

Early nutritional intervention is essential in the management of chronic IBD. Individual nutrient deficiencies may occur in children, but more frequently there is a generalized protein-energy malnutrition complicated by progressive growth retardation. The etiology of malnutrition in disease is multifactorial. Patients at risk for developing malnutrition or its complications are those individuals with long-standing disease and weight-for-age deficits. Nutritional intervention provides support during active inflammatory disease, treatment of individual deficiencies, reversal of malnutrition, and stimulation of growth. Prevention of nutritional disorders and their complications in IBD is possible by carefully monitoring appropriate anthropometrics and

laboratory indices, and by promptly instituting enteral or parenteral nutrition rehabilitation as soon as indicated.

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# Commentary: Abortion, Politics, and Science

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Of the inheritable diseases, Lesch-Nyhan syndrome is one of the most devastating. Affected boys are not only profoundly mentally retarded, they engage in compulsive self-mutilating behavior, chewing on their fingers, shoulders, lips—whatever they can reach. Lesch-Nyhan syndrome is especially frustrating because, unlike many inherited illnesses, we understand its pathogenesis—the sex-linked disorder is caused by lack of hypoxanthine phosphoribosyl trans-

ferase—but there is little we can do to help these children. Current efforts at gene therapy to correct the enzyme deficiency have shown promise in vitro, but have yet to solve the problem of how to deliver the enzyme (or its gene) to the appropriate brain cells and induce activity or expression at the appropriate time in development.

Lacking treatment, families with the Lesch-Nyhan gene often seek to have daughters, each of whom stands a 50% chance of being a carrier like her mother, and a 50% chance of being neither carrier nor affected. In contrast, each son has a 50% chance of having the illness. To increase their chance of having a daughter, parents can seek to avail themselves of one of three methods.

Artificial insemination with enrichment for X-bearing sperm can shift the odds somewhat in favor of a girl. Alternatively, parents can turn to in vitro fertilization (IVF).

With DNA amplification via the polymerase chain reaction, the DNA of a single blastomere each from several zygotes can be amplified and exposed to Y-specific DNA probes, in an attempt to identify the Y chromosome. If a female zygote is identified, it can then be transferred to the woman's uterus by IVF.

However, until recently, the usual route followed by most parents was simply to abort any male fetuses identified by chorionic villus sampling or amniocentesis. Of course, Lesch-Nyhan syndrome was not the only sex-linked disorder in which prenatal diagnosis ended in abortions of healthy male fetuses half the time; pregnancies involving Duchenne muscular dystrophy or hemophilia presented the same dilemma.

Fortunately, progress has been made in several sex-linked disorders, including Lesch-Nyhan, so that parents may no longer have to

face the terrible choice of aborting potentially healthy males. DNA amplification and polymerase chain reaction techniques used in the first trimester can now help distinguish between Lesch-Nyhan-affected and unaffected males. The next frontier is to push diagnosis back even further to the pre-embryo stage, and thus preclude many of the emotional issues involved in abortion.

Pre-embryos, tiny spheres whose cells have not yet even "decided" whether they are to become part of the embryo proper or of an extra-embryonic membrane, can be manipulated to reveal information of great benefit to families with sex-linked disorders. Moreover, understanding the metabolic requirements of pre-embryos during those crucial first few days may explain why IVF fails far more often than it succeeds; why millions of zygotes conceived the conventional way do not implant into the endometrium properly; why for every 100 conceptions only 31 survive to be born. We still know comparatively little about this period of the zygote. This is a time in development that has remained shielded from the increasingly commonplace tools of obstetrics; eg, at-home pregnancy kits, ultrasound, chorionic villus sampling and amniocentesis, and alpha-fetoprotein testing.

These are compelling questions, whose answers are not likely to be discovered in the United States. Due to the many ethical/legal issues, research on pre-embryos, embryos, and fetuses is currently blocked by the US government's disregard for federal regulations that in fact provide a mechanism for consideration of such projects. Since 1980, the federal government has made progress in this field virtually impossible because abortion politics, rather than scientific considerations, dominate policy concerning the prenatal human. The result is a bizarre "catch-22" situation: Federal regulations governing human subjects for research ban any federal funding of projects

involving the pre-embryo and sharply restrict any project entailing more than "minimal risk" to the fetus—unless a Health and Human Services (HHS) Ethical Advisory Board (EAB) recommends a waiver of the rules.

But in 1980, HHS officials, fearing political controversy, let the EAB be disbanded—leaving researchers in the incredible predicament of being obliged by law to have their project approved by an entity that does not exist. Outgoing HHS Secretary Otis Bowen did not approve the charter of a new EAB before leaving office. Thus, for the past 9 years, HHS has been in violation of its own regulations.

The United States' reticence toward exploring the biology of the unborn extends to the fetus as well. In May 1988, HHS imposed a moratorium on federally supported human fetal tissue transplant research aimed at relieving conditions such as Parkinson's disease and juvenile diabetes. In early January 1989, an advisory panel appointed by the National Institutes of Health (NIH) recommended that funding be restored. But despite the NIH report, the moratorium has now become a ban on federal funding for research with fetal tissue obtained after induced abortion. This ban was extended by Assistant Secretary for Health James O. Mason.

Meanwhile, talented US researchers are being forced to put on hold or abandon their ideas, or to seek private funding. Consider Oliver H. Lowry, a Washington University biochemist whose experimental protocol utilizing very early human zygotes received a high priority rating by NIH. He was subsequently denied funding because an EAB was not in place. Dr. Lowry, struggling to continue on short-term private funding, is investigating the metabolites and enzymes needed by, and hazardous to, fertilized human ova and early zygotes developing *in vitro*. Says Dr. Lowry, "The advent of IVF has raised a problem and created an opportunity. The problem is that the abnormal fertilized ova that are

now discarded could be used for scientific study. With modern techniques, these discarded ova could provide answers to what may be wrong with present *in vitro* procedures. But even more importantly, such studies could give clues to the cause of early pregnancy loss and fetal malformations in general."

In Great Britain in 1982 a committee of inquiry of the Department of Health and Social Security was convened. It was chaired by Mary Warnock, a philosopher, and consisted of scientists, social workers, lawyers, ethicists, and health administrators. Unlike many other groups charged with answering the eternal question, "When does life begin?" this one actually set some boundaries, concluding, "A human embryo cannot be thought of as a person, or even as a potential person. It is simply a collection of cells, which, unless it implants in a human uterine environment, has no potential for development." Although implantation generally occurs on days 5 to 7 post-fertilization, the committee set day 14 as the time before which research would be permitted. The reason for this cut-off point is anatomical: day 14 is the time of appearance of the primitive streak, the first rudimentary inkling of a central nervous system. This certainly contrasts with the position of a Maryville, Tennessee, judge, who recently bestowed upon the frozen zygotes of a divorced couple the status of "children."

In West Germany, the sort of work being done in Great Britain would be a criminal offense punishable by up to 5 years in prison, if a law drafted in July 1989 is passed in early 1990, as is expected. The country's two main financial providers for scientific research have vowed to refrain from funding embryo work. Behind the Germans' restraint lie several factors: memories of the horrors of Nazi eugenic experiments, the volatile German politics of today, and Chancellor Kohl's need to show that he hears the opposition of the Green Party and religious groups to this research.



No one wants to see women intentionally becoming pregnant in order to sell harvested fetal parts. Every type of research involving human embryos or fetuses requires and should always require approval by appropriate committees. The British, for example, forbid research fusing a human cell with a nonhuman cell and allowing a chimera to develop, or implanting a human fertilized ovum or zygote in the uterus of a nonhuman. Both of these cross-species experiments have been done in nonhuman mammals.

The potential scientific and health benefits of research involving the embryo (and the fetus under some conditions) are now threatened by the debate over the morality of abortion and the nature of the embryo. Without a sensible and coherent national policy on fetal research, infertile couples, families at high genetic risk, fetuses with disorders that may be correctable, and patients afflicted with brain disorders will continue to suffer. So will scientific knowledge.

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## Special Report

### Third International Conference on the Control of the Onset of Puberty, May 7-10, 1989, Amsterdam

Robert M. Blizzard, M.D.

*Chairman*

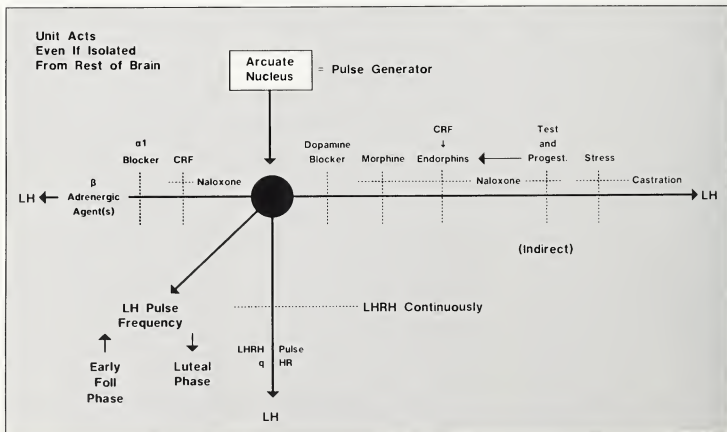
*Growth, Genetics, and Hormones*

The last International Conference on Puberty was held in 1981. In the intervening 8 years much emphasis in investigation has been placed on molecular biology. The content of this 4-day meeting was approximately 80% basic physi-

ology or microbiology and 20% clinical investigation of the onset of human puberty. The proceedings of this conference, which should be available by the time this issue of *Growth, Genetics, and Hormones* arrives on your desk, will provide a valuable resource for all basic scientists and endocrinologists who have an interest in the physiology and pathophysiology

of adolescence.

A highlight of the meeting was an outstanding review by Professor Ernest Knobil of the past and present knowledge regarding luteinizing hormone (LH) pulsatility in the primate. Professor Knobil emphasized that the gonadotropin-releasing hormone (GnRH) pulse generator continues to work when the arcuate nucleus-pituitary



axis is isolated from the rest of the brain in vivo by surgery and that destruction of the arcuate nucleus in the intact monkey negates the pulse generator. Electrodes in the arcuate nucleus block only the release of LH and FSH; the production and secretion of other pituitary hormones remain intact. Down-regulation of LH receptors occurs with constant infusion of luteinizing hormone releasing hormone (LHRH), but if pulses of LHRH are given every hour, ovulatory cycles are induced. This establishes the physiological necessity for pulses of LHRH to occur. Estrogens do not seem to affect the pulse frequency of LH, as both post-menopausal women and younger

women during the midfollicular (estrogen) phase of the cycle have the same frequency of pulsations. The acceleration of the pulse generator during the early follicular phase may be attributed to a release from the inhibitory action of progesterone. Knobil discussed the roles played by neurotransmitters and hormones in the release of LH, and these roles are graphed for easier assimilation (Figure).

Dr. T. Plant of Pittsburgh presented a scholarly paper regarding the ontogeny of GnRH secretion in the rhesus monkey. The differences in basal LH and FSH and in the time of onset of pulsatile LH and FSH between males and fe-

males were discussed. The explanation of these differences remains obscure. A fascinating part of Plant's presentation dealt with the administration of estrogen, testosterone, and dihydrotestosterone to female and male pre-pubertal monkeys. All stimulated body weight and length gain, but only the first two accelerated skeletal maturation. Plant conjectured that this may be because dihydrotestosterone is not aromatizable.

There were many other excellent presentations and readers may wish to consult the proceedings, which are published in a supplement to *Acta Scandinavica*.

## Special Report

### The 4th National Cooperative Growth Study Conference November 18-21, 1989, Palm Springs, California

Robert M. Blizzard, M.D.

*Chairman  
Growth, Genetics, and Hormones*

Among the most important topics covered at this conference were those that pertained to growth hormone (GH), insulin-like growth factor (IGF) binding proteins, and related receptors. The GH receptor is found in large quantities in rabbit liver but also in kidney, muscle, bone, and brain (hypothalamus). Its gene is on the short arm of chromosome 5. The receptor is one of a family of a new type of receptors (prolactin and GH) that serves as a binding protein in plasma and as a receptor in tissue. These receptors do not act through tyrosine kinase and are unrelated in amino acid sequence to other known receptors. The plasma binding protein (BP) is probably the external portion of the GH receptor, which extends outside the cell membrane. This BP and the receptor have both been reported to be absent in Laron dwarfism. In order to analyze the receptor gene in patients with Laron dwarfism, nine patients with this entity were studied; two had a deletion of a large portion of the extracellular hormone-binding do-

main of the receptor gene.

The IGF receptors (IGF-1 and IGF-2) and the insulin receptor are frequently considered together, because the receptors for insulin and IGF-1 are closely related and bind IGF-1, IGF-2, and insulin in various proportions. The receptor for IGF-2, which is also the mannose phosphate receptor, is of a completely different structure and binds IGF-1 and IGF-2 but not insulin. IGF-2 promotes growth by acting through the IGF-1 receptor. IGF-2 *does not* promote growth through its interaction with the IGF-2 receptor, and what role that interaction does play remains obscure.

There are at least 3 IGF BPs. The major BP is BP-3, which comprises 98% of the circulating IGF BP and which is under GH control. IGF BP-3 increases and decreases concomitantly with GH production. This BP is produced in both breast and liver cancer as well as in intact liver. It is a large glycoprotein complex (140 kDa) that has a non-binding alpha subunit (acid labile) and a binding beta subunit (acid stable). These two BP-3 subunits, along with IGF, which is bound to the beta subunit, comprise the large BP.

The three BPs may be responsible for the true autocrine functions of IGF-1 and IGF-2. Because of its binding characteristics for IGF-1, BP-3 may protect the individual against the hypoglycemic effect of IGF-1 and increase the half-life of IGF-1. In uremia this BP-3 is increased to very high levels, possibly because the kidney clears this protein. With chronic renal disease the marked increase in BP-3 may act as the "inhibitor" described for IGF-1 in kidney disease: The excess BP-3 may bind IGF-1 so there appears to be only a small amount of IGF-1 present, which is then misinterpreted as the presence of an inhibitor.

The presenters who addressed these issues were Dr. Michael Ranke of Tübingen, FRG, Dr. Ron Rosenfeld of Stanford University, Palo Alto, CA, and Dr. William Wood of Genentech, South San Francisco, CA.

Robert M. Blizzard, M.D.

**Editor's note:** Dr. Ranke will be contributing a lead article titled "IGF BPs" in a forthcoming issue of *Growth, Genetics, and Hormones*. In it, Dr. Ranke will discuss how measuring IGF BP-3 may assist in diagnosing GH deficiency.

## Prenatal Diagnosis for Pediatricians: Committee on Genetics of the AAP

Rapid advances in technology have prompted this Committee to prepare a new report to inform pediatricians and others in the medical profession about the current status of antenatal diagnosis of genetic disorders and guidelines for parental counselling. The recommendations of the new report are as follows:

*Fetal chromosome analysis* should be offered when:

- maternal age is advanced.
- a previous offspring has a trisomy condition.
- a chromosome abnormality is present in a parent.
- the fetus is at risk for a serious X-linked condition and specific intrauterine diagnosis is unavailable.
- a parent is a "fragile X" carrier.

- a fetal abnormality (eg, omphalocele, hydrocephalus, etc.) has been identified by ultrasound, which might indicate an increased risk for karyotypic abnormalities.

*Biochemical studies* are indicated when:

- a previous child is affected with a biochemical condition.
- couples are at risk because of possible carrier status related to their ethnic origin (eg, sickle cell disease, Tay-Sachs disease).
- neural tube defects are present in a parent or sibling.
- couples are at increased risk for having an infant with a neural tube defect (eg, a low alpha-fetoprotein level is found in maternal serum at screening).

*Molecular genetic studies* are of potential benefit when:

- hemoglobinopathies, hemo-

philia-A, Duchenne or Becker muscular dystrophy, or cystic fibrosis may reasonably be suspected.

Techniques for tissue sampling, including discussion of amniocentesis, chorionic villus sampling, fetal blood sampling, fetal skin sampling, and organ biopsies are also discussed, as are the techniques for fetal visualization, such as ultrasound, fetoscopy, magnetic resonance imaging, and radiography.

A copy of the report should be kept readily available in every pediatrician's office and reviewed frequently.

Committee on Genetics of the AAP  
*Pediatrics* 1989;89:741-744.

Robert M. Blizzard, M.D.

## Biopsy of Human Preimplantation Embryos and Sexing by DNA Amplification

With improvement of in vitro fertilization techniques, major advances in handling preimplantation embryos, and the advent of the polymerase chain reaction (PCR), it was just a matter of time until nondestructive biopsy of a human embryo allowed diagnosis before implantation. In this report, single cells were removed from 38 human embryos at the 6-10 cell cleavage stage (3 days after in vitro fertilization). The individual blastomeres were then subject to amplification of a Y-specific repeat sequence through 60 cycles. On day 6 the embryos were analyzed cytogenetically to determine chromosomal sex using fluorescent Y chromosomes and in situ hybridization with Y-specific probes. Of the 15 "normal" embryos (ie, possessing two pronuclei), all were correctly sexed by

means of DNA amplification. Results were available within 8 hours, which means a "normal," sexed embryo could be transferred to the uterine environment the day of diagnosis. Biopsied embryos appeared to have normal development to the blastocyst stage in the same proportion as unmanipulated embryos, and good morphologic development to a mean of 35.6 cells occurred. M. Monk (*BioEssays* 1988;8:184-189) has previously reported similar techniques for the purpose of preimplantation diagnosis.

Handyside A, Penketh R, Winston R, et al. *Lancet* 1989;1:347-349.

**Editor's comment**—This technique is readily applicable to any disorder for which the gene has been identified and PCR primers developed that can distinguish the mutant gene(s). Preimplantation diagnosis is technically possible and appears to be relatively safe

for the embryo; however, more work concerning safety is needed in animal models. This technique is valuable because it allows the selective implantation of an XX karyotype embryo into the female of a couple at risk for male infants with an X-linked inherited disease such as hyperphosphatemic hyperphosphatemic rickets. Early work on a mouse model for Lesch Nyhan syndrome is very promising.

Judith G. Hall, M.D.

## Gross and Fine Motor Development in 45X and 47XXX Girls

Results of this study indicate that gross and fine motor developmental delays are associated with a mild to moderate sensory-motor integration dysfunction in 45X and 47XXX girls followed over many years. In contrast, near-normal functioning in these parameters was seen in 45X/46XX mosaic girls. The incidence of these chro-

## Oocyte Donation as a Means of Achieving Pregnancy in Women with Primary or Secondary Ovarian Failure

Two groups providing oocyte donation for achieving pregnancy report promising results. A total of 77 patients (from both studies), including women with Turner syndrome, premature ovarian failure, or with a 46 XY karyotype, underwent oocyte donation. Pregnancy rates were in the range of 35% per cycle (rates similar to other forms of in vitro fertilization). Multiple pregnancies (twins/triplets) and preeclamptic toxemia were frequent complications, but all newborns were normal [one with intrauterine growth retardation (IUGR)] and the miscarriage rate was low. Nine women with Turner syndrome (with a variety of karyotypes) had

successful pregnancies. Best results were achieved in women who were regularly cycling prior to attempting pregnancy.

Serhal PF, Craft IL. *Lancet* 1989;1: 1185-1187.

Hens L, Devroey L, Van Waesberghe M, et al. *Clin Genet* 1989; 36:81-91.

**Editor's comment**—*This is good news indeed for Turner syndrome women. It means they have options for reproduction "just like everyone else." The data do suggest, however, that regular monthly cycling prior to attempted pregnancy gives the best results, so appropriate monthly cycling is recommended for Turner women considering this option at any time in the future.*

Judith G. Hall, M.D.

mosome abnormalities in 20,000 consecutive apparent female infants was 1:1,000. The defects in gross motor function included running speed and agility, balance, bilateral coordination, strength, and upper limb coordination. Defects in fine motor function included abnormalities related to upper limb speed and dexterity, visual motor control, and speed of response. Walking late—between 15 and 22 months—was present in 9 of the 15 45X and 47XXX girls studied.

Of these 15, 11 manifested moderate to severe language dysfunction and 12 were referred independently for special educational services. The mean IQ for those in the 45X and 47XXX groups was 83.0, whereas the mean IQ for the mosaic group was 102.0, which was the same as for the normal control group.

The authors conclude that sex chromosome aneuploidy in girls is associated with an increased risk for sensory-motor integration dys-

function. This is likely to be an additional factor that negatively influences classroom performance along with the language delays and depressed cognitive abilities frequently found in these girls. Regular developmental assessments are recommended to provide anticipatory guidance through early identification and intervention. Neuromuscular status and sensory-motor integration should be ongoing as part of the evaluation of children with sex chromosome aneuploidy.

Salbenblatt JA, Meyers DC, Bender BG, et al. *Pediatrics* 1989; 84:678.

**Editor's comment**—*The authors have added significantly to our knowledge and understanding of the mental and physical performance of girls with sex chromosome aneuploidy. I, for one, will be more attentive to these frequent problems in such girls.*

Robert M. Blizzard, M.D.

## Hyponatremia and Inappropriate Secretion of Vasopressin in Patients with Hypopituitarism

Significant and symptomatic hyponatremia is reported in five patients with hypopituitarism that included adrenocorticotrophic hormone (ACTH) deficiency. These patients, although adults, had severe hyponatremia without significant dehydration. Vasopressin [antidiuretic hormone, (ADH)] levels were increased and believed to be the etiology of the inappropriate ADH syndrome. Glucocorticoids are believed to suppress vasopressin production and release, and all patients were restored to the normal osmolar state following physiological doses of hydrocortisone.

The author concludes that hypopituitarism (actually hypocortisolism) should be considered in the differential diagnosis of hyponatremia without dehydration because, given the correct diagnosis, treatment with glucocorticoids is much more effective than treatment with hypertonic saline. The author notes that it is possible to have low basal cortisol levels in this syndrome, and such levels should not preclude suspicion of its presence.

Oelkers W. *N Engl J Med* 1989; 321:492.

**Editor's comment**—*An abstract of this excellent article is appropriate because many pediatric endocrinologists are unaware that hyponatremia results from cortisol deficiency. Cortisol replacement is very much needed in patients with hypopituitarism that includes relative or actual ACTH deficiency. Cortisol replacement is highly desirable in such patients although, in dealing with children, we must be careful not to exceed physiological replacement, in order not to produce growth inhibition.*

Robert M. Blizzard, M.D.



## Growth, Genetics, and Hormones Index for Volume 5, 1989

### Volume 5, Number 1:

"Sexual Precocity, GnRH Analogs, and Growth"

Paul A. Boepple, M.D.  
William F. Crowley, Jr., M.D.

### Special Report:

David W. Smith Workshop on  
Malformations and Morphogenesis,  
August 3-7, 1988, Oakland, California

### Subject Review:

GH Neuroregulation and Its Alterations  
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### Abstracts:

Serum IGF-I and Serum Growth-  
Promoting Activity During the First  
Postnatal Year in Infants with IUGR  
Copper Deficiency Impairs Growth of  
Infants Recovering from Malnutrition  
The Frequency of Genetic Disorders in  
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Knemometry in Childhood: Accuracy  
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### Index for Volume 4, 1988

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Cause of Short Stature"  
Asaria Ashkenazi, M.D.  
"The Remarkable Catch-up Growth of  
American Slaves"  
Richard H. Steckel, Ph.D.

### Editorial Comment:

Slow Grows the Child: Psychosocial  
Aspects of Growth Delay

### Abstracts:

Decreased Height Velocity in Children  
and Adolescent Boys Before the  
Diagnosis of Crohn's Disease  
Birth Weight and Childhood Growth  
Natural History of Williams' Syndrome:  
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Report  
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**Letters to the Editor:**  
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Low-Dose Testosterone in Boys with  
Constitutional Delay of Growth

### Volume 5, Number 3:

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Growth Factors and the Mutations  
Causing Disorders of Growth"  
Victor A. McKusick, M.D.

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Acromegaly in an Infant  
Verification of the Fetal Valproate  
Syndrome Phenotype  
Development of Human Palmar and  
Digital Flexion Creases  
Transient Growth Deceleration in  
Normal Short Children: A Potential  
Source of Bias in Growth Studies  
Atlantoaxial Instability in Down  
Syndrome  
New Concepts of the Growth Spurt of  
Puberty  
Identification of the Molecular Defect in  
a Family with Spondyloepiphyseal  
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### Letters to the Editor:

Effects of Anabolic Steroids  
A Rare Form of Inherited Lipodystrophy

### Volume 5, Number 4:

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Current Concepts"  
Barbara C. McGilivray, M.D.  
"The Final Phase of Growth in Stature"  
Alex F. Roche, M.D., Ph.D., D.Sc.

### Special Reports:

7th International Symposium on Growth  
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1989, Rome, Italy

The American Diabetes Association  
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Alternate-Day Steroid Treatment  
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Glucose Turnover in Growth-  
Hormone-Deficient Children Before  
and After Growth Hormone  
Administration

### Letter from the Editor

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# GROWTH

## Genetics & Hormones

Vol. 6 No. 2

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### The Biology of Bone Growth

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Linear bone growth is a very complex biologic process. Although much is known about the circulating factors that influence it, much less is understood about the process itself, especially in humans. Indeed, to a large extent it has been viewed as a "black box," which when appropriately stimulated generates longer bones. However, as non-growth hormone deficient types of growth deficiency, such as the chondrodysplasias, receive greater attention, it becomes necessary to dissect the black box and examine its mechanisms.

The genesis of the embryonic skeleton and its subsequent linear growth arise from the same three fundamental phenomena: chondrogenesis, cartilage hypertrophy, and osteogenesis. Chondrogenesis is responsible for the formation of a cartilage model of the skeleton and most of its subsequent physical lengthening. Hypertrophy contributes to some extent to lengthening, but its role is mainly to facilitate the transition of the cartilage model to bone. Osteogenesis produces the final skeletal form. After embryogenesis all three processes integrate in a smoothly functional unit that structurally corresponds to the growth plate. A closer examination of the process reveals how this occurs.

The current biologic model of skeletogenesis is derived from

studies of limb development in lower vertebrates, especially the chick. Skeletogenesis begins early in embryogenesis, with the outgrowth of limb buds composed of mesenchymal tissue covered by a layer of ectoderm (Figure 1A). The extracellular matrix produced by the poorly differentiated mesenchymal cells contains noncarti-

laginous molecules such as types I and III collagen, fibronectin, and small proteoglycans. Soon after the bud is formed, in areas destined to become bone, these cells form cellular condensations coincident with the appearance of mRNAs for cartilage-specific proteins, including type II collagen and cartilage (large aggregating)  
continued on page 2

### Autocrine and Paracrine Aspects of Bone Metabolism

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One of the major functions of bone is to provide mechanical support to the body. The strength of bone depends on its volume, which in turn is determined by the balance between two opposing processes, osteoblastic bone formation and osteoclastic bone resorption. Faulty regulation of this balance

leads to disease states, eg, osteoporosis, making studies of the mechanisms of this regulation essential.

Two mechanisms have been postulated for the maintenance of bone volume: (1) systemic regulation by calcium- and phosphate-regulating hormones (eg, parathyroid hormone [PTH], vitamin D, calcitonin) and (2) local regulation. Because all parts of the skeleton are not used equally, local mechanisms are required for appropriate local adaptation. For example, professional tennis players may have a 30% higher bone density in their dominant arm than in the non-dominant one. Local mechanisms are thought to involve growth factors, which stimulate bone formation by increasing osteoblast proliferation and matrix biosynthetic activity.<sup>1</sup> This is not to say that systemic regulation of bone does not utilize growth factors. Indeed, there is evidence that the skeletal effects of at least some hormones (eg, growth hormone and PTH) are  
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proteoglycan core protein. Overt chondrogenesis begins shortly after this condensation, with the synthesis and secretion of matrix rich in these macromolecules and others including types IX and XI collagen, proteoglycan link protein, and several less well characterized noncollagenous matrix proteins. Synthesis of the noncartilaginous proteins such as type I collagen ceases. This process produces the models, or so-called anlagen, of the future skeleton (Figure 1B).

### The Current Biologic Model

Soon after the cartilaginous models are formed, chondrocytes in the center of the anlagen begin to synthesize matrix molecules that are atypical of cartilage. These include type X collagen, fibronectin, and osteopontin. These changes signal the expression of a different type of chondrocyte, the hypertrophic chondrocyte (Figure 1C). More accurately, they indicate a switch in the phenotype expressed by the chondrocytes: Those synthesizing typical cartilage molecules are said to express the differentiated chondrocyte phenotype, whereas those synthesizing the atypical molecules express the hypertrophic chondrocyte phenotype. Other characteristics of the hypertrophic chondrocyte phenotype include a dramatic increase in cell size; expression of the activities of the enzymes alkaline phosphatase and carbonic anhydrase; and reduced synthesis of type II collagen and, possibly, of cartilage proteoglycans, proteoglycan link protein, and protease inhibitors that prevent vascular invasion. The net effect of these changes is that the matrix in the vicinity of the hypertrophic chondrocytes becomes susceptible to invasion by vascular cells penetrating from outside the cartilage model. As the cartilage matrix is degraded, the hypertrophic chondrocytes die and osteoblasts accompanying the vascular invasion begin to deposit bone matrix on fragments of incompletely degraded cartilage—i.e., osteogenesis (Figure 1D). This

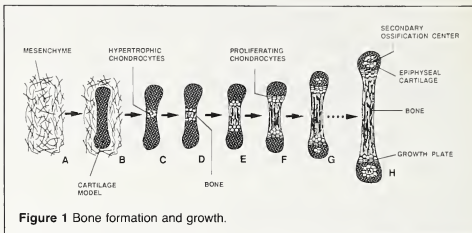


Figure 1 Bone formation and growth.

produces hybrid trabecular structures containing a core of cartilage matrix and a surface of bone matrix. As the trabeculae are remodeled to complete the osteogenesis, the cartilage is degraded. Thus, chondrocyte hypertrophy initiates a cascade of events in which a space originally occupied by cartilage is completely replaced by bone.

As the center of the anlage is converted to bone, an ossification front is created between this newly formed bone and the remaining cartilage. The cartilage side of the front consists of hypertrophic chondrocytes preparing the matrix for vascular invasion, and the bone side is composed of osteoblasts depositing osteoids on spicules of incompletely degraded hypertrophic cartilage. This front spreads centripetally as progressively more of the chondrocytes hypertrophy and in turn more of the anlage is converted to bone (Figure 1D, 1E). Most of the cartilage anlage and resident differentiated chondrocytes are consumed by this process; however, as this front nears the ends of a bone, a new element emerges. The chondrocytes distal to the front begin to proliferate and elaborate typical cartilage matrix before they hypertrophy (Figure 1F). This occurs directionally along the growth axis of the bone and pushes apart the cartilaginous ends of the bone, which are now known as epiphyseal cartilages (Figure 1F-1H). In other words, *de novo* chondrogenesis provides a new and continuous source of cartilage to be converted to bone as the ossification front progresses linearly. With

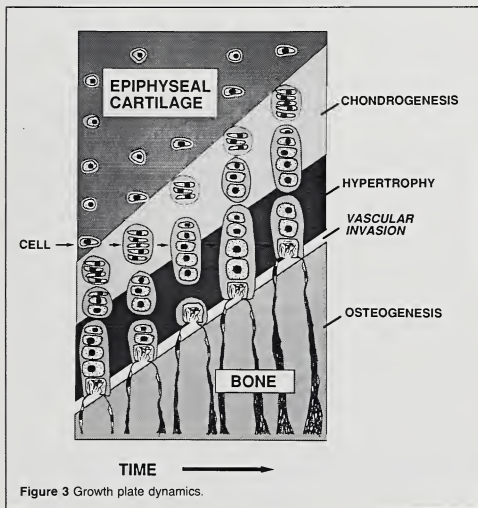
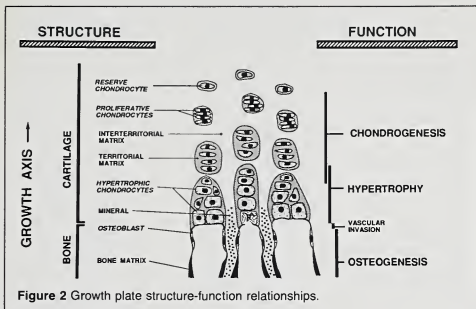
time the *de novo* chondrogenesis (chondrocyte proliferation and matrix production) become incorporated into the ossification front as its leading edge, thereby creating an active growth plate. The bone thereafter grows because new cartilage is formed, hypertrophies, is degraded, and eventually is replaced by bone within the growth plate, which is only a few millimeters thick. The structure and functional correlates of the growth plate are depicted in Figure 2. The dynamic nature of the growth plate is illustrated in Figure 3.

Structural elements of the growth plate can be identified in humans as early as at 16 weeks' gestation. As long as *de novo* chondrogenesis continues, the growth plate remains active and

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the bone continues to grow. However, when it ceases, ie, at the end of puberty, the remaining epiphyseal cartilage undergoes hypertrophy and is replaced by bone.

The epiphyseal cartilages also give rise to secondary ossification centers during late fetal life and early childhood (Figure 1H). These develop in much the same way

that the primary ossification centers arise in the center of the cartilage anlagen. They enlarge slowly as the cartilage is converted to bone and correspond to the "epiphyses" seen on X-ray.

Thus, the growth plate is a linear and dynamic structure in which de novo chondrogenesis provides a cartilage model that is modified through hypertrophy and eventu-

ally replaced by bone through osteogenesis. The coordinate regulation of these processes is not well understood, and a thorough discussion of the subject is beyond the scope of this brief review. Nevertheless, several important points can be made.

First, although osteogenesis alters the structural and mechanical properties of the skeleton, only chondrogenesis and hypertrophy contribute to physical lengthening. Second, these two processes reflect the progression of cells down a differentiation pathway (Figure 4). At the single cell level they correspond to the expression of the differentiated and the hypertrophic chondrocyte phenotypes, to which is added the element of proliferation. Thus, the factors that ultimately regulate bone growth are those that at the cellular level affect chondrocyte proliferation, expression of the two chondrocyte phenotypes, and progression of cells down the differentiation pathway.

Many factors have been shown to influence one or more aspects of this scheme through endocrine, paracrine, and possibly autocrine mechanisms. Indeed, at least three types of receptors have been either demonstrated or postulated to exist on chondrocytes. The first type includes cell-surface receptors that bind protein/peptide growth factors, such as growth hormone, insulin, insulin-like growth factor (IGF)-I, IGF-II, parathyroid hormone (PTH), epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor (TGF)- $\beta$ . These receptors modulate signals across the plasma membrane that influence events within the cytoplasm and generate new intracellular signals. The second type of receptors, cytoplasmic receptors, respond to several steroid hormones (ie, glucocorticoids), estrogens, vitamin D metabolites, and retinoic acid derivatives. Activated hormone receptor complexes in the nucleus act by binding to specific DNA sequences and regulating gene transcription. The third type of receptor binds extracellularly to specific recogni-



tion sequences on matrix macromolecules, such as collagens, coupling them to intracellular cytoskeletal proteins. Some proteoglycans also function in this manner as do a large group of transmembrane molecules (integrins). These cell adhesion receptors permit the extracellular matrix, which is secreted and organized by the cells, to feed back to the cells and thereby affect their cytoskeletal organization.

Considerable interplay seems to occur among these receptor signalling mechanisms. For example, growth hormone is thought to modify the density of receptors for a number of peptide growth factors. Likewise, TGF- $\beta$  may regulate the expression of integrin receptors. Furthermore, the signalling mechanisms are not distinct. EGF-like domains have been identified on several extracellular matrix molecules, and recognition sequences for integrin receptors have been demonstrated on one of the IGF-I binding proteins (BP-2).

The cellular regulation of chondrogenesis and chondrocyte hypertrophy is extremely complex. Although many hormones and growth factors are known to bring about various responses from chondrocytes, such as mitosis or synthesis of matrix molecules, the manner in which the many signals are integrated and related to each other and to the regulation of the overall developmental scheme is largely unknown.

This discussion provides an abbreviated and simplified view of how bones develop and grow. It also provides a framework in which to view the chondrodysplasias, inherited disorders of bone growth of which well over 100 distinct clinical entities are currently recognized. However, considering the complexity of the above scheme and the diversity of mutations that can occur within a single gene, the number of potential disorders, or more appropriately clinical phenotypes, is much larger. Candidate genes for mutations causing chondrodysplasias include those that are either expressed as components of one or

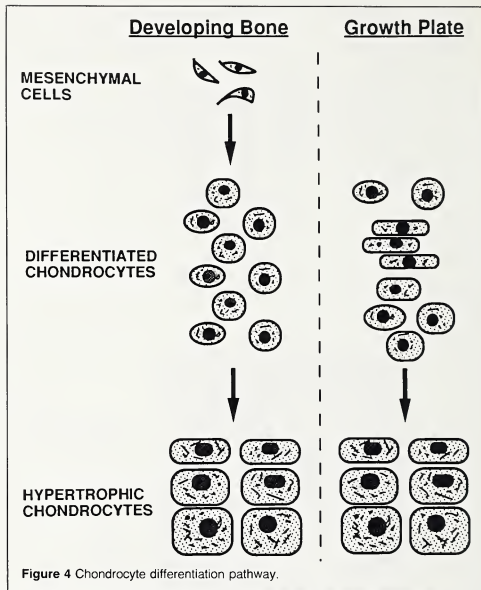


Figure 4 Chondrocyte differentiation pathway.

more of the chondrocyte phenotypes or whose expression is involved in regulating chondrocyte proliferation or the progression of cells down the differentiation pathway. Much attention is currently focused on detecting mutations of the genes encoding cartilage matrix macromolecules, especially

#### Suggested Readings

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type II collagen. However, as the cellular and molecular biology of the overall process and its local regulation becomes better understood, the search will broaden, ultimately leading to results that will provide the basis for future approaches to therapy for short stature.

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## Autocrine and Paracrine Aspects of Bone Metabolism

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mediated by production of local growth factors under the influence of these systemic hormones. Recent findings—that skeletal tissue is a major storage site for growth factors and that bone cells in culture produce and respond to bone growth factors—support the concept that regulation of bone volume may depend on the local growth-promoting activities of bone-derived growth factors.

We will evaluate the potential role(s) of human bone-derived growth factors as determinants of local bone formation by discussing (1) growth factors stored in human bone; (2) growth factors produced by human bone cells; and (3) the biologic actions of human bone-derived growth factors.

### Growth Factors Stored in Human Bone

Bone has the unique ability of self-regeneration in response to mechanical injury or tissue wasting. In recent years it has become apparent that these self-regenerative properties may result from the

presence of bioactive polypeptide factors in the extracellular matrix of bone. Our studies during the past few years have centered on one such bioactive factor, skeletal growth factor (SGF).

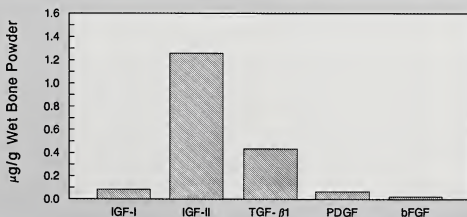
SGF is present in high-molecular-weight forms in nondissociated extracts of both human bone and serum-free human bone cell-conditioned medium, apparently in complexes with binding proteins. Subsequently human SGF has been dissociated from these high-molecular-weight complexes and purified to homogeneity.<sup>2</sup> Structural studies of human SGF have revealed that the amino acid sequences of the amino terminal region and several tryptic fragments of human SGF were identical to the corresponding sequences of insulin-like growth factor II (IGF-II) from human serum, thus suggesting that SGF is very similar, if not identical, to IGF-II. During the purification of SGF from human bone matrix extract, we found evidence for the presence of additional growth factors. Characterization of

these additional growth factor activities (Figure 1) revealed that:

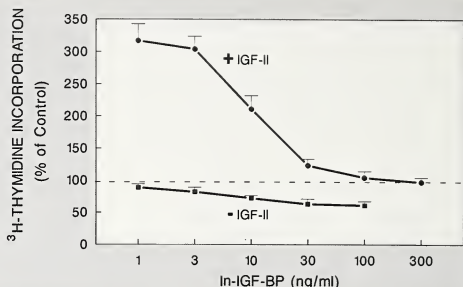
1. Human bone matrix contains multiple growth factors, including IGF-I, IGF-II, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF).
2. Human bone matrix does not contain detectable amounts of epidermal growth factor.
3. IGF-II and TGF- $\beta$ 1 are the two most abundant growth factors present in human bone matrix. IGF-I, PDGF, and bFGF are several-fold less abundant.<sup>3</sup>

The majority of TGF- $\beta$ 1 is present in an inactive (latent) form in human bone matrix extract under nondenaturing conditions. Latent TGF- $\beta$ 1, however, can be activated by treatment with acid, by certain proteases, or by deglycosylation. Several bioactive factors have also been identified in dissociative extracts of bovine and rat bones. These include bone morphogenic proteins, osteoinductive factor, osteogenin, and chemo-

**Figure 1** Relative distribution of growth factors in human bone. Matrix proteins were extracted from human bone powder by demineralization in a solution of 10% EDTA containing 4 M guanidine-HCl and protease inhibitors. The extracts were desalted and used for growth factor measurements using specific assays.



**Figure 2** In-IGF-BP inhibits both basal and IGF-II-induced chick bone cell proliferation. The incorporation of [ $^3$ H]thymidine into DNA of chick calvarial cells was determined in the presence or absence (basal) of 3 ng/mL IGF-II and varying concentrations of In-IGF-BP. The values are means  $\pm$  SD of six replicate wells. Basal and IGF-II-stimulated [ $^3$ H]thymidine incorporation was significantly inhibited ( $P \leq 0.001$ ) by In-IGF-BP at 10, 30, 100, and 300 ng/mL.



tactic factors. Thus, skeletal tissues may constitute the single largest storage site for growth factors in the body.

#### Growth Factors Produced by Human Bone Cells

Human bone cells in culture produce a number of growth factors, many of which are known to be stored in human bone matrix, including IGF-I, IGF-II, TGF- $\beta$ 1, and PDGF. Bovine bone cells have been shown to produce bFGF, and since bFGF is found in human bone matrix, it seems likely that human bone cells also produce bFGF. Of these growth factors, IGF-II seems to be the most abundant mitogen produced by human bone cells. IGF-I is produced by human bone cells at 50- to 100-fold less concentration than IGF-II. As mentioned earlier, systemic hormones may modulate local bone formation at least in part through regulation of synthesis and release of bone growth factors. For example, PTH (which can also act as a bone cell mitogen) was shown to increase the release of IGF-I in rat bone cell cultures and the release of both IGF-I and IGF-II in mouse bone cultures.<sup>4,5</sup> Recent experiments have also shown that steroid hormone, 17 $\beta$ -estradiol increased the release of IGF-I, IGF-II, and TGF- $\beta$ 1 in the rat osteosarcoma cell line, UMR106.<sup>6</sup> These findings suggest that agents that stimulate bone formation may modulate their effects

by altering the production of one or more bone growth factors in local skeletal sites.

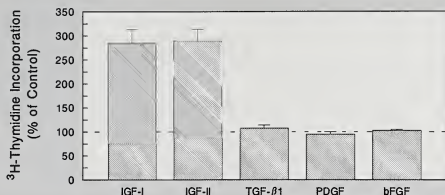
We have recently shown that human bone cells produce, in addition to IGF-II, a binding protein known as inhibitory IGF-binding protein (In-IGF-BP). Studies on the N-terminal amino acid sequence and amino acid composition have revealed that In-IGF-BP purified from human bone cell-conditioned medium is unique, with limited sequence similarities to other known IGF-binding proteins.<sup>7</sup> Studies on the biologic actions of In-IGF-BP have revealed that it acts by inhibiting the binding of IGF-II to bone cell receptors. Figure 2 shows that 3 ng/mL of IGF-II stimulated bone cell proliferation (determined by [ $^3$ H]thymidine incorporation into DNA) 2.2-fold over controls in chick calvarial cells. The 3 ng/mL of IGF-II-stimulated bone cell proliferation was inhibited dose dependently with increasing concentrations of In-IGF-BP, and at 100 ng/mL, In-IGF-BP completely inhibited stimulation by 3 ng/mL IGF-II. In addition, In-IGF-BP also inhibited basal chick bone cell proliferation in a dose-dependent manner, with maximal inhibition of 40% at 100 ng/mL In-IGF-BP ( $P < 0.001$ ), thus emphasizing the importance of local (paracrine and autocrine) IGFs in bone cell proliferation. These findings are consistent with previous results showing that bone cells in culture produce IGF-I and IGF-II. Our recent find-

ings have also shown that the production of In-IGF-BP by bone cells is regulated. For example, prostaglandin E<sub>2</sub> and dibutyl cyclic AMP stimulated production in a dose-dependent manner.<sup>7</sup> These findings together suggest that In-IGF-BP may act as an important local regulator of IGF-II actions.

#### Biologic Actions of Human Bone-Derived Growth Factors

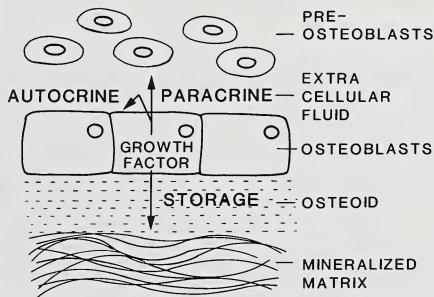
In recent years it has become evident that bone cells from non-human species may respond quite differently than human bone cells to growth factors. For example, TGF- $\beta$ 1 has been shown to be a potent mitogen for chick bone cells but has not been shown to stimulate human bone cell proliferation. Thus, for in vitro studies to be relevant to human physiology and pathology, human bone cells must be used. On the other hand, human bone cells are difficult to grow and thus there is a dearth of published reports on them. Work from our lab shows the effects of known bone-derived growth factors on proliferation of human bone cells isolated from trabecular bone of femoral head samples (Figure 3): 30 ng/mL of IGF-I or IGF-II doubled the stimulation of human bone cell proliferation, compared with control, with identical dose-response curves for IGF-I and IGF-II. The concentration required for half-maximal stimulation was estimated to be 25 ng/mL for either factor.<sup>8</sup> IGF-II also increased syn-

**Figure 3** Effect of growth factors found in human bone on human bone cell proliferation. Values are means  $\pm$  SEM of six replicate wells. Stimulation by 30 ng/mL IGF-I or IGF-II was significant at  $P \leq 0.001$ . TGF- $\beta$ 1, PDGF, or bFGF at 5 ng/mL had no effect on human bone cell proliferation under the culture conditions tested in this study.<sup>8</sup>



thesis of type I collagen and thus stimulated the differentiation of human bone cells. TGF- $\beta$ 1, PDGF, and bFGF had no effect on human bone cell proliferation under the conditions tested in this study. However, more recently, with a different set of culture conditions, we have found that PDGF and bFGF each stimulated human bone cell proliferation, whereas TGF- $\beta$ 1 did not stimulate human bone cell proliferation under any of the culture conditions we tested. TGF- $\beta$ 1 did stimulate production of type I collagen in human bone cells. Furthermore, *in vivo* stimulatory effects of TGF- $\beta$ 1 on bone formation have been reported by two different groups. Thus, each of the bone-derived growth factors identified in human bone matrix has been shown to increase proliferation and/or collagen synthesis in cells of osteoblastic lineage. We and others have proposed that these growth factors may act individually or in concert to stimulate the local bone formation.

We speculate that the bone-derived growth factors may act in an autocrine, paracrine, or delayed paracrine manner in the bone microenvironment. These growth factors are either incorporated into bone matrix or they diffuse to the extracellular fluid (Figure 4). On the other hand, growth factors secreted into extracellular fluid will have an acute autocrine or paracrine action on osteoblast-like cells. The finding that cells of the murine clonal osteoblastic cell line, MC3T3-E1 (representing relatively mature osteoblasts) both produce and respond to IGF-II



**Figure 4** Model illustrating both storage of growth factors in bone and autocrine and paracrine actions of these factors on bone cells. Growth factors produced by osteoblasts can diffuse toward, and be deposited in, bone, or diffuse into the extracellular fluid to act on the same cell in an autocrine manner or on a nearby cell in a paracrine manner.

supports an autocrine action of IGF-II in bone cells. On the other hand, growth factors secreted into extracellular fluid could also act on nearby cells in a paracrine manner. For example, IGF-II produced by mature osteoblasts may act on nearby preosteoblasts as a paracrine agent to stimulate cell proliferation.

In contrast to these acute effects, growth factors stored in bone may also function as delayed paracrine agents, coupling bone formation to bone resorption.<sup>9</sup> Hence growth factors may be deposited for a time in bone and then released by osteoclastic bone resorption in a bioactive form to act on preosteoblasts and mature osteoblasts, thus allowing for site-

specific replacement of bone that was lost to resorption (Figure 5).

### Future Directions

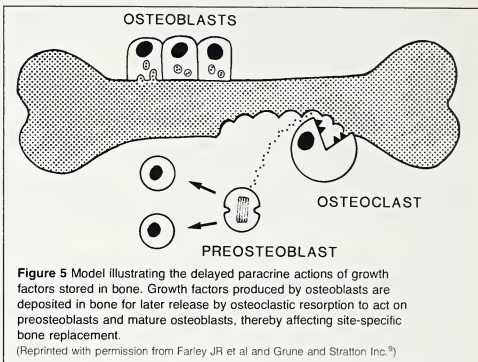
In terms of what we know now and how this knowledge should be extended by future investigations, there is a considerable body of data on the regulation of serum IGF-I. In contrast, we know much less about the regulation of serum IGF-II or of the family of IGF binding proteins. The binding proteins probably function not only as carriers for the IGFs in serum but also as modulators of their actions. It would seem prudent to pursue the serum regulation of In-IGF-BP inasmuch as it can completely abolish the stimulatory effects of both IGFs. However, before embarking



on extensive studies of the serum IGFs and their binding proteins, methods must be developed that allow measurement of the IGFs specifically, without the potential artifacts of the binding proteins, and conversely, of the binding proteins without measuring the IGFs. It seems likely that in the past we have interpreted serum changes in IGFs when actually the changes were at least in part due to changes in the amounts of circulating binding proteins.

In past decades, efforts have concentrated on determining the regulation of hormones and hormone-like molecules in the circulation. More recently, the autocrine and paracrine actions of those hormones, which are actually local messenger molecules as well, have been studied. For example, vitamin D may have different effects when it behaves as a hormone and when it behaves as an autocrine or paracrine agent. The active metabolite to vitamin D,  $1,25(\text{OH})_2\text{D}_3$ , is synthesized in the kidney and circulates as a hormone to regulate calcium metabolism and specifically to increase calcium absorption. However,  $1,25(\text{OH})_2\text{D}_3$  is not exclusively produced in the kidney. It is also produced in several other tissues, where it is thought to behave as an autocrine or a paracrine agent to facilitate the process of cell differentiation. Thus, as a hormone it regulates calcium metabolism at the level of both the gut and bone, whereas, as an autocrine and a paracrine agent, it may well act as a differentiation promoter.

Similarly, IGFs may also act as both hormones and local messengers. It is thought that the majority of the circulating IGFs are synthesized in the liver, however we now know that many other organs produce the IGFs, perhaps for use as autocrine and paracrine messenger molecules. Regulation of liver production of IGFs for hormonal use may be quite different from the autocrine/paracrine regulation of the IGFs. Thus we cannot understand the IGFs by looking only at serum; we must also examine the local effectors of IGF secretion in



**Figure 5** Model illustrating the delayed paracrine actions of growth factors stored in bone. Growth factors produced by osteoblasts are deposited in bone for later release by osteoclastic resorption to act on preosteoblasts and mature osteoblasts, thereby affecting site-specific bone replacement.

(Reprinted with permission from Farley JR et al and Grune and Stratton Inc.<sup>9</sup>)

various tissues. It is possible that the regulation of IGFs at all local levels, including the gene level, may be different in the liver than in other tissues. Such studies of the paracrine/autocrine actions of the IGFs will be technically difficult, because for true clinical relevance, they must be studied *in vivo*; unfortunately, technology now permits their study only *in vitro*.

Thus far we have discussed general opportunities for future investigations of the IGFs and their corresponding binding proteins. More specifically, we would suggest that some emphasis should be placed on examining the role of the IGFs in mediating the tissue-promoting (anabolic) activities of physical exercise. It is now well established that exercise promotes large changes in growth hormones. It is also possible that locally, exercise somehow signals an increase in IGF production or action. If so, it could well be that exercise increases tissue anabolism, not only by the hormonal actions of circulating IGFs, but also by the actions of locally produced IGFs in response to local mechanical loads.

Another important area in which growth factors may be operative is in the determination of peak bone mass, which occurs at about 25 to 30 years of age. The higher the

peak bone mass, the less likely a patient will, during aging, lose bone down to a level where fractures begin to occur. Thus, one way to mitigate osteoporosis would be to promote a high peak bone mass. It seems likely that growth factors, and perhaps the IGFs, are involved in determining peak bone mass. These possibilities could be explored by observing restriction fragment length polymorphism (a clinical study). Another approach would be to correlate bone density to either serum or bone IGFs; individuals with a high peak bone mass would be expected to have high IGF levels in either serum or bone.

IGFs may also play a role in coupling bone formation to resorption. The hypothesis is that whenever there is an increase in bone resorption, there will, after a brief delay, be a corresponding increase in bone formation to maintain a constant appropriate bone mass. On the other hand, during estrogen deficiency after menopause, there is a large increase in bone resorption with an inadequate increase in bone formation, causing a progressive loss of bone. *In vitro* studies in rats suggest that bone cells make IGFs as well as TGF- $\beta$ 1 in response to estrogen, leading to the hypothesis that during estrogen deficiency, growth factors drop to lev-

els insufficient to mediate a coupled increase in bone formation. The importance of estrogen deficiency in the development of osteoporosis cannot be overemphasized, and any clarification of the role of estrogens on growth factor and binding protein production by bone cells would be an important advance.

Similarly, we know that androgen deficiency causes a marked bone loss in males and that androgens are potent mitogens for bone cells. However, the mechanism of growth factor involvement in the androgen response is large-

ly unknown. These androgenic actions are not only relevant to the aging male in whom androgen deficiency probably contributes to bone loss, but also to adolescent children during the growth spurt. In the same manner, estrogen deficiency accounts for menopausal bone loss as well as decreased adolescent growth spurt.

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## GnRHa Therapy: Questions and Answers

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#### Editor's note:

*There is considerable interest today in the use of GnRH analogs for the treatment of sexual precocity. There is also much confusion about how to monitor for suppression of LH and FSH when the various analogs are used. In an attempt to clarify this confusion, Dr. Paul Boepple succinctly answered several questions that I posed to him. Readers may benefit from these responses, with which I agree. Dr. Boepple previously published an article titled, "Sexual Precocity, GnRH Analogs, and Growth" in this journal (Vol. 5, No. 1, March 1989).*

Robert M. Blizzard, M.D.

*How do the potency and pharmacokinetics of different gonadotropin-releasing hormone (GnRH) analogs correspond with clinical observations?*

To date only GnRH agonist (GnRHa) analogs are used in clinical situations. These agents

stimulate the release of leutinizing hormone (LH) and follicle-stimulating hormone (FSH) and induce gonadotropin desensitization with continuous exposure of high concentrations to the pituitary. Desensitization thus depends on the potency of the LHRHa, the frequency of administration, bioavailability (eg, only a small fraction of an intranasal dose is absorbed), and clearance. Less potent and more rapidly cleared agonists are less effective in inducing complete suppression. Importantly, some agonists given intranasally or subcutaneously are cleared so rapidly that the pituitary is not completely suppressed and responds to the next agonist dose with increased release of LH and FSH.

#### *What methods of monitoring do you recommend?*

"Undetectable" levels of estradiol in serum are insufficient proof of suppression since most radioimmunoassays are not sensitive enough to measure small but clinically significant levels. The best evidence for suppression is failure of the LH to rise when the pituitary is "challenged," either with LHRH or LHRHa, intravenously. FSH measurement is desirable but not absolutely necessary. The physician must ensure that the LH assay

used *does not* measure  $\alpha$ -subunits, as  $\alpha$ -subunits continue to be released even when LH and FSH are suppressed, and LH assays that have cross-reactivity between LH and  $\alpha$ -subunits can be misinterpreted.

#### *What about comparisons of the dose-effectiveness of the various analogs?*

Relative potencies have been determined primarily in *in vitro* assays. While these cannot be translated directly for use in humans, the doses required clinically follow the rank order of these potency determinations. For each LHRHa the dosage must be established by monitoring for complete suppression in each patient.

#### *Must suppression be complete or is it acceptable for small amounts of estrogen or testosterone to be secreted?*

Opinions vary; however, our group believes that complete suppression is exceedingly important. The failure of medroxyprogesterone acetate or cyproterone acetate to have a significant impact on final height in precocity may very well be a result of their inability to achieve complete suppression of gonadal activity.

*Is the timing for biochemical evaluation of complete LH suppression in relation to the time of agonist administration important?*

Yes. Randomly measured gonadotropin and sex steroid levels may be low, even in incompletely suppressed patients. LH, FSH, and sex steroids may rise for several hours after the daily dose of LHRHa and then return to "suppressed" levels before the next dose. However, since the time course of the pituitary and gonadal responses are different, apparent discrepancies may arise (eg, in a single random sample, estradiol or testosterone may be increased after LH and FSH have returned to baseline). Monitoring must be done after a "pituitary challenge" to prove that the axis is suppressed.

*Is there a difference in the way children of various height ages or bone ages (BAs) respond to the analog?*

LHRHa can produce complete suppression of LH secretion re-

gardless of age, if an adequate dosage is used. However, children with BA  $\leq 12$  years will grow more rapidly with treatment than children with BA  $> 12$  years. It stands to reason that the growth patterns will be different. Without sex steroids younger children have a decrease in growth velocity but grow at age-appropriate rates, and their BA maturation slows to a normal rate but does not stop. Growth is slower in children with BAs in the late pubertal range, but their BAs show very little progression when sex steroids are removed. Even though growth velocity is  $< 4$  cm/yr in these patients, epiphyseal fusion is de-

layed and final heights surpass pretherapy predictions.

*Is LHRHa indicated in growth hormone (GH)-deficient patients who have entered adolescent development and who are short?*

Theoretically this might be of benefit. However, I cannot advocate this now outside an investigational protocol. The data to support its use are not available. Similarly, its use in other causes of short stature, with or without GH, cannot be currently approved. Appropriate protocols to answer these questions are needed.

In Future Issues

**IGF-Binding Proteins:  
Their Physiological and Clinical Importance**

by Michael Ranke, M.D.

**Genomic Imprinting**

by Judith G. Hall, M.D.

**Complications of Excessive GH in Acromegaly**

by Mark Hartman, M.D.

**Robinow Syndrome: An Update**

by Meinhard Robinow, M.D.

**Childhood Obesity**

by William Dietz, M.D.

## Special Report

### American Society of Human Genetics Meeting

November 12-15, 1989, Baltimore, Maryland

Judith G. Hall, M.D.

*Associate Editor*

*Growth, Genetics, and Hormones*

Among many outstanding symposiums and presentations, some highlights of this meeting included a report by Tsui of the isolation of the cystic fibrosis gene on chromosome 7. According to Tsui, 70% of individuals carrying the cystic fibrosis gene have the same defect (allele). Recent work has attempted to characterize the other 30%. With the isolation of the gene, work on its function and on the pathogenesis of cystic fibrosis becomes the central issue, along with the question of whether newborn screening should be adopted.

Nicholls and co-workers reported several patients with Prader-Willi syndrome who, instead of

having deletions of chromosome 15, had inherited two copies of chromosome 15 from their mothers. Both isodisomy and heterodisomy of maternal chromosome 15 were reported. This suggests strongly that it is the *absence* of the specific locus in the p11-p13 region on chromosome 15 that is responsible for producing the syndrome.

Verlinsky and co-workers presented a new approach to pre-conception prenatal diagnosis, using in vitro fertilization techniques prior to fertilization. After removing the first polar body, they were able to analyze its DNA using the polymerase chain reaction, to see whether it carried an abnormal allele. They were looking for the abnormal allele of the  $\alpha$ -1 antitrypsin gene, but almost any other characterized gene could be analyzed in

the same way. If the abnormal allele is in the polar body, the egg will be left with the normal gene; thus prenatal diagnosis can be accomplished prior to fertilization and implantation. The problem with the technique is that (1) crossover occurs with meiosis, and (2) the polar body may be heterozygous.

A large symposium was held on the status of the human genome project. Both the NIH and the Department of Energy are advocating an improvement of techniques for mapping and isolating genes, particularly with regard to technology, management of large amounts of information, and communication between researchers. In addition, a number of issues have arisen relating to the ethics of the research itself and to the ethical uses of the information that is obtained.

## Effects of Chronic Overproduction of GH and IGF-I in Transgenic Mice

An animal model of gigantism was created a few years ago by developing a transgenic mouse that expressed high levels of growth hormone (GH). The animals exhibited a dramatic increase in size and weight as well as a variety of complications (*Nature* 1982;300: 611-615). Because nonmurine GH genes were used and also because the GH was expressed in many organs, it was not known if the pathologic effects were due to chronically high levels of circulating GH or to other factors. To resolve this question, another transgenic mouse model was created in which hypothalamic growth hormone releasing factor (GRF) was overproduced. This caused hyperplasia and hypertrophy of pituitary somatotrophs with secretion of excessive amounts of endogenous GH in the transgenic mice (*Nature* 1985;315:413-416). Since many of the effects of GH are mediated by insulin-like growth factor I (IGF-I), Quaife et al produced another transgenic mouse in which IGF-I is overproduced. They also compared a number of parameters in the three transgenic mouse models and controls.

In general, animals with high levels of GH exhibited similar features regardless of the source of the "trans" GH gene (rat, human, bovine), the promoter that regulated its expression (metallothionein or albumin promoter), or whether the GH excess was endogenous from GRF overstimulation or from expression of a foreign GH gene. When these animals (high-GH animals) were compared to animals with high IGF-I levels that resulted from IGF-I transgene expression (high IGF-I animals), several differences were detected. Although both animals weighed much more than controls, linear skeletal growth was increased in the high-GH animals

but not in the high-IGF-I animals. In addition to GH, insulin levels were greatly increased in the high-GH animals, whereas both were subsequently reduced in the high-IGF-I animals. Hepatic and renal pathologic lesions were seen in the high-GH animals but not in the high-IGF-I animals. The lesions consisted of hyperplasia, hypertrophy, and sclerosis in the liver and increased glomerular size, mesangial hypercellularity, and glomerular sclerosis in the kidneys. The renal lesions resembled those found in diabetes. Thickening of the skin due to an increase in dermal and subdermal fat was observed only in the high-IGF-I animals. Cholesterol tended to be elevated in the high-GH animals, whereas triglycerides were elevated in the high-IGF-I animals. Finally, survival was reduced in the high-GH animals; 60% were alive at 6 months of age compared with 100% of controls. The deaths were attributed to renal disease. Survival was not examined in the high-IGF-I animals.

The authors concluded that chronically elevated GH has detrimental effects on a number of organ systems and that many of these effects are not mediated by IGF-I alone. They acknowledged many differences between transgenic models of GH elevation and the clinical administration of GH in humans but cautioned that the long term effects of GH treatment in children must be carefully evaluated.

Quaife CJ, Mathews LS, Pinkert CA, et al. *Endocrinology* 1989; 124:40-48.

**Editor's comment**—As the authors point out, the experimental models employed in this study of the effects of chronic GH and IGF-I stimulation differ both qualitatively and quantitatively from the clinical setting in which GH is administered to children. Nevertheless, as temptation grows to use higher and more frequent doses of GH to treat short stature, especially short stature not due to GH deficiency, the caution urged by the authors should be remembered.

One of the more interesting observations from the study is that even though IGF-I levels were increased 1.5-fold and body weight 1.4-fold over controls in the mice expressing the IGF-I transgene, linear skeletal growth was not increased. These results differ from those reported by Guler et al (*Proc Natl Acad Sci USA* 1988;85: 4889-4893), who infused GH or IGF-I into hypophysectomized rats and found increased linear bone growth in both cases. Because of differences in design, the results of the two studies cannot be directly compared, but both sharpen the debate over how GH acts to promote linear skeletal growth.

William A. Horton, M.D.

## Do Extracellular Matrix Proteins Exhibit Growth Factor Activity?

Historically, growth factors were identified as circulating proteins and peptides that influenced cell division and differentiation. It was later determined that many growth factors are generated and act locally, ie, paracrine and autocrine growth factors. There is now growing evidence that many extracellular matrix proteins contain func-

tional domains with growth factor activity.

Engel recently reviewed the situation with regard to epidermal growth factor (EGF) domains in several large matrix proteins. EGF is a small peptide (53 amino acid residues) that is known to promote mitosis in many cell types through interaction with a specific cell



**Do Extracellular Matrix Proteins Exhibit Growth Factor Activity?** continued from page 11

membrane receptor. Three large multidomain extracellular matrix proteins contain EGF-like domains. They are typically repeated manyfold in accessible regions of the molecules. The first protein is laminin, which is found in basement membranes; the second is tenascin, a widely distributed matrix protein; and the third is thrombospondin, found in platelets and vascular walls. When these proteins are used as culture substrates, cells generally grow even in the absence of other growth factors, eg, those supplied by serum.

Engel suggests that these pro-

teins may stimulate growth early in development, before the EGF-like domains are covered up by other components of extracellular matrix, and especially during tissue repair, when they are exposed by the tissue damage. In this fashion they are able to provide highly specific and very localized signals for growth and repair that cannot be achieved by diffusible growth factors.

Engel J. *FEBS Lett* 1989;251:1-7.

**Editor's comment**—This article brings attention to three relatively

new concepts. The first is that large proteins often exhibit modular construction with different modules having different functions, ie, cell adhesion, molecular interaction, structural integrity, growth promotion, etc. Second, the same module may be shared by different molecules. Third, the extracellular matrix does more than occupy space between cells. Overall, this article contributes to a more complete picture of how the growth and differentiation of individual cells are regulated.

William A. Horton, M.D.

### The Half-Life of Exogenous GH After Suppression of Endogenous GH Secretion with Somatostatin

Suppression of endogenous growth hormone (GH) secretion by an infusion of somatostatin (SRIF, IV, 50  $\mu\text{g}/\text{m}^2/\text{hour}$ ) permitted measurement of the half-life of exogenously administered GH. Fourteen studies were performed in six male subjects (five normal adult males, one adolescent with GH deficiency following cranial irradiation). One hour after the start of the SRIF infusion, a bolus of monomeric biosynthetic GH (Nordisk, Gentofte, Denmark) was injected intravenously at a dose of either 500 mU ( $n=9$ ) or 50 mU ( $n=5$ ). Serum GH was measured over three consecutive 30-minute periods at intervals of 1, 5, and 10 minutes, respectively. Both immunoradiometric assay (IRMA) and enzyme-linked immunosorbent assay (ELISA) were used for the GH measurements. The half-life of GH was calculated from the logarithm of serum GH concentrations during the 90 minutes. A control study with GH, 500 mU, after 1 hour of saline infusion was performed twice in three subjects.

The serum GH was undetect-

able at the end of the first hour of SRIF. The distribution phase of injected GH was complete by 6 minutes. The mean half-life of GH was  $9.3 \pm 1.45$  min after 500 mU and  $8.5 \pm 1.5$  min after 50 mU. Combining the data from both studies gave a mean half-life of  $8.9 \pm 1.5$  min. Replacing SRIF with saline did not change the results.

Hindmarch PC, Matthews DR, Brain CE, et al. *Clin Endocrinol* 1989;30:443-450.

**Editor's comment**—There have been many discrepant studies suggesting that the half-life of circulating GH was more than 15 minutes. These previous studies measured the decay of either a small bolus of radiolabelled GH or a very large bolus of unlabelled GH in subjects whose endogenous secretion of GH had not been suppressed. The technical conditions of the present study—no interference from endogenous secretion; use of monomeric hGH at physiologic doses; serum GH measured by two sensitive and reliable methods—are clearly more appropriate.

Knowing that the half-life of the circulating GH is around 8 to 9 minutes is of clinical importance. It suggests that a 10-minute sampling interval may be necessary to properly evaluate the profile of en-

dogenous GH secretion and that the usual 20-minute interval of sampling may be insufficient.

Jean-Claude Job, M.D.

**Second editor's comment**—The authors note that the data reported in the above abstract are at variance with other reports. Using variable techniques, the half-lives of circulating GH have been found to be between 7 to 51 minutes; at least five previous articles reported that the half-life is greater than 15 minutes. The authors attribute the difference in their results to the use of SRIF. However, in an article by Faria et al (*J Clin Endocrinol Metab* 1989;68:535) in which SRIF and endogenous secretion of GH under GH releasing hormone stimulation were studied, the *in vivo* half-life was found to be  $18.9 \pm 0.8$  min by monoexponential analysis, and  $3.5 \pm 0.78$  min and  $20.7 \pm 0.7$  min by biexponential curve fitting. Both studies tested normal young adults except for one patient in Hindmarch's study who was GH deficient. The reason for differences in the results in these studies is unclear. The reader needs to be aware that a consensus has not been reached regarding the half-life of circulating GH.

Robert M. Blizzard, M.D.

## Partial GH Deficiency in Short Prepubertal Children with Intra-uterine Growth Retardation

Three European groups of pediatric endocrinologists have recently emphasized the frequency with which a low or abnormal secretion of growth hormone (GH) is found in children with intrauterine growth retardation (IUGR), with or without Silver-Russell syndrome (SR).

Albertsson-Wikland reports data on 16 IUGR children with lengths 3 SD below normal at birth. These children were studied between 2 to 6 years of age, when their heights were 2.7 to 5.5 SD below normal. (In addition, 6 children had features of SR.) Their mean GH response to an arginine-insulin test was  $15.7 \pm 7.2$  ng/mL; five of these had peak responses below 10 ng/mL. A 24-hour GH profile (withdrawals every 30 min) in 3 of the 6 SR patients and in 2 of the 10 other IUGR children showed low spontaneous secretion. Most of the other children showed minor disturbances in their circadian rhythm of GH secretion. All were treated with GH, 0.1 IU/kg/day, resulting in an average increase in growth velocity of 3.7 cm and 3.0 cm in the SR and the other IUGR children, respectively, during the first year of treatment. The gain in height was negatively correlated with the 24-hour GH secretion, evaluated by the area under curve ( $r = -0.56$ ,  $P < 0.05$ ), but not with the peak result of the arginine-insulin stimulation test.

Rochiccioli et al studied 24 prepubertal IUGR children born with lengths below the 10th percentile for gestational age. At the time of the study their mean age was 5.5 years and their mean height -3.3 SD. One or two GH stimulation tests (glucagon-betaxolol, clonidine-betaxolol, or arginine-insulin) and a 24-hour (20-30 min sampling) profile of serum GH were performed in each patient. Of the 24, 7 had both a 24-hour integrated concentration of GH below 1.5 ng/mL and GH peaks not exceeding 5

ng/mL at the two stimulation tests. Another 9 had low integrated circadian concentrations, with either normal ( $n = 4$ ) or low ( $n = 5$ ) peak responses to stimulation. Only 8 had both normal responses to the stimulation tests and normal spontaneous GH secretory profiles. Of the 24, 9 (unclear which children) were treated with GH, 0.4 IU/kg/week, and had an increase in growth velocity from  $3.5 \pm 0.8$  cm/year before GH to  $7.0 \pm 0.9$  cm/year during the first year of treatment.

Stanhope and associates report data on 31 IUGR prepubertal children with mean age 6.0 years, mean height -2.84 SD, mean birth weight -2.82 SD, and mean growth velocity -0.76 SD, during the year preceding the study. Seventeen had signs of SR. GH secretion (15 min sampling) was determined overnight (8 P.M. to 8 A.M.): 4 of 31 had no spontaneous GH peak above 10 ng/mL and thus were considered to be GH deficient. Nine (8 with SR) had a single nocturnal pulse of GH. A therapeutic trial of GH was performed in 23 patients, with randomization to two clinically similar groups receiving either 15 IU/m<sup>2</sup>/week or 30 IU/m<sup>2</sup>/week of GH, by daily SC injections (approximately 0.45 and 0.90 IU/kg/week). Short-term mean results were: in the low-dose group, an increase of height velocity from -0.61 to +1.09 SD for 0.82 year; in the high-dose group, an increase from -0.61 to +3.48 SD for 0.92 year. The authors conclude 1) that GH deficiency—mainly abnormal rhythm of nocturnal GH secretion—is apparently common in growth retarded children with IUGR; 2) that the short-term effect of GH in these patients is positive and dose dependent; 3) that these initial results cannot determine whether GH treatment may improve the final height of IUGR patients, some of whom may have an accelerated skeletal mat-

uration and an early onset of puberty.

Albertsson-Wikland K. *Acta Paediatr Scand (Suppl)* 1989; 349:35-41.

Rochiccioli P, Tauber M, Moisan V, Pienkowski C. *Acta Paediatr Scand (Suppl)* 1989;349:42-46.

Stanhope R, Ackland F, Hamill G, et al. *Acta Paediatr Scand (Suppl)* 1989;349:47-52.

**Editor's comment**—Although these three studies differ in terms of protocol, their results are similar. They clearly show that some degree of abnormality in the secretion of GH is found, more often than previously reported, in very short children born small-for-date, irrespective of whether they have the features of Silver-Russell syndrome. In these children frequent circadian or nocturnal measurement of the serum GH levels is perhaps a better way to evaluate GH secretion than the usual stimulation tests. However, nothing is known at present about the long-term usefulness of GH therapy in non-GH-deficient IUGR children. A dose dependency may exist during the first year of treatment, but beyond this time data do not exist. We can conclude that studies such as these in IUGR children are extremely useful, but that they must be developed and conducted in long-term, controlled protocols. We cannot at present extend the data from these trials to conclude that the use of GH in endocrinologically normal children with short stature of prenatal onset is efficacious.

Jean-Claude Job, M.D.

## Homeotic Gene Expression in Vertebrates

A fundamental question in developmental biology concerns how a cell knows where it is, relative to other cells and to the overall body plan. Position signalling (as it is called) is very important during early embryologic development and also in linearly ordered processes such as skeletal growth. Much is known about position signalling in lower species, such as *Drosophila*, in which so-called homeotic selection genes appear to serve as master genes controlling expression of many other genes in the developing embryo. These master genes contain highly conserved (homeobox) sequences that code for DNA binding protein domains and are organized as clusters of contiguous genes on chromosomes. Their expression is segmentally distributed, providing information about the anterior-posterior position of cells within an embryo. Interestingly, there is a spatial relationship between the chromosomal order

of the genes and the location of their expression in the embryo, such that the genes within a homeobox are sequentially expressed congruently with their anterior to posterior expression in the body.

Homeotic genes and gene clusters have been identified in higher species, including humans, but their functional similarity has been questioned because genesis of vertebrate and insect bodies differs so much. In particular, segmentation, which demarcates the limits of expression of the insect homeotic genes, has been thought to occur in a different fashion in vertebrates. However, a report by Wilkinson et al suggests that segmental expression of homeotic genes does occur in vertebrate embryos. Using *in situ* hybridization, these investigators demonstrated that expression of four contiguous murine homeotic genes (Hox 2.1, 2.6, 2.7, 2.8) exhibited a segmental distribution in the hindbrain of 9.5-day-old mouse embryos. The anterior limits of expression jumped by two

segment intervals and the order of segmental expression corresponded to the chromosomal order of the gene loci. Hence, as with *Drosophila*, there is a physical relationship between the chromosomal order of gene loci and their segmental expression along the anterior-posterior axis of the early embryo.

Wilkinson DG, Bhatt S, Cook M, et al. *Nature* 1989;341:404-409.

**Editor's comment**—The segmental expression of homeotic genes in the mouse hindbrain seems far removed from growth in humans. However, as one strives to understand human growth and development at the cellular and molecular levels, one becomes more dependent on knowledge acquired from lower organisms. Finding similarities between man and distant species in fundamental processes, such as positional signalling, greatly facilitates this task.

William A. Horton, M.D.

## Strategies for Optimizing Growth in Children with Kidney Transplants

In an attempt to diminish the growth failure that occurs post-organ transplantation in children with graft acceptance, but who are on low-dose steroids, the authors attempted to use cyclosporine as the primary immunosuppressant. Of 53 patients, 23 were able to discontinue prednisone and be maintained on cyclosporine monotherapy. Of these, 9 had to return to prednisone after a mean of 9 months (3-24 months). The other 14 remained off prednisone without an episode of rejection.

L-DOPA stimulation was used to evaluate growth hormone (GH) release. All patients were receiving >5 mg of prednisone daily; four patients had peak values <10 ng/mL GH. Standard deviations for height were evaluated in 15 pa-

tients who were off prednisone for at least 6 months; the SD scores improved in all.

Four pubescent children with growth retardation, requiring prednisone, received recombinant human GH (rhGH) in an attempt to stimulate growth. Three of these were believed to have accelerated growth.

The authors concluded that cyclosporine can produce long-term graft survival when used alone in some patients. Catch-up growth occurs in patients able to discontinue prednisone, and the potential of rhGH to improve post-transplantation growth in children needs further exploration.

Tejani A, Butt KM, Rajpoot D, et al. *Transplantation* 1989;47:229.

**Editor's comment**—The observations of Tejani et al conform with

data collected through the years concerning catch-up growth that occurs when glucocorticoids are discontinued. The surprising observation is the increase in growth that occurred in three of the four patients on low-dose steroids who received rhGH. The effect of GH administration to patients with chronic renal disease and its growth promoting effect has previously been reported by Koch (*Pediatr Res* 1988;23:541A).

The data in this report are preliminary, and more such studies are needed to clarify the usefulness of rhGH therapy in patients with chronic renal disease. Significant new data will be forthcoming within the next year or two. In the meantime every effort should be made to minimize the amount of steroid used in such patients.

Robert M. Blizzard, M.D.

## Effects of Different Oestrogen Doses on Final Height Reduction in Girls with Constitutional Tall Stature

Gruters et al have studied the effects of two different dosages of ethinylestradiol (EE) on final height reduction in German girls whose final height prediction exceeded 3 SD ( $>180$  cm) above the mean. Group 1 (38 girls) at the University Children's Hospital in Göttingen received a daily dose of 0.3 to 0.5 mg EE, while 44 girls (group 2) at the University Children's Hospital in West Berlin received 0.1 mg EE daily. Both treatment protocols utilized daily estrogen administration in conjunction with 10 mg medroxyprogesterone acetate for 5 to 7 days every 4 weeks to induce cyclic bleeding. Subjects were examined every 3 months and bone age (BA) was determined by the Greulich and Pyle method every 6 months.

Treatment was discontinued at BA-15 years in group 1 and after two successive BA determinations  $\geq 15$  years in group 2. Final height was measured in all girls  $\geq 18$  years (mean, 20.2 years). Standing height was measured with a calibrated stadiometer and predicted height was estimated according to Bayley and Pinneau tables.

At the onset of treatment there were no differences in chronologic age, BA, height, growth velocity, or height prediction between the two groups. Growth velocity was significantly reduced by estrogen in both groups.

Although duration of treatment was longer in group 2, the cumulative estrogen dose was lower in group 2 than in group 1. From the predicted final height the mean reduction was  $4.9 \pm 2.6$  cm in group 1 and  $5.1 \pm 2.4$  cm in group 2. Final height was reduced more in each group when the treatment was started at BA  $<13$  years. No

side effects were observed in either group.

Gruters A, Heiderman P, Schludter H, Stubbe P, Webber B, Helge H: *Eur J Ped* 1989;149:11-13.

**Editor's comment**—This article reports that in a large sample of tall girls, different doses of EE had similar effects on final height reduction. Thus, as the authors point

out, it would seem prudent to utilize the lowest possible dose of estrogen in an attempt to minimize possible side effects—such as thromboembolism, hypertension, and increased body weight—that are known to be dose dependent. A prospective randomized clinical trial to determine the lowest effective dose is needed.

William L. Clarke, M.D.

## Effects of Oestrogen Treatment on the Proportionality of Growth in Tall Girls

Hermanussen et al studied the effects of estrogen therapy (conjugated estrogen, 7.5 mg/d, plus cyclic gestagens) on standing height and lower leg length in 17 girls with tall stature and compared those results to a control group of 17 healthy untreated tall girls. The heights of all girls exceeded 2 SD for age, or their predicted adult height exceeded 182 cm. All were measured weekly or monthly using a Harpenden stadiometer. Knemometry, a noninvasive technique, was used to measure the lower leg length in the sitting position. Growth rates were calculated using linear regression analysis.

Estrogen treatment led to a significant reduction of lower leg length increment in the treated girls. Standing height velocity dropped from 150 to 122  $\mu\text{m}/\text{d}$  in the estrogen-treated girls. The decrease in standing height velocity was explained by a marked inhibition of lower leg growth velocity, from 42 to 30  $\mu\text{m}/\text{d}$ . No differences in trunk growth velocity were detectable. According to the authors, these findings suggest that pharmacologic doses of estrogen act locally at the level of epiphyseal growth and, therefore, girls who have passed mid-puberty—when most peripheral growth has been completed—would not be expected to benefit significantly from high estrogen treatment.

Hermanussen M, Geiger-Benoit K,

Burmeister J. *Euro J Ped* 1989;149:14-17.

**Editor's comment**—This interesting paper suggests that treatment for excessively tall stature in healthy girls should be initiated prior to mid-puberty if maximal benefit is to be attained. In addition, the finding that high-dose estrogen works primarily at epiphyseal growth centers leads to speculation about the use of estrogen therapy in agonadal girls. It would be of interest to evaluate the effect of low-dose estrogen on lower limb length and growth velocity in normal girls and in girls with Turner syndrome, particularly because others have suggested that sex steroids play no role in the growth rate of agonadal children prior to puberty. Similarly, the effects of low- and high-dose testosterone therapy in agonadal and constitutionally delayed-growth boys should also be pursued (with knemometry).

William L. Clarke, M.D.

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## A Preliminary Report on the Role of Somatostatin Analog (SMS 201-995) in the Management of Children with Tall Stature

This paper presents preliminary results obtained in seven children with excessive height and height velocity leading to a height prediction (TW2 method) >180 cm in girls (n=5) and >200 cm in boys (n = 2) treated for more than 6 months with one daily injection of the long-acting somatostatin analog SMS 201-995. Two of the participants were prepubertal, two at pubertal stage 2-3, and three at stage 4.

Growth hormone (GH) secretion, measured as the sum of the amplitudes of the GH pulses during 24 hours, deeply decreased after the first dose of the analog and was still low following 1 year of therapy in patients who were re-tested. No change occurred in serum thyroxine levels or in glucose and glycosylated hemoglobin A1C values. A small, nonsignificant decrease of serum insulin values was observed.

Mean growth rate decreased significantly from 8.3 (range, 5.5-12.2) to 3.0 (range, 0.2-4.5)

cm/yr at the end of 6 months of treatment. It remained <5 cm/yr after 1 year in the four patients who were still receiving therapy. The effect of the treatment on predictable adult height was measurable in five patients, with a reduction of -2.1 to -6.3 cm in three, and no reduction in two (-1.1 and +0.7 cm), although this predicted height reduction was only borderline significant.

The authors point out, from this preliminary work, that SMS 201-995 effectively reduced the secretion of GH, with no important side effects, during treatment for 6 months to 1 year, but that it also had no constant or significant effects on the predictable adult height. They suggest that SMS 201-995 may have a role in the management of excessively tall children, but the optimum mode and timing for its use remain to be established.

Hindmarsh PC, Pringle PJ, DiSilvio L, Brook CGD. *Clin Endocrinol* 1990;32:83-91.

**Editor's comment**—*Avoiding excessive adult height remains a challenge, as predictions and results for the treatments that have been proposed remain uncertain.*

*This new approach deserves consideration, mainly because no harmful effects have been observed and the drop in GH secretion has been well documented. Everyone will agree with the authors that the data are preliminary and allow no more than continuing clinical research with somatostatin analogs as a possible treatment for extremely tall children.*

Jean-Claude Job, M.D.

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# GROWTH

## Genetics & Hormones

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### The Adverse Systemic Effects of Growth Hormone in Acromegaly

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Chronic hypersecretion of growth hormone (GH) in adults results in acromegaly. Growth hormone is secreted in a pulsatile fashion in both normals and acromegalic patients, but whereas GH concentrations remain detectable in serum throughout the 24-hour measurement period in acromegalic patients,<sup>1</sup> in normal subjects GH concentrations decay to undetectable levels several times during a 24-hour period.

A GH-secreting pituitary adenoma is the cause in 99% of cases of acromegaly. Rare causes include eutopic (eg, hypothalamic gangliocytoma) or ectopic (eg, carcinoid or islet cell tumors) hypersecretion of GH-releasing hormone. After epiphyseal fusion, excessive GH causes a gradual enlargement of the acral skeleton, most notably the hands, feet, nose, and mandible. However, these changes occur very slowly and may not become recognizable for 10 to 20 years. In addition to its effects on the skeleton, GH hypersecretion adversely affects most organ systems. In a retrospective review of 194 acromegalic patients treated between 1939 and 1967, Wright and coworkers observed a twofold increase in mortality rates compared to the general population of England and Wales, particularly from cardiovascular, respiratory, and cerebrovascular deaths.<sup>2</sup> This paper will review recent advances

in our understanding of the consequences of GH hypersecretion observed in acromegalics.

#### Endocrine Complications

Insulin resistance induced by GH hypersecretion results in glucose intolerance in 29% to 45% of acromegalic patients and frank diabetes mellitus in 10% to 20%.<sup>3-5</sup> Insulin response to an intravenous glucose challenge is exaggerated in patients with normal glucose tolerance and is decreased and delayed when glucose tolerance is abnormal. In the latter group, lowering GH concentrations usually results in improved, although possibly still abnormal, glucose tolerance.<sup>3</sup> The risk factors for development of diabetes in acromegalics have not been well defined. Higher serum GH levels are associated with a higher incidence of diabetes, but HLA phenotype, family history of diabetes, and dura-

tion of acromegaly do not have predictive value.<sup>4,5</sup> Pancreatic islet cell antibodies are negative in diabetes caused by acromegaly.<sup>5</sup>

Hypertriglyceridemia occurs in 19% to 44% of acromegalic patients, especially those with abnormal glucose-insulin response,<sup>6</sup> and improves with reduction of serum GH concentrations. Hepatic triglyceride lipase and lipoprotein lipase activities are decreased; they increase when serum GH levels are lowered.<sup>7</sup> The effect of GH hypersecretion on serum cholesterol concentrations is not clear, as current reports conflict.<sup>6,7</sup>

Hypogonadism in acromegaly may result from either destruction of gonadotrophs by a pituitary tumor or by alterations in gonadotropin releasing hormone secretion as a result of coexistent hyperprolactinemia. Menstrual disorders occur in 32% to 87% of women under 45;

#### Letter From the Editor

Pediatricians seldom see patients who produce excessive growth hormone (GH). However, as GH is given to children for an increasing number of indications, the signs and symptoms associated with excessive GH become of increasing academic interest. Internists who are endocrinologists deal with the problem of acromegaly frequently, and we have capitalized on their experience. Dr Mark Hartman has very nicely summarized the complications of acromegaly in the article beginning on this page.

For the reader with limited experience in the administration of GH to children, please understand that the complications found in adult acromegaly are highly unlikely to occur in children at the doses of GH currently used.

Robert M. Blizzard, MD

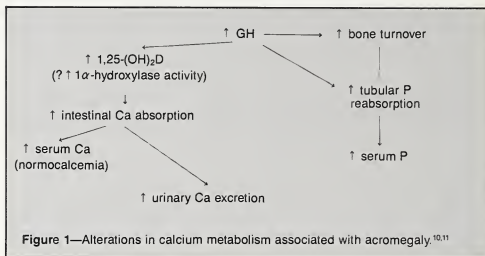
27% to 46% of men have decreased libido or impotence.<sup>4</sup> Galactorrhea occurs in approximately 20% of women, of which 10% to 28% may have normal serum prolactin concentrations. In the latter case, galactorrhea has been postulated to arise from specificity spillover effects of GH on prolactin receptors.<sup>8</sup>

Thyromegaly is observed on examination in 25% to 53% of acromegalic patients. Thyroid ultrasonography reveals a higher incidence of increased thyroid volume (71%), as well as a high incidence of multiple thyroid nodules (65%). Serum thyroglobulin concentrations are increased in 47% of acromegalics. These effects may be mediated by increased concentrations of insulin-like growth factor I (IGF-I). Thyroid volume and serum thyroglobulin levels decrease with effective therapy of acromegaly.<sup>9</sup>

### Calcium and Bone Metabolism

The alterations in calcium metabolism in acromegaly are depicted in Fig 1.<sup>10,11</sup> These changes occur apart from any changes in serum concentrations of parathyroid hormone or calcitonin. The major adverse consequence of hypercalciuria is an increased incidence of urolithiasis, which ranges from 6% to 12.5%.<sup>12</sup> All of these abnormalities reverse when serum GH concentrations are normalized.

Bone formation and resorption, as assessed by quantitative microradiography, are both increased in acromegaly. However, bone formation exceeds resorption in rib cortex, whereas the reverse occurs in the iliac crest. These findings suggest that bone remodeling is increased in acromegaly, with a possible redistribution of bone mass from trabecular to cortical bone.<sup>13</sup> Although bone mineral density (BMD) in the radius is consistently increased in acromegaly, there are conflicting



**Table 1**—Forearm and spinal bone mineral density in acromegaly

| Ref | No. of patients | Acromegaly status | Gondal status | Forearm BMD (P v ctrl) | Vertebral BMD (P v ctrl) |
|-----|-----------------|-------------------|---------------|------------------------|--------------------------|
| 13  | 26              | active            | normal        | ↑ (P < 0.05)           | ND                       |
| 14  | 7               | active            | normal        | ↑ (NS)                 | ↑ (P < 0.01)             |
| 15  | 12              | active            | normal        | ↑ (P < 0.001)          | ↓ (NS)                   |
|     | 5               | active            | hypo          | ↑ (P < 0.05)           | ↓ (P < 0.05)             |
|     | 7               | inactive          | hypo          | ↓ (P < 0.01)           | ↓ (P < 0.05)             |

BMD, bone mineral density; ND, not done; NS, not statistically significant; ctrl, control subjects.

data about the BMD of the vertebral bodies (Table 1).<sup>13-15</sup> However, in the only study that documented decreased spinal BMD, hypogonadism was a confounding factor in half of the patients.<sup>15</sup>

### Gastrointestinal Neoplasms

Table 2 summarizes the findings of 3 studies that have documented an increased incidence of gastrointestinal neoplasms in acromegalic patients, including colonic polyps and adenocarcinoma of the colon and stomach.<sup>16-18</sup> Since adenomatous colonic polyps are considered a premalignant condition, these authors recommend that colonoscopy be performed in acromegalic patients over age 50, especially if more than 6 skin tags are present.

### Cardiovascular Complications

Cardiovascular disease is the most common cause of death in acromegalic patients, with mortality rates twofold above those expected for men, but these are not increased in women. This increased mortality is associated with hypertension.<sup>2</sup>

Idiopathic hypertension occurs in 13% to 50% of acromegalic patients. It is associated with higher mean GH concentrations and with longer duration of acromegaly. It is usually mild, uncomplicated, and responds well to a variety of antihypertensive medications.<sup>19</sup>

Sodium retention, expansion of extracellular fluid volume, and suppression of the renin-angiotensin-aldosterone axis are observed in both hypertensive and normotensive acromegalics. Overactivity of the sympathetic nervous system may also be a possible etiologic factor.<sup>20</sup> However, there are conflicting data on whether hypertension improves with therapy of GH hypersecretion.

The existence of a specific type of heart disease in acromegaly is con-

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### Robinow Syndrome: An Update

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troversial. In a recent retrospective review of 256 consecutive patients, 10 acromegalics were identified with heart disease without evidence of hypertension, diabetes, thyroid disease, or coronary or valvular heart disease. Patients with active acromegaly uniformly developed worsening cardiac function. Among patients cured of acromegaly, equal numbers experienced improvement, stabilization, and deterioration of cardiac function.<sup>21</sup> Pathologic findings in the hearts of acromegalics include myocardial hypertrophy (93%), interstitial fibrosis (85%), and lymphomononuclear myocarditis (59%).<sup>22</sup> Approximately half of acromegalic patients have left ventricular hypertrophy (LVH), and about one quarter have evidence of abnormal left ventricular function, as assessed by echocardiography and radionuclide imaging studies (Table 3). While most of these studies included hypertensive patients, LVH was seen in 43% to 64% of normotensive patients as well.<sup>23-29</sup>

The cardiac complications of acromegaly may improve or stabilize with therapy of GH hypersecretion, although left ventricular function may continue to deteriorate in some patients with long-standing disease.

### Skin and Soft Tissue Changes

The earliest manifestations of acromegaly include oily skin, hyperhidrosis, and soft tissue swelling of the hands, fingers, and feet. After successful transphenoidal surgery, these signs resolve almost immediately. Increased skin thickness results in exaggerated facial wrinkles and nasolabial folds; these resolve more slowly with therapy. Multiple skin tags, increased skin pigmentation, and coarsened and darker scalp and body hair may be seen. Women may have mild hirsutism. The lips, tongue, nose, and ears are

**Table 2—Gastrointestinal neoplasms in acromegaly**

| Ref | Study type | No. of pts | No. pts with colonic polyps | No. pts with GI malignancy | O/E ratio for GI malignancy |
|-----|------------|------------|-----------------------------|----------------------------|-----------------------------|
| 16  | Pr         | 17         | 9 (53%)*                    |                            |                             |
|     | R          | 44         |                             | 4 colon (9.1%)             | ND                          |
| 17  | Pr/R       | 12         | 4 (33%)**                   | 3 colon (25%)              | > 3 ( $P < 0.001$ )         |
| 18  | R          | 48         | ND                          | 5 (10%)                    | 4.6 ( $P < 0.05$ )          |
|     |            |            |                             | (3 colon, 2 gastric)       |                             |

Pts, patients; Pr, prospective; R, retrospective; ND, not done; O/E, observed/expected.

\* 8 pts with polypectomies: 5 had adenomatous and 3 had hyperplastic polyps.

\*\* 3 pts with polypectomies: 2 had adenomatous and 1 had hyperplastic polyps.

enlarged; enlargement of the vocal cords, larynx, and sinuses results in a deeper, more resonant voice. These signs are important since they occur early in the natural history of acromegaly and are reversible with therapy, with the exception of growth of cartilaginous structures.

### Acromegalic Arthropathy

Arthralgias are a common presenting complaint of acromegaly, occurring in 62% to 75% of patients. Objective arthropathy is observed in 16% to 62% of patients; 10% to 40% experience arthropathy severe enough to limit activities of daily living. Among peripheral joints, the knees, hips, and shoulders are more frequently affected; the elbows and ankles are less commonly involved. Although the entire spine may be involved, the lumbosacral spine is usually more affected than the cervical or thoracic spine. Subcu-

taneous thickening of periarticular tissues causes the earliest symptom of joint tightness, especially in the hands. This symptom may resolve with effective therapy of GH hypersecretion. Significant joint pain usually indicates that irreversible cartilage degeneration has occurred.<sup>30,31</sup>

Early in acromegaly, joint spaces are increased, secondary to cartilage proliferation. Synovial and periarticular swellings result in joint swelling without effusion. As cartilage proliferation continues, ulcerations develop at weight-bearing sites, resulting in abnormal joint geometry. New bone formation and remodeling ensues, with development of osteophytes and, ultimately, narrowed joint spaces. The end stage is a debilitating, severe osteoarthritis, which may require artificial joint replacements. There is no evidence for an inflammatory component. In the spine, disc spaces are

**Table 3—Echocardiographic and radionuclide studies of the heart in acromegalic patients**

| Ref   | No. of patients | LV hypertrophy* | Abnormal LV function** | Correlation with GH |
|-------|-----------------|-----------------|------------------------|---------------------|
| 23    | 10              | ND              | 7 (70%)                | —                   |
| 24    | 16              | 7 (44%)         | 6 (38%)                | +                   |
| 25    | 23              | 13 (57%)        | 4 (17%)                | +                   |
| 26    | 25              | 20 (80%)        | 3 (12%)                | +                   |
| 27    | 27              | 14 (52%)        | 6 (22%)                | +                   |
| 28    | 16              | 6 (38%)         | 3 (19%)                | ND                  |
| Total | 117             | 60/107 (56%)    | 29/117 (25%)           |                     |

ND, not done; LV, left ventricular.

\* Increased LV mass or wall thickness, concentric LVH, asymmetric septal hypertrophy.

\*\* Abnormal systolic time intervals, systolic shortening fractions, ejection fractions.

### Erratum

In *Growth, Genetics, and Hormones* Vol. 6, No. 1 (March 1990), an error on page 14 incorrectly linked neural tube defects with a low alpha-fetoprotein level found in maternal serum screening. In fact, neural tube defects are associated with a high maternal alpha-fetoprotein level.



increased and dorsal kyphosis and anterior osteophytes are common. Although patients frequently complain of backaches, spinal mobility is usually normal or increased, apparently because the discs remain resilient and the paraspinous ligaments become hypertrophied and somewhat lax.<sup>30,31</sup>

Results of 2 recent studies suggest that arthropathy is worse in patients with long duration of acromegaly.<sup>32,33</sup> These reports also indicate that therapy is most likely to improve joint symptoms if it is initiated early in the course of the disease and if serum GH concentrations are rapidly lowered. If GH levels remain elevated for many years, irreversible cartilage degeneration occurs, and the arthropathy is less likely to respond to therapy.

### Neuromuscular Complications

Carpal tunnel syndrome occurs in 30% to 64% of patients with acromegaly. Symptoms include paresthesias in the median nerve distribution and hand pain at night or with prolonged use. On physical examination, a positive Tinel's sign or Phalen's sign, or thenar muscle atrophy strongly suggests the diagnosis. A nerve conduction study may be helpful to confirm the diagnosis. Hyperplasia of ligaments and tendons in the carpal tunnel with synovial edema lead to compression of the median nerve. The syndrome usually resolves within 6 weeks of normalization of serum GH concentrations.<sup>34</sup> In 1 study, electroneurographical findings improved within 1 week of transphenoidal surgery in 12 of 28 patients (43%).<sup>35</sup>

Table 4 outlines the clinical features of the proximal myopathy of acromegaly. Muscle biopsies in these patients have revealed hypertrophy of type I and II muscle fibers,

muscle fiber necrosis, an increased number of sarcolemmal nuclei, and increased glycogen and lipofuscin deposits. Proximal myopathy is associated with a longer duration of acromegaly and improves very slowly after reduction of serum GH concentrations.<sup>34,36</sup>

### Respiratory Complications

Respiratory mortality rates are increased threefold in acromegalic patients.<sup>2</sup> Two studies have reported that abnormal pulmonary function tests correlated with the duration of acromegaly.<sup>37,38</sup> Whether any of these changes are reversed by successful therapy is unknown.

Three major clinical presentations of upper airway problems occur in acromegalic patients. The most unusual is an acute exacerbation of upper airway narrowing by an upper respiratory infection, resulting in the acute onset of dyspnea and inspiratory stridor. A flow-volume loop study may be helpful in making the diagnosis.

A second, more common syndrome is difficulty with intubation. Careful preoperative assessment, the use of fiberoptic laryngoscopy, and the "sniffing position" may facilitate intubation in these patients. Occasionally, tracheostomy may be required. In all cases, the patient should be closely monitored following extubation for the development of upper airway obstruction.

The third and most recently recognized manifestation of upper airway narrowing in acromegalics is the obstructive sleep apnea syndrome (OSAS). Symptoms include excessive daytime sleepiness, habitual snoring, restless nocturnal sleep, and apneic episodes. Polysomnography is required for diagnosis since pulmonary function tests, including flow-volume loops, may be

normal in these patients. The diagnosis is made when more than 5 apneas occur per hour of sleep. The prevalence of OSAS in 3 series was 40% to 50% and 0% of patients with active and long-standing inactive disease, respectively (Table 5).<sup>39-41</sup> Men were affected more commonly than women. Both the prolapse of an enlarged tongue and the inspiratory collapse of the hypopharynx have been implicated by endoscopic studies.<sup>42,43</sup> Short-term studies in a limited number of patients have indicated that lowering GH levels results in improvement or resolution of OSAS in approximately 50% of patients after 6 days to 12 months of follow-up. However, OSAS may persist in some patients whose serum GH levels have been normalized by successful therapy, at least for periods up to 1 year (Table 6).<sup>41,43-45</sup> These preliminary observations suggest that the reversibility of obstructive sleep apnea in acromegaly may depend on which anatomical structures are most affected in a given patient. For patients in whom sleep apnea persists despite therapy, effective treatments include nasal continuous positive airway pressure (CPAP) at night, surgical reduction of the tongue and/or other pharyngeal tissue, and tracheostomy.

### Conclusions

Excessive GH secretion in acromegaly adversely affects most organ systems, leading to increased morbidity and mortality. As many of these complications correlate with the duration of acromegaly, it is important to make the diagnosis early in the course of the disease and to initiate therapy to rapidly decrease serum GH concentrations to the normal range. The measurement of serum IGF-I concentration is a sensitive screening test for

Table 4—Proximal myopathy in acromegaly

| Ref | No. of patients | History | Weakness PE | CPK or aldolase | Abnormal EMG | Abnormal biopsy |
|-----|-----------------|---------|-------------|-----------------|--------------|-----------------|
| 34  | 17              | 76%     | 41%         | 18%             | 46%          | 1/3             |
| 36  | 11              | 55%     | 55%         | 45%             | *            | 5/9             |

CPK, creatine phosphokinase; EMG, electromyography; PE, physical exam.

\* Mean action potential duration significantly decreased compared to control subjects.

acromegaly. The diagnosis may be confirmed if the serum GH concentration is greater than 2 µg/L, 60 minutes after a 100-g oral glucose load. Random serum GH levels are not helpful, as GH is secreted in a pulsatile fashion in normal subjects. Transsphenoidal surgery by a neurosurgeon experienced in pituitary surgery is the initial treatment of choice, since it will rapidly decrease serum GH concentrations. If serum GH levels are not normalized by surgery, therapeutic options include pituitary irradiation and medical therapy with either octreotide (a somatostatin analogue) or bromocriptine.

**Table 5—Prevalence of the obstructive sleep apnea syndrome in acromegaly**

| Ref | No. of patients | Acromegaly status* | No. with OSAS | Mean serum GH concentration (ng/mL) |
|-----|-----------------|--------------------|---------------|-------------------------------------|
| 39  | 6               | active             | 3 (50%)       | 21 ± 18                             |
|     | 5               | inactive           | 0             | 2.7 ± 1.5                           |
| 40  | 10              | active             | 4 (40%)       | 62                                  |
|     | 11              | inactive           | 0             | 3.2 ± 2.2                           |
| 41  | 11              | active             | 5 (45%)       | 41 ± 38                             |

OSAS, obstructive sleep apnea syndrome.

\* Acromegaly was defined as inactive by the authors if the fasting serum GH concentration was <5 ng/mL.

**Table 6—Effect of treatment of acromegaly on the obstructive sleep apnea syndrome**

| Ref   | No. of patients | Treatment | No. OSAS resolved | No. OSAS improved | No. OSAS unchanged | No. with normal GH but OSAS unresolved |
|-------|-----------------|-----------|-------------------|-------------------|--------------------|--|
| 41    | 5               | TSS       | 1                 | 1                 | 3                  | 2                                      |
| 43    | 1               | TSS       | 0                 | 0                 | 1                  | 1                                      |
| 44    | 1               | SMS       | 0                 | 1                 | 0                  | 1                                      |
| 45    | 1               | BC        | 0                 | 1                 | 0                  | 0                                      |
| Total | 8               |           | 1 (12%)           | 3 (38%)           | 4 (50%)            | 4 (50%)                                |

OSAS, obstructive sleep apnea syndrome; TSS, transsphenoidal surgery; SMS, SMS 201-995 (a somatostatin analogue); BC, bromocriptine.

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# Robinow Syndrome: An Update

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In 1969, Silverman, Smith, and I reported a family consisting of 3 siblings, mother, and grandmother with "a previously unrecognized dwarfing syndrome."<sup>1</sup> The major features were: moderately short stature, characteristic facial dysmorphism, genital hypoplasia, and

mesomelic brachymelia. The facial characteristics (Fig 1) included hypertelorism; short, upturned nose; long philtrum; broad, triangular mouth; and dental malalignment (Fig 2). The genital hypoplasia consisted of micropenis in the males (Fig 3) and hypoplastic clitoris and labia minora in the females. Somewhat later, I proposed the more descriptive term "fetal face syndrome," since the face resembles that of the human fetus at 8 weeks<sup>2</sup> (Fig 4).

In 1973, Wadlington et al<sup>3</sup> published 4 more cases of the syndrome and added vertebral segmentation anomalies to the clinical spectrum. In the more than 30 publications on the syndrome,<sup>4</sup> a number of other, less constant anomalies have been described, some nonspecific, others more nearly unique and thus of greater diagnostic value (Table).

In most cases, the diagnosis is obvious on inspection. In "atypical" cases, when some of the major features are missing, the diagnosis must remain in doubt. Atypical cases have not been reported in relatives of "classic" cases.

## Genetics

In the index family,<sup>1</sup> transmission of the syndrome was autosomal dominant. Two of the patients of Wadlington et al,<sup>3</sup> boy-girl siblings, were children of normal parents, strongly suggesting autosomal recessive

inheritance. Since then, both modes of inheritance have been amply documented. As to be expected, pedigrees indicating recessive inheritance have been encountered more often in populations with high rates of consanguinity, eg, Arabs.

*I believe characteristic dominant and recessive phenotypes can be delineated.<sup>5</sup>* Individuals with the dominant form seem to have normal stature or only mild dwarfing, no vertebral abnormalities or, at most, a single butterfly vertebra, and only mild forearm brachymelia. Patients with the recessive form seem to have more severe dwarfing, more extensive vertebral segmentation defects, and more severe forearm brachymelia and dysplasia (Fig 5). Intrafamilial variability has been slight while interfamilial variability has been considerable, so that further genetic heterogeneity seems likely.

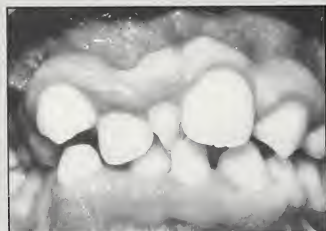
Unfortunately, all the diagnostic criteria for this syndrome are morphologic. No metabolic defect has been identified; no animal model is known. Chromosome studies have yielded uniformly normal results, although chromosome mapping has not been attempted.

## Teratogenic Period

Since the face and the external genitalia attain their final shape at around 10 weeks of gestation, some authors have speculated that this may also be the teratogenic period



**Figure 1**—Index patient (dominant type). Face at 7 years. Large forehead, hypertelorism, short upturned nose, long philtrum, broad mouth.



**Figure 2**—Same patient. Dental malalignment and gingival hyperplasia.



**Figure 3**—Extreme micropenis in newborn. Note normal-sized scrotum and testicles.

**Table—Anomalies in Robinow syndrome**

**Nonspecific Abnormalities**

Cryptorchidism  
Inguinal hernia  
Hypospadias  
Congenital heart disease  
Cleft lip/palate  
Brachydactyly, clinodactyly  
Mental retardation (rare)

**Syndrome-specific Abnormalities**

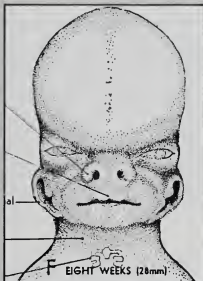
Midline indentation of the lower lip  
with tongue tie and bilobed  
tongue tip  
Gingival hyperplasia (Fig 2)  
Partial or complete duplication of  
distal phalanx of thumb or big toe  
Distal ulnar and proximal radial  
hypoplasia with proximal radio-  
ulnar dislocation (Fig 5)  
Apparent exophthalmus due to  
hypoplasia of the lower lids

**The Future**

Further studies of the phenotype are not likely to add much to present understanding. Progress will have to await metabolic and molecular genetic studies. Once the gene abnormality(ies) has (have) been identified, we can derive a better classification and may gain a better insight not only into the teratogenesis of the syndrome but also into mechanisms of normal embryogenesis.

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**Figure 4—The "fetal face." Human embryo at 8 weeks.<sup>2</sup> Reproduced with permission of McGraw-Hill Book Co.**



**Figure 5—Autosomal recessive type. Severe acromesomelic brachymelia, short metacarpals and phalanges, hypoplastic distal ulna and proximal radius, and radio-ulnar dislocation.**

for the syndrome. However, vertebral segmentation is normally completed at 4 weeks, suggesting that teratogenesis is more extended, at least in the recessive type.

**Course**

Approximately 10% of patients have died in infancy, most of them of pulmonary disease or cardiac malformations. Probably all the deaths occurred in the recessive type. The remaining 90% seem to have enjoyed good health. The facial features become less striking at puberty, which may explain the fact that almost all index cases have been in

infants and young children.

**Endocrine Aspects**

Sexual maturation occurs at the usual age in both sexes. In males, the penis remains abnormally short but may attain normal circumference, permitting sexual function. Endocrine studies by Lee et al<sup>6</sup> suggested partial primary hypogonadism. Androgen receptors and 5 $\alpha$ -reductase in genital skin fibroblasts were normal. Females with both the dominant and recessive forms have reproduced, as have males with the dominant type. Reproduction by males with the recessive form has not yet been documented.

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## Phenotype Abnormalities Seen with 45X/46XY Mosaicism

With the advent of prenatal diagnosis, 45X/46XY mosaicism is ascertained on a fairly regular basis. The question is whether 45X/46XY mosaicism is associated with Turner syndrome, infertility, ambiguous genitalia, or any other problems. Until recently, selection for testing for 45X/46XY mosaicism has been based on the presence of unusual postnatal features.

The authors have taken advantage of current prenatal testing procedures to conduct an unbiased survey of 45X/46XY incidence by sending a questionnaire to an international sample of 730 cytogenetic laboratories. A total of 92 cases of prenatal diagnosis of 45X/46XY mosaicism were reported. There was good clinical information on 76 cases; 75 were phenotypically male and 1 was female. Three of the phenotypic males had hypospadias, and the phenotypic female had clitoromegaly. Many of the cases had been terminated prenatally at the parents' request, and gonad histology was done in 11 cases. Of these, 3 (27%) had abnormal testicular development, but only 1 had abnormal external genitalia. Of the 75 "males," 5 had other congenital abnormalities of consequence. The authors found no relationship between the degree of mosaicism observed at prenatal diagnosis and the severity of abnormalities.

The percentage of 45X cells ranged from 1% to 98%; the majority had less than 50% 45X cells and (probably for this reason) presented with a normal male phenotype rather than a Turner phenotype.

Long-term follow-up of 45X/46XY patients is not available, and information concerning long-term stature, pubertal development, tumor risk, and fertility is needed. However, this study suggests that most patients with 45X/46XY karyotype (95%) have normal male genitalia, in contrast with previous postnatal studies. Dysgenetic gonads appear to occur in about 25% of cases, but whether this figure would be higher by

puberty is not yet known. Dysgenetic gonads in normal-appearing males who have never had chromosome studies may be a source of infertility or gonadoblastomas in the general population.

Chang HJ, et al. *Am J Hum Genet* 1990;46:156-167.

**Editor's Comment**—*This is an important study in view of the frequent use of prenatal diagnosis and concomitant finding of 45X/46XY karyotypes. This study suggests that most cases will do well, but clearly long-term follow-up is needed. Also of interest, it appears that approximately half of the families where this diagnosis was made prenatally have terminated the fetus. These decisions were probably based on expectations of poor outcome ensuing from previous, biased, reports, whereas the actual prognosis may not be so unfavorable for such children.*

Judith G. Hall, MD

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## In Future Issues

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### Obesity in Childhood and Adolescence, Part II: Pathophysiology, Associations, and Complications

by W.H. Dietz, MD, and L.G. Bandini, MD

## The Polymerase Chain Reaction

During the last 4 years, the technique of polymerase chain reaction (PCR) has revolutionized the way in which molecular genetics is done. PCR is a relatively simple method to amplify or increase the number of copies of a specific segment of DNA, to obtain sufficient DNA for further evaluation. The segment of DNA to be amplified may come from many sources or from DNA in an intact cell. It is possible to amplify a short segment, 50 to over 2,000 base pairs in length, to more than a million copies in just a few hours. Furthermore, the process has been automated and is presently being used for detecting DNA on fixed pathologic specimens, from single cells (lymphocytes, sperm cells, skin cells, etc), from forensic material such as hairs or blood cells, from ancient archeological specimens,

and for many molecular genetic studies and diagnostic tests.

The basis of the technique, developed by the Cetus Corp, lies in targeting the DNA segment to be amplified by identifying its boundaries with 2 single-stranded oligonucleotide primers. A heat-stable DNA polymerase is used to catalyze the duplication reaction. The native double-stranded DNA to be amplified is denatured by heat, and once the DNA has been liberated as single strands it can then be duplicated by the polymerase using the primers. Thus, the process moves very rapidly as new copies become templates for more copies. By rapidly alternating the temperature—causing separation of the double strand, allowing duplication—large amounts of double-stranded DNA of the specific short segment

are produced.

The technique has endless research applications, including the study of specific mutations, use in genomic cloning, analysis of protein-DNA interactions, a variety of genetic therapies, rapid diagnosis (both prenatal and prior to implantation), unique identification of tissues, diagnosis of infectious states such as HIV, etc; and there will undoubtedly be additional major applications in the years to come.

Eisenstein BI. *N Engl J Med* 1990; 332:178-183.

**Editor's Comment**—This is an excellent review. All individuals in medicine should understand the PCR technique; if you don't, read the article!

Judith G. Hall, MD

## Nontraditional Inheritance: Genomic Imprinting in Glomus Body Tumors

The most striking finding in this report was that the familial form of this disorder is *transmitted by males only*, either directly or through unaffected females, but is *not an X-linked condition*. Interestingly, these tumors are very much like other familial tumors, in that when they are familial, they are of early onset, occur at multiple sites, and have more severe symptoms and arise at a younger age than nonfamilial tumors.

This recent review by van der Mey et al of patients with glomus tumors identified 69 patients, of whom 34 had no family history and 35 had a family history. Another 82 patients were found within the families of the familial cases. Overall, there was a female excess, but this was entirely in those patients without a family history. This was not an X-linked condition because there was male-to-male transmission. The number of affected males and females in the familial cases was the same. The most appealing explanation for

these observations is *genomic imprinting*, in which a condition expresses itself only when inherited from a parent of one sex. The maternally derived gene is apparently inactivated during oogenesis in the mother but can be reactivated during spermatogenesis in her male offspring. In the case of glomus tumors, the family history suggests that when the gene is inherited from the mother, the genetic information is somehow suppressed; but when inheritance is from the father, the genetic information allows expression of the tumors over time. These are slow growing, benign, single or multiple tumors. They are known as chemodectomas or nonchromaffin paragangliomas and are derived from the glomus body tissue. They are most often found in the carotid body but also sometimes in the adventitia of the carotid bifurcation, the glomus jugulare, or the vagal body. Genomic imprinting seems to be common among familial and congenital tumors.

van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ, et al. Genomic imprinting in hereditary glomus tumors: evidence for a new genetic theory. *Lancet* 1989; 2:1291-1294.

**Editor's Comment**—The concept of genomic imprinting is important and exciting. It may explain patterns of inheritance that have not previously been easily understood. Reexamination of a large pedigree, looking for differences in expression when the disorder is inherited from the mother versus the father, presents a whole new way of looking at information and understanding mechanisms of genetic expression.

Judith G. Hall, MD

## Steroids and Bowel Rest Versus Elemental Diet in the Treatment of Patients With Crohn's Disease: The Effects of Protein Metabolism and Immune Function

The traditional management of acute attacks of inflammatory bowel disease (Truelove regimen) consists of bowel rest, intravenous fluids, steroids, and antibiotics. However, there have been studies suggesting that an elemental diet is as effective as steroids in inducing remission from an acute attack of Crohn's disease. This study was undertaken to investigate the metabolic and immunological effects of these 2 disparate therapies.

Six patients with chronic Crohn's disease who met the inclusion criteria of a palpable inflammatory mass, elevated erythrocyte sedimentation rate (ESR), nausea, abdominal cramps, weight loss, and absence of obstruction were randomly assigned to receive a 1-week course of either steroids (400 mg/dL) plus bowel rest and intravenous fluids, or an elemental diet alone. At full strength, the elemental formula (Elental ED), infused via a nasogastric tube, provided 2,000 calories from glucose polymers, MCT oil, and 84 g of amino acids. Amino acid and protein turnover ratios, assessed by  $^{14}\text{C}$ -labeled tracers, plus immunological status were assessed initially and again on day 7 of treatment. Total nitrogen losses were estimated by adding 2 g of nitrogen to the 24-hour nitrogen excretion.

Clinical and symptomatic improvement occurred in all patients. Improvement was also reflected by more normal ESR values, platelet counts, and serum albumin and globulin concentrations. The steroid therapy resulted in higher levels of glucose, insulin, and cortisol, but lower T lymphocyte counts, immunoglobulin concentrations, and IgG synthesis rates. Plasma amino acid concentration, protein breakdown, and albumin synthesis increased in the steroid-treated patients whereas they fell in the patients who received the elemental formula. Nitrogen excretion increased in both groups over the duration of the study, but the mean nitrogen balance on day

7 was  $+2.4$  g/d for the group receiving the elemental diet and  $-8.9$  g/d for those who received the steroid regimen. Both therapies were associated with increased rates of plasma amino acid flux, amino acid oxidation, whole body protein turnover, and suppressed lymphocyte subsets, lymphocyte transformation, and serum complement concentrations.

The authors conclude that the primary difference with the steroid therapy was greater immunosuppression and higher nitrogen loss.

O'Keefe SJD, Ogden J, Rund J, et al. *J Parent & Ent Nutr* 1989; 13:455-460.

**Editor's Comment**—This study is very important; it is the first well-designed scientific study that attempts to evaluate the effects of bowel rest with steroid therapy versus an elemental diet for the management of an acute attack of Crohn's disease. In this prospective, blinded study, the authors demonstrate that both treatments induced clinical and symptomatic improvement of the disease; however, the treatment with steroids plus bowel rest was associated with greater immunosuppression and a more severe loss of nitrogen than occurred with the elemental diet. With the steroid-based therapy there was a cumulative loss of 55 g of body nitrogen (equivalent to 360 g of protein or 1.5 kg of lean body mass). This high catabolic state persisted even after the disease was in remission, suggesting that steroids may interfere with normal adaptation to fasting. In contrast, an elemental diet reversed this process and resulted in a small gain in body nitrogen.

The data clearly show that from a nutritional point of view, it is difficult to support the use of a starvation regimen in patients with acute disease. Additionally, the data point to a possible role of dietary proteins in the perpetuation of the inflammatory

process of patients with Crohn's disease. For the individual patient, the Truelove regimen of bowel rest, steroids, and antibiotics should always be complemented at least with IV nutrition or preferably with an elemental diet. The prompt reversal of the negative nitrogen balance with an elemental diet during acute relapses of the disease may allow children with Crohn's disease to sustain more normal growth. Therefore, I agree with the authors that diet restriction is contraindicated and that there are times when it may be advantageous to avoid the use of steroids.

Fima Lifshitz, MD

## Germ-line Mosaicism in Osteogenesis Imperfecta

Germ-line mosaicism is the presence of more than 1 population of germ cells within a gonad. It is suspected when multiple children affected with an autosomal dominant disorder or a disorder that results from a new mutation of an X-linked gene are born to normal parents. Although evidence for such mosaicism is usually circumstantial, Cohn et al document the phenomenon in a family with lethal osteogenesis imperfecta (OI) type II. Two affected sons were born to an "unaffected" father by 2 separate wives. Electrophoretic abnormalities typical of OI type II were detected in type I collagen synthesized by skin fibroblasts from both affected infants, but not from the father or from 2 unaffected sisters of the second son. Further analysis pointed to an abnormality in the  $\alpha 1(I)$  collagen chain; ultimately, a single nucleotide change resulting in a substitution of aspartic acid for glycine at position 883 of the triple helix was detected. Since the base change disrupted a restriction endonuclease cleavage

site, it allowed the normal gene to be distinguished from the mutant gene, which was exploited to search for the mutation in the germ cells and somatic cells of the father.

A small (225 base pair) fragment containing the exon harboring the mutation was amplified by polymerase chain reaction from genomic DNA isolated from the father's sperm, white blood cells, and hair root bulbs. The mutation was found in approximately 12% of sperm and in about 40% of the somatic cells. Thus, in addition to germ-line mosaicism, the father exhibited somatic mosaicism for the mutation, despite being clinically unaffected. The authors mention that they are aware of several other cases of undocumented germ-line mosaicism in OI type II and point out that it appears to be more common in OI type II (estimated 6% to 7%) than in most other genetic conditions. They con-

clude that the clinical phenotypes produced in genetic disorders reflect not only the qualitative effects of the mutation but also quantitative effects determined by the abundance and distribution of the cells expressing the mutation.

Cohn DH, Starman BJ, Blumberg B, et al. Recurrence of lethal osteogenesis imperfecta due to paternal mosaicism for a dominant mutation in a human type I collagen gene (COL1A1). *Am J Hum Genet* 1990; 46:591-601.

**Editor's Comment**—Determining recurrence risks for Mendelian (single gene) disorders, such as OI type II, used to be simple and straightforward. Standard risk figures are given in any genetics textbook. However, there are a growing number of

phenomena that complicate such calculations. Germ-line mosaicism is a good example. In the past, the father in the above case would have been given a negligible recurrence risk considering his normal clinical phenotype and especially his normal collagen electrophoretic studies. However, as demonstrated, his actual risk was substantially higher. Uniparental disomy, in which a child receives 2 copies of a particular chromosome from 1 parent and none from the other, and genomic imprinting, in which the expression of a mutation (and the disease phenotype) is influenced by which parent transmitted the mutation, are 2 other examples. It seems likely that more will be heard about these phenomena that distort Mendelian risk figures as their investigation receives more attention.

William A. Horton, MD

## Natural History of Premature Thelarche in Olmsted County, Minnesota, 1940 to 1984

Van Winter et al report a population-based study of the incidence of premature breast development in girls between the ages of 6 months and 6 years in Olmsted County, Minnesota, for 1940 to 1984. Because of the dossier-type recording by the Mayo Clinic and other health providers in this community, diagnoses are indexed so that the details of medical care for the entire community are available for review. The authors identify cases of unilateral or bilateral benign breast development occurring between the ages of 6 months and 8 years if other signs of sexual maturity had not developed by 8 years of age. A total of 66 girls were identified, for an incidence rate of 21.2 per 10<sup>5</sup> patient-years; 48 of these had early breast development as an isolated finding and 43 of the 48 could be followed through age 8. Of the 48 girls, 23 had bilateral breast development ranging from 1 to 6.5 cm in diameter. Of the 48, 43 were located and 39 responded to a survey concerning the development of early puberty, breast cancer,

gynecologic malignancy, or autoimmune disease. Of 25 respondents between the ages of 16 and 42 years, all had attained an adult height between 155 and 173 cm, their mean age of menarche was 12.6 years, and 10 women had attempted pregnancy and conceived.

The authors point out that this is the first population-based study to show that premature thelarche is self-limiting and has a low incidence. In most of the patients, the premature thelarche disappeared before the onset of puberty and was followed by normal puberty, including menarche and normal reproduction.

Van Winter JT, Noller KL, Zimmerman D, et al. *J Pediatrics* 1990; 116:278-280.

**Editor's Comment**—Although there have been reports of the prevalence of premature thelarche, there have been no studies that sys-

tematically followed these girls through puberty, young adulthood, and the reproductive years. There have been various reports in the literature of clusters of cases of premature thelarche, but as the authors point out, their significance is unclear because it is unknown whether the observed cases were more numerous than might have been expected by chance alone. The Olmsted County, Minnesota, and Mayo Clinic data provide a unique opportunity to perform such a population-based study. However, the causes of thelarche may be multiple. In addition, epidemics do occur, as for example those reported in Puerto Rico. Therefore, the incidence of thelarche will vary from geographic site to geographic site, and possibly from year to year. For example, between 1940 and 1960, estrogens were frequently found in vitamins, meats, and other ingestible products, but no ingestion of contaminating estrogens has been found in the patients in Puerto Rico (*J Pediatr* 1985;107:393-396). While



this short paper is an important addition to our understanding of the benign course of this disease, the statistics regarding incidence must be regarded as applying only to Olmsted County, Minnesota.

William L. Clarke, MD

## Growth in Hemophilic Boys After HIV Infection

Pasi et al measured height and weight 3 times yearly in 26 boys with hemophilia A who became HIV positive during the period from 1981 to 1986. Ten of the boys presently have AIDS-related complex. Height and weight recordings were analyzed over a mean period of 9.2 years, with a mean duration of HIV seroconversion of 4.5 years. Mean growth (height and weight) before and after seroconversion were analyzed in this group by the Wilcoxon matched pairs signed rank test. No significant change in growth or weight was observed after HIV seroconversion. One boy who developed clinical AIDS continued to grow along his respective percentile, and 1 boy with constitutional short stature continued to grow along his respective percentile. Only 1 boy failed to grow along the original percentile, but his growth retardation began 3 years before HIV seroconversion.

Pasi KJ, Collins MA, Ewer AK, et al. *Arch Dis Child* 1990;65:115-118.

**Editor's Comment**—This short descriptive paper is the first to document growth in children with asymptomatic chronic HIV infection. As noted by the author, growth failure has been described previously in children with chronic symptomatic HIV infection. The preservation of linear growth in the present sample (up to 6 years) demonstrates the heterogeneity of the complications seen in this syndrome.

William L. Clarke, MD

## Increase in Serum Concentration of Keratan Sulfate After Treatment of Growth Hormone Deficiency With Growth Hormone

Pachman et al measured the serum concentrations of keratan sulfate (KS) in 2 groups of children with short stature: 1 group with constitutional delay and the other with growth hormone deficiency (GHD). The study populations consisted of 14 children between 8 and 11 years of age with constitutional delay and 9 children, ages 8 to 15, with GHD, defined as a peak GH  $\leq 10$  ng/mL with insulin-induced hypoglycemia, oral L-dopa, or glucagon. The GHD children were growing at a rate  $< 4$  cm/yr whereas the children with constitutional delay were growing  $> 5$  cm/yr, which was nevertheless below the fifth percentile.

In children with constitutional delay, KS averaged  $414 \pm 118$  ng/mL, compared with  $505 \pm 126$  ng/mL in children from a control population (which consisted of 33 children 8 to 11 years old with normal growth). In the GHD children, KS levels were determined at the time of initial evaluation and after 3 to 15 months of GH therapy. These levels initially ranged from 239 to 587 ng/mL, encompassing the levels in the children with constitutional delay. However, 7 of the 9 children with GHD had a rise in KS ranging from 64 to 192 ng/mL during GH therapy. This increase in KS was correlated with an increase in growth velocity.

The authors point out that KS is a glycosaminoglycan that is almost exclusively derived from the metabolism of cartilage proteoglycans and that the amount of KS in the blood is directly proportional to the rate of degradation of cartilage proteoglycans. They previously reported that serum levels of KS rise from a low level in infancy to reach a plateau by age 4 to 5 years. The measurement of serum KS is felt to be an indicator of the response of chondrocytes to IGF-I. The relationship demonstrated between increased growth and increased serum KS suggests that KS may be a reasonable indicator of cartilage proteoglycan metabolism during growth. The authors note that

2 patients with GHD who did not increase their KS levels after GH therapy, despite increases in growth velocity, had pretreatment KS levels at the upper range of normal for age.

Pachman LM, Green OC, Lenz ME, et al. *J Pediatrics* 1990;116:400-403.

**Editor's Comment**—Measurement of keratan sulfate (KS) may be a useful indicator of the activity of GH/IGF-I in bone metabolism. These data are somewhat confusing, however, as the increase in KS was relatively modest despite marked increases in growth velocity in the GHD children on GH therapy. This may be due to the heterogeneity of the pretreatment KS levels in this group of children and also to the fact that KS levels plateau in early childhood. The authors correctly point out that an increase in KS indicates a change in the metabolism of proteoglycans, but it cannot be used to predict changes in growth velocity with GH therapy. It is important to remember that IGF-I levels also do not always correlate with response to GH therapy. Nevertheless, it is both interesting and useful to evaluate metabolic changes in bone as a consequence of GH therapy in our attempts to gain a better understanding of how children grow.

William L. Clarke, MD

## Characterization of Dimeric Forms of Human Pituitary Growth Hormone by Bioassay, Radioreceptor Assay, and Radioimmunoassay

Seven highly purified dimeric forms of human pituitary (extracted) growth hormone (hGH) were characterized

from the monomeric forms of 20-, 22-, and 24-kilodalton (kDa) hGH linked together by covalent or non-covalent bonds. Each was studied using 3 different assays: (1) a solid-phase radioimmunoassay (RIA) with rabbit anti-hGH antiserum, the results being expressed in mIU/L by reference to the WHO First IRP 66/217; (2) a radioreceptor assay (RRA) on solubilized bovine liver receptors; and (3) a bioassay (BA) measuring the growth effect on culture of Nb2 lymphoma cells.

These assays produced strikingly different results. In the RIA, all dose/response curves were parallel, except those of the 20-kDa monomeric and the 20/20-kDa dimeric forms. In the RRA, considerable differences appeared in the ability to displace labeled monomeric recombinant hGH from its ligand, with maximal effectiveness for 2 isomers derived from the 22-kDa hGH. The mitogenic effect in the BA was maximal with a non-acidic 20/22-kDa dimer, and minimal with the 20/20-kDa dimer, all the regression lines (number of cells versus log of hormone concentration) being parallel.

Brostedt P, Luthman M, Wide L, et al. *Acta Endocrinol* 1990;122: 241-248.

**Editor's Comment**—The general sense of the study is that the various molecular forms of GH found in the pituitary—both monomeric (little) and dimeric (big)—have different mobilities in the 3 types of assays used. It is likely that similar observations would be made for the circulating forms of GH. The authors also note that some variants of hGH occur in the pituitary, mainly in dimeric forms. We may conclude from this that measuring hGH is difficult; that the various types of assays may give discrepant results, depending on the molecular forms of the hormone; and that bioassay with Nb2 cells may be relevant for clinical studies.

Jean-Claude Job, MD

## Insulin-like Growth Factors I and II in Healthy Man: Estimations of Half-Lives and Production Rates

The authors measured the half-life of insulin-like growth factors (IGFs) in 2 normal young adult males after a bolus injection of radio-iodinated IGF-I and IGF-II, with measurement of the serum levels of both the free IGFs and the IGFs bound to their specific carrier proteins. They found a half-life of 10 to 12 minutes for free-labelled IGF-I and -II, 20 to 30 minutes for the 50-kDa bound complex, and 12 to 15 hours for the 200-kDa complex.

In a second step of the study, they infused recombinant IGF-I, 20 µg/kg per hour intravenously, during 6 days in the same subjects and measured the different circulating forms of IGFs by RIA after chromatographic separation. By this means, the calculated production rates were found to be 10 mg/d for IGF-I and 13 mg/d for IGF-II.

This agrees with the earlier findings, by the same group and by others, that the 200-kDa complex contains the major pool of IGF in human serum, and confirms that this

complex is mainly responsible for the relatively long half-life of IGF in humans. It suggests that, besides the main pool of 200-kDa, the free and the 50-kDa IGF pools, which have a rapid turnover and could account for daily IGF production, are the source of a shift toward the 200-kDa pool.

Guler HP, Zapf J, Schmid C, et al. *Acta Endocrinol* 1989;121:753-758.

**Editor's Comment**—These physiological data in adult humans are possibly of great importance for the interpretation of measurements of IGFs, mainly of IGF-I, in growing children and adolescents. Probably measurement of free IGF-I and of the 2 main IGF-I carrier protein complexes could reduce the difficulty in correlating the results of routine IGF-I assays with such clinical data as height or growth rate.

Jean-Claude Job, MD

## Comparison of Education and Occupation of Adults With Achondroplasia With Same-Sex Sibs

A common concern to parents of children with achondroplasia is that the children will suffer occupational discrimination when they grow up. To investigate the issue, Roizen and colleagues compared education and occupation levels in adults with achondroplasia to those of same-sex sibs. Information was gathered by interview or questionnaire from 8 affected men and 32 unaffected brothers and from 12 affected women and 35 unaffected sisters. An occupational score was calculated from a subscale of the Hollingshead Four Factor Index of Social Status. No significant differences in age or education were noted between the patients and their same-sex sib. The occupation score for affected men was not statistically different from that of their brothers;

however, the score for affected women was significantly lower than that of their unaffected sisters. Education level was the single most important variable affecting occupation level for both sexes. The authors speculate that physical deformity accompanying achondroplasia (eg, large head size) may be more detrimental in the workplace to women than to men. They stress the need for more research in this area and the need for parents and educators to invest heavily in educating achondroplastic children.

Roizen N, Ekwo E, Gosselink C. *Am J Med Genet* 1990;35:257-260.

**Editor's Comment**—As pointed out by the authors, this is a small study, and the data are not sufficient to

address certain issues, such as how well patients advance in their careers relative to their unaffected sibs. Moreover, the relatively low response rate, 23%, may have introduced bias into the results, eg, patients with low occupation scores may not have returned the questionnaires. Nevertheless, the final conclusion that education is a very important, if not major, determinant of adult success in the workplace is worth underscoring. This should be reassuring to average-statured parents of achondroplastic infants who are typically the most concerned about their child's occupational potential and what can be done to enhance it.

William A. Horton, MD

### Clinical Variation of Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy in 68 Patients

This relatively rare entity, also called polyglandular endocrinopathy type I (PGE-I), is characterized by at least 2 of the following: hypoparathyroidism, hypoadrenalism, and mucocutaneous candidiasis. Other autoimmune diseases are often associated with this basic triad, including alopecia, pernicious anemia, gonadal failure, vitiligo, hypothyroidism or hyperthyroidism or chronic lymphocytic thyroiditis, and hepatitis. Ahonen et al have analyzed the interrelationships of these entities, as well as ungual dystrophy (pitted nails), steatorrhea, and keratopathy (Table).

Periodic malabsorption was observed in 12 patients, which was intensified with hypocalcemia, but some patients had steatorrhea when they were not hypocalcemic, and malabsorption preceded hypoparathyroidism in some. Neither keratopathy, pitted nails, nor enamel hypoplasia correlated with hypoparathyroidism or hypocalcemia, suggesting strongly that these were independent entities.

Table —Incidence of autoimmune diseases in PGAD-I

|                                     | No. of Patients | Incidence % |
|-------------------------------------|-----------------|-------------|
| Moniliasis (candidiasis)            | 68              | 100         |
| Hypoparathyroidism                  | 54              | 79          |
| Hypoadrenalism                      | 49              | 72          |
| Diagnostic triad                    | 35              | 51          |
| Ovarian failure (age > 13 yr)       | 16 (of 29)      | 56          |
| Testicular failure (adults)         | 3               | 12          |
| Insulin-dependent diabetes mellitus | 8               | 12          |
| Pernicious anemia                   | 9               | 13          |
| Alopecia                            | 20              | 29          |
| Vitiligo                            | 9               | 13          |
| Keratopathy                         | 24              | 35          |
| Thyroid autoimmunity                | 3               | 4           |
| Enamel hypoplasia                   | 23              | 35          |
| Calcified tympanic membranes        | 14/42           | 33          |
| Ungual dystrophy (nails)            | 26/50           | 52          |
| Mucocutaneous candidiasis           | 33/50           | 66          |

With the exception of candidiasis, none of these entities was manifest before the age of 12 months. Although most of the organ diseases occurred in childhood, some patients developed autoimmunity of some organs, including hypoparathyroidism, as adults. Interestingly, patients who developed Addison's disease as the first disease other than candidiasis tended to develop far fewer associated diseases.

Ahonen C, Myllarianni S, Sipila I, et al. *N Engl J Med* 1990; 332:1829.

**Editor's Comment**—This report greatly augments previous data on patients with PGE-I. The association of diseases and their time of appearance is surprising to me in that keratopathy and hypoplastic enamel are apparently not related to hypocalcemia or hypoparathyroidism. The relatively good prognosis of patients with only candidiasis and Addison's disease is worth noting. Unfortunately, the presumed autoimmune process in the gut that causes steatorrhea remains an enigma. Hypocalcemia, when present, needs to be controlled to minimize the

steatorrhea. Interestingly, in the patients with Addison's disease, the aldosterone deficiency was evident before the cortisol deficiency in approximately 50% of the cases.

The authors have published several previous reports of this syndrome, concerning mode of inheritance, oral findings, diagnosis and staging of hypocortisolism in progressive autoimmune adrenalitis, effective use of ketoconazole against candidiasis, the presence of adrenal and steroidal cell antibodies in evaluating risk of adrenocortical and ovarian failure, and the expression of PGE-I in association with human leukocyte antigen (HLA)-A, but not HLA-DR. The interested reader will find these and other pertinent references in the extensive bibliography of this article.

Robert M. Blizzard, MD

## Prenatal Treatment of Females With Congenital Adrenal Hyperplasia Due to 21-Hydroxylase

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency has been well defined on a pathogenetic basis during the last few years. There are actually 2 genes linked to the HLA loci next to the C4B gene of the major histocompatibility complex on chromosome 6. Prenatal diagnosis is possible in the first trimester by chorionic villus sampling and DNA analysis or HLA linkage. Because congenital adrenal hyperplasia is the most common cause of female pseudohermaphroditism, the possibility of in utero therapy has been raised. This paper reports a pregnancy in which a female was recognized to be affected with the salt wasting form of congenital adrenal hyperplasia at 10 weeks of gestation. However, in order to suppress the adrenal, dexamethasone therapy had already been introduced during the eighth week. The child was born at term with minimal masculinization of her external genitalia in spite of being a severe salt loser.

The article summarizes the published total of 14 such cases of female infants who had been prenatally treated. Five newborn girls whose mothers received dex-

amethasone (starting between 5 and 8 weeks) had normal external genitalia. Five newborn girls whose mothers received hydrocortisone starting from 3 to 9 weeks had mild or partial virilization. Four female newborns whose mothers were treated with dexamethasone starting at 5 to 10 weeks had marked virilization.

It would appear that prenatal treatment has varying effectiveness. The reasons for this variation are not clear, but they may include familial variation in response to therapy, problems with transplacental passage of glucocorticoids, variations in maternal metabolism of glucocorticoids, variations in the clearance of exogenous glucocorticoids, fetal adrenal steroidogenic functional differences, and differences in the pituitary adrenal feedback mechanism.

Pang S, Pollack MS, Marshall RN, et al. *N Engl J Med* 1990;322:111-115.

**Editor's Comment**—This report demonstrates not only the power of DNA techniques to diagnose pre-

nately, but also the problems with intrauterine therapy: The therapy needs to be started earlier than it is possible to diagnose the presence of the biochemical abnormality. Since chorionic villus sampling is not available until approximately 9 weeks, the process of masculinization would have started prior to our ability to make the diagnosis. In addition, the range of masculinization among treated fetuses makes it clear that we do not really understand individual differences in response or the processes that lead to masculinization. It is clear that additional cases need to be followed carefully and reported so that we may ultimately arrive at the best therapies both in utero and ex utero. Follow-up information is also needed on those infants who are treated early but found not to be affected females. The assumption is that no harm has been done, but we need to be sure. A prospective collaborative study is very much needed. Hopefully, those pediatric endocrinologists with a special interest in congenital adrenal hyperplasia will establish such a study.

Judith G. Hall, MD

## Sex Steroids and Somatic Growth in Childhood

This is a short but provocative summary of the linear growth characteristics of 18 anatomically or functionally agonadal children with normal sex chromosomes, in an attempt to determine the importance of sex steroids to prepubertal growth. The children in this study included those with gonadal agenesis, gonadal dysgenesis, vanishing testes syndrome, surgical gonadectomy, gonadal destruction from radiation and chemotherapy, and biosynthetic defect in sex steroid production (17  $\alpha$ -hydroxylase deficiency). The agonadal status of these children was confirmed after surgical exploration, by determina-

tion of luteinizing hormone and follicle-stimulating hormone values, and plasma estradiol levels.

None of the 18 patients studied had heights or growth velocities greater than 2 SD below the mean of normal children. Thus, the authors suggest that gonadal steroids do not influence somatic growth during childhood and that it is highly unlikely that estrogen deficiency is responsible for growth failure of girls with Turner's syndrome.

**Editor's Comment**—This short report is interesting and provocative. It stands, however, in sharp contrast to studies in pubertal children. To confirm these findings, it would be useful to evaluate a larger group of children, more homogeneous as to diagnosis. In addition, it is not clear that all the patients had completed their growth at the time they were studied. Hence, it would be useful to determine the effect of full physiologic replacement of sex steroids on final height in similar patients.

William L. Clarke, MD

Campos S, MacGillivray M. *Am J Dis Child* 1989;143:942-943.



## MEETING CALENDAR

**October 16-20, 1990** The 41st Annual Meeting of the American Society of Human Genetics. Dr Albert B. Sabin Cincinnati Convention Center, Cincinnati, Ohio. Contact: American Society of Human Genetics, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1825)

**October 28-November 1, 1990** 42nd Annual Postgraduate Assembly of the Endocrine Society. Sheraton Waikiki, Honolulu, Hawaii. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1802)

**January 9-12, 1991** 38th Postgraduate Course, American Diabetes Association, Marriott Hotel and Marina, San Diego, Calif. Contact: American Diabetes Association, 1660 Duke St, Alexandria, VA 22314 (800-232-3472)

**January 12-16, 1991** 2nd International Symposium on Insulin-like Growth Factors/Somatomedins. The Grand Hyatt, Union Square, San Francisco, Calif. Contact: Sarah Burke, Extended Programs in Medical Education, Room C-124, University of California School of Medicine, San Francisco, CA 94143-0742. (Registration information 415-476-5808; program information 415-476-4251; fax: 415-476-0318)

**February 6-9, 1991** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, Calif. Contact: Marilyn Jones, MD, Children's Hospital, 8001 First St, San Diego, CA 92123 (619-576-5840)

**February 9-13, 1991** 18th Annual Seminar in Pediatric Nephrology: Current Concepts in Diagnosis and Management. Diplomat Resort and Country Club, Hollywood, Fla. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960, Miami, FL 33101 (305-549-6726)

**March 16-21, 1991** Spring Session, American Academy of Pediatrics. San Diego Convention Center, San Diego, Calif. Contact: Department of Education, American Academy of Pediatrics, PO Box 927, Elk Grove Village, IL 60007 (800-433-9016)

**April 29-May 30, 1991** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric Association. Riverside Hilton, New Orleans, La. Contact: Society for Pediatric Research, 2650 Yale Blvd SE, Suite 104,

Albuquerque, NM 87106 (505-764-9099)

**May 12-15, 1991** International Symposium on Epidemiology and Etiology of IDDM in the Young. Chantilly-Gouvieux, France. Contact: Dr. Allen Drash, Children's Hospital, Pittsburgh, PA 15213

**June 19-22, 1991** 73rd Annual Meeting of the American Endocrine Society. The Sheraton Washington, DC. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (Tel: 301-571-1802; fax: 301-571-1869)

**June 19-22, 1991** Combined ADA Council on Youth/ISGD Satellite Conference. "New Developments in the Etiology and Treatment of Childhood Diabetes." Williamsburg, VA. Contact: William L. Clarke, MD, Box 386, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA 22908

**June 24-27, 1991** 30th Meeting of the Teratology Society. Boca Raton Club, Boca Raton, Fla. Contact: Teratology Society, 9650 Rockville Pike, Bethesda, MD 20814 (301-571-1841)

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# GROWTH

## Genetics & Hormones

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## Nontraditional Inheritance

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**Judith G. Hall, MD**

*Professor of Medical Genetics  
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During the last few years, a body of evidence has accumulated suggesting that many kinds of genetic phenomena occurring are not explained by traditional Mendelian concepts. The purpose of this review is to bring to attention the genetic phenomena of mosaicism, uniparental disomy and genomic imprinting.

### **Mosaicism**

A growing body of evidence suggests that *mosaicism* is a common, possibly universal phenomenon.<sup>1</sup> X-inactivation and tissue differentiation occur regularly, producing functional mosaicism. It has been calculated that all individuals must have some mutant cells.<sup>2</sup> Since the human body has approximately  $10^{13}$  cells and since the mutation rate for known genes is in the order of 1 in 50,000, it follows that every individual will probably have mutations in some cells for every gene. Specific examples of mosaicism have been found in germ line and somatic cells now that DNA markers are available. For instance, there are individuals with neurofibromatosis who have a patchy or segmental form of neurofibromatosis and who appear to be somatic mosaics, but their affected children are fully affected in all cells, suggesting that the parent

has both germ line and somatic mosaicism.<sup>2</sup> Similarly, there are known cases of several disorders where the parents appear clinically normal but have more than 1 affected child. This has been demonstrated on a DNA level in osteogenesis imperfecta<sup>3</sup>: 1 father was found to have 20% of his sperm carrying a gene for collagen with a specific deletion that led to perinatal lethal osteogenesis imperfecta. The accumulating evidence suggests that as many as 5% of new mutations are actually occurring while the parental germ line is developing. On a practical level, this means that the risk of recurrence in a "new" mutation may be much higher than previously predicted.<sup>1</sup> Similarly, it means that in rare situations in which there are 2 or more affected children with normal appearing parents, the affected children may represent a new dominant mutation in 1 parent's germ line rather than an autosomal recessive condition.

Chromosome mosaicism has been demonstrated for a long time but only recently has the recognition of patchy or streaky pigment as an indicator of mosaicism led to careful studies of fibroblast chromosomes in individuals with mental retardation and patchy pigment.<sup>4,5</sup> Thus, many individuals with hypomelanosis of Ito and a large number of patients with

asymmetric growth have been found to be chromosomally mosaic.

Now that chorionic villus sampling is frequently being used as a means of prenatal diagnosis, it has been a surprise to find that as many as 5% of chorionic villus samples demonstrate chromosome mosaicism.<sup>6</sup> This suggests that chromosome mosaicism is a very common phenomenon during early embryonic development.

### **Uniparental Disomy**

Uniparental disomy occurs when 2 copies of a particular chromosome come from 1 parent and none from the other parent. Isodisomy occurs where there are 2 copies of exactly the same chromosome. Heterodisomy is defined as the presence of 2 different copies in a chromosome pair. In uniparental heterodisomy they are both inherited from the same parent. With the new DNA markers it is possible to determine whether each of the 2 chromosomes of a particular pair are inherited from each parent as they usually are. To the surprise of most investigators many examples of uniparental disomy are being found. In 2 cases out of approximately 1,000 cases of cystic fibrosis, for example, both chromosome 7s have come from the mothers,<sup>7,8</sup> and

the fathers apparently are not carriers for cystic fibrosis. What this means is that the usual concept of an autosomal recessive disease, where both parents are carriers, may be incorrect for some cases of a particular disease. In other words, in these examples of cystic fibrosis, both copies of the abnormal gene came from the mother. This may be an explanation for "nonfamilial" recessive diseases. It seems likely that these cases of cystic fibrosis may have started as trisomy 7 until 1 of the chromosomes was lost, an event that actually allowed the survival of the individual since trisomy 7 is a lethal condition. Similarly, in the case of uniparental (maternal) disomy of chromosome 15, which produces Prader-Willi syndrome,<sup>9</sup> we have learned that an individual must have a paternal complement of 15q11-q13 to be normal. These cases probably started off as trisomy 15, which is lethal. If this assumption is correct, then survival occurs because 1 chromosome 15 is lost early in development. If after the loss 2 maternal chromosomes are left, the individual apparently develops Prader-Willi syndrome.

It is unclear how common a phenomenon uniparental disomy is. However, in specific disorders it may be a relatively frequent occurrence, eg, it accounts for 20% to 30% of Prader-Willi cases. There is reason to infer from research with mouse models that uniparental

disomy of chromosome 7 in humans may be involved in producing the intrauterine growth retardation observed in the 2 cases of cystic fibrosis with uniparental disomy. Possibly, uniparental disomy is involved in other cases of intrauterine growth retardation or overgrowth.<sup>10</sup>

### Genomic Imprinting

The concept of genetic imprinting holds that modifications of genetic material take place depending upon whether genetic information is derived from the mother or the father. These modifications are observed as differences in phenotype. An accumulating body of compelling evidence from research with animals and humans suggests that imprinting occurs in some parts of some chromosomes and to some genes, and thus must be taken into consideration when evaluating inheritance patterns in humans.<sup>10</sup>

The evidence for genomic imprinting includes observations made in mice reproduced by pronucleus transplantation or parthenogenesis. Zygotes are constructed in which all the genetic information comes from either the mother or the father.<sup>11,12</sup> These constructs are nonviable. Interestingly, when there is only paternally derived chromosome material, relatively normal development of membranes and placentas occurs, but very poor development of embryonic structures. In contrast, there is relatively good

embryonic development but poor development of membranes and placentas in those zygotes with only maternally derived chromosomes. These 2 experimental situations are very similar to the naturally occurring human placental tumor, the hydatiform mole, in which there are 2 parental sets of chromosomes and overgrowth of the placenta, and also very similar to human ovarian teratomas, which are primitive tumors made up of all embryonic tissue types, but that are derived entirely from maternal chromosome complements.

The effects of genomic imprinting are also suggested by human triploids. Human triploids occur in 2 categories: those with 2 paternal chromosome sets and 1 maternal chromosome, and those with 2 maternal chromosome sets and 1 paternal. When there are 2 sets of chromosomes from the father, there is usually a typical well-grown cystic placenta (the typical cystic placenta of triploids) but poor embryonic growth. When there are 2 maternal complements, the pregnancy is almost always miscarried at an early stage, with very poor placental growth.<sup>4</sup> Therefore, paternal chromosomes appear to influence placental development more than maternal chromosomes and the reverse appears to occur in the development of the embryo.

Disomic experiments in mice support the concept of genomic imprinting.<sup>13-15</sup> Mice can be constructed where a segment of a chromosome or a whole chromosome will come only from 1 parent. A normal amount of chromosome material is present, but in a particular chromosome both copies will have been derived completely from either the mother or completely from the father. By working through the mouse chromosomes it has been found that there are 8 or 9 chromosome

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segments in which very different phenotypes are produced depending upon whether all the chromosome material comes from the male or female.<sup>16</sup> The changes that are seen are effects on growth, behavior, and survival.

As mentioned above, there are 2 human situations that are homologous. These are situations which have been recognized to represent uniparental disomy (ie, both chromosomes of a pair have come from 1 parent). There are 2 known cases of cystic fibrosis in which both chromosome 7s came from the mother. In these cases, the children with cystic fibrosis also have intrauterine growth retardation. There are also now several cases of Prader-Willi syndrome<sup>9</sup> that have occurred because the children have 2 chromosome 15s from the mother but no chromosome 15 from the father. Thus it appears that deficiency of a chromosome from 1 parent can lead to congenital abnormalities even though the correct number of chromosomes are present.

There are many chromosome deletion syndromes. Recently, it has been recognized that in the case of Prader-Willi and Angelman syndromes, it is usually the chromosome derived from a particular parent which is deleted. Prader-Willi and Angelman syndromes are deletions of the same area of chromosome 15. It is not clear whether they are deletions of exactly the same area of the chromosome; however, in the Prader-Willi syndrome, the chromosome 15 which is deleted is always the paternally derived one, and in the Angelman syndrome it is always the maternally derived one.<sup>17</sup> Interestingly, the "opposite" phenotypic effects seen in Prader-Willi and Angelman (hypotonic versus hyperactive) are very similar to those observed in the mouse disomy of distal chromosome 2.<sup>16</sup>

In a number of cases of congenital cancers, loss of heterozygosity is associated with the tumors but differential parental origin of the chromosome which is lost has been observed.<sup>10</sup> Thus, in sporadic Wilms' tumor, there is often loss of part or all of chromosome 11. It is almost always the maternal chromosome 11 that is lost.<sup>18</sup> These findings suggest that the maternal chromosome 11 plays some role in tumor suppression not compensated for by the paternal chromosome 11. Interestingly, familial Wilms' tumor is not linked to chromosome 11 but is usually transmitted by fathers. By contrast, examinations of sporadic sarcomas associated with loss of the retinoblastoma gene indicate that the chromosomal loss of chromosome 13 is almost always maternal.<sup>20</sup> However, in sporadic retinoblastomas of the eye, this kind of preferential loss from 1 parent or the other is not seen, suggesting that there may be differential imprinting in different tissues.

Transgene expression in some transgenic mice also seems to be modified depending upon the parent transmitting the gene in about a quarter of the cases examined.<sup>11,21</sup> In these situations, the DNA of the transgene has been integrated into the mouse genome and is passed from generation to generation, but the expression of the gene differs depending upon whether it is paternally or maternally transmitted. Interestingly, nonexpression is associated with methylation of the gene while expression is associated with nonmethylation, suggesting that the modifications are dependent upon methylation.<sup>22</sup>

Finally, there are a number of specific genes in humans and in mice in which there are unusual manifestations depending upon the sex of the parent from whom the gene is inherited.<sup>10</sup> For instance, in juvenile

Huntington's disease, inheritance is almost always from the father; in the congenital onset form of myotonic dystrophy, inheritance is almost always from the mother. Seizures, cerebellar ataxia, spinocerebellar ataxia, Beckwith-Wiedemann syndrome, fragile-X syndrome, and neurofibromatosis type 2, all seem to have unusual preferential transmission expression.<sup>10</sup>

The concept of imprinting needs to be reexamined carefully and always considered when evaluating a particular disorder, since traditionally we have been trained to think that the sex of the parent of origin of a particular gene has no effect. It would appear from research with mice that as many as 10% to 20% of genes have the type of modification that depends upon the parent of origin. In human studies, it is important to reexamine specific diseases, chromosomal syndromes, and malformation syndromes in order to determine whether a differential parental effect is associated with severity, age of onset, and a particular manifestation. In particular, chromosome anomalies need to be reexamined to ascertain whether the differences in manifestations observed in a particular syndrome are actually related to parent of origin.

If one looks at the areas of human chromosome that are homologous to the areas of mice chromosome involved with genomic imprinting,<sup>10</sup> there are a number of very interesting human genes that lie in these areas, including genes having to do with atherosclerosis, gastrointestinal diseases, tumorigenesis, growth factors, and congenital anomaly syndromes. The possible role that genomic imprinting plays in humans with diseases related to these genes<sup>10</sup> is under study.

In summary, uniparental disomy occurs when both chromosomes of a pair or segments of



chromosomes of a pair are derived from the same parent. Several examples (Prader-Willi syndrome, 2 cases of cystic fibrosis, and sporadic cases of Wilms' tumor) have been cited. Imprinting is the modification of expression of genetic material that occurs depending upon whether the genetic material is derived from the mother or the father. For example, paternally derived material (genes) have a positive effect on placental development and maternally derived material (genes) have a positive effect on embryonal development. Combinations of mosaicism, uniparental disomy, and imprinting may explain a variety of conditions and unusual patterns of inheritance not previously understood in human disease processes.

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# Mosaicism in Turner Syndrome

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As described by Henry Turner, the original clinical criteria for the diagnosis of Turner syndrome were: growth retardation, sexual infantilism, nuchal webbing and low posterior hairline, and cubitus valgus.<sup>1</sup> As others reported their experiences, the list of typical clinical features grew to include, among others: congenital lymphedema, a shield-shaped chest, multiple acquired and deeply pigmented nevi, cardiac and renal malformations, a high arched palate, recurrent otitis media, ptosis, short fourth metacarpals, and hypoplastic nails. The basis for the sexual infantilism was determined to be gonadal dysgenesis.<sup>2</sup> In 1959, Ford and

colleagues demonstrated monosomy X in a typical patient.<sup>3</sup> Subsequently, many different karyotypic alterations were recognized to result in the clinical features of the Turner syndrome. Today, it is generally held that it is monosomy for the short arm of the X chromosome (in the absence of a normal Y) that is responsible for the phenotype we associate with the diagnosis of Turner syndrome. Deletions of the long arm will not be discussed in this review.

Among cytogenetic surveys of liveborn individuals with the Turner syndrome,<sup>4,7</sup> a variety of chromosomal aberrations are reported with consistent frequency. Monosomy X (45,X) is found in approximately half of the patients with Turner syndrome. Patients with 46,X,i(Xq), in which there is a duplication of the long arm of one X and loss of the short arm, account for another 5%. In the remainder, there is mosaicism for 1 or

more abnormal cell lines. An individual who is mosaic for a chromosomal abnormality has 2 or more cell lines that originate from a single zygote. This arises from mitotic nondisjunction occurring after fertilization. Patients who are mosaic for Turner syndrome may have a normal 46,XX cell line and a second population of 45,X, 46,X,i(Xq), 46,X,+r(X), or some other sex chromosome aberration. There may be mosaicism for 2 abnormal cell lines, eg, 45,X/46,X,i(Xq). Mosaicism for 3 or more cell lines also occurs. Some patients with Turner syndrome will be mosaic with a 45,X/46,XY or 45,X/46,X, or abnormal Y karyotype.

Clinical surveys of patients with Turner syndrome show that no single clinical feature is invariably present, although short stature is most constant. With a few exceptions, some of which are discussed below, there are no reliable pheno-

type-karyotype correlations in Turner syndrome, and the full-blown syndrome can be seen in patients mosaic with a normal cell line. Conversely, fertility and relatively normal stature in individuals with pure 45,X have been reported. These occurrences may possibly represent mosaicism only in the affected tissue and not in unaffected tissues.

While mosaicism for a normal or isochromosome X cell line is relatively common among live-born females with Turner syndrome, it is infrequently found in abortuses with X chromosome abnormalities.<sup>6</sup> It is the 45,X chromosome complement that is associated with high fetal mortality and that is responsible for 10% of recognized embryonic and fetal loss at 5 weeks of gestational age.<sup>9</sup> It is estimated that less than 1% of 45,X conceptuses survive to birth.<sup>6</sup> It has been suggested that all liveborn individuals with 45,X are mosaic to some degree for a cell line with 2 sex chromosomes, and that it is this occult mosaicism that has allowed them to survive. A single study<sup>10</sup> attempted to address this hypothesis. In none of 10 patients karyotyped (skin and blood) was a normal cell line detected. Nonetheless, it is still possible that the mosaicism exists in tissues other than peripheral lymphocytes or fibroblasts, that there is mosaicism limited to the placenta that allows for survival, or that a normal cell line is present long enough in embryogenesis to ensure that the infant is carried to term, and then is lost prior to birth.

The phenomenon of mosaicism in Turner syndrome presents several problems for the clinician. The first is the influence of a normal 46,XX cell line on phenotype and prognosis, ie, how hard should one search for a normal cell line? The second is the converse: how and when should mosaicism for an

abnormal cell line be pursued in patients whose clinical findings suggest the clinical diagnosis of Turner syndrome and yet who appear karyotypically normal on initial testing of peripheral blood? Third, how should the presence of mosaicism for a 45,X cell line be interpreted in older women, given that loss of the second sex chromosome in tissue culture appears to be a normal feature of aging?<sup>11</sup> Fourth, mosaicism for part or all of the Y chromosome presents a unique problem in patients with Turner syndrome and is responsible for one of the few reliable phenotype-karyotype correlations in the disorder: the risk for gonadoblastoma. Finally, one other problem caused by mosaicism for sex chromosome aneuploidy is its significance when it is detected prenatally.

Mosaicism in Turner syndrome in which there is a 45,X cell line and a second cell line containing an X chromosome that is also abnormal, eg, 45,X/46,X,i(Xq);45,X/46,X+r(X), will not be addressed here. These patients do not differ phenotypically from 45,X individuals.

An adequate number of cells need to be evaluated to rule out mosaicism. Most laboratories will count between 25 and 50 cells to rule out mosaicism of 2% to 5% or more. Simpson<sup>7</sup> hypothesized that mosaicism for Turner syndrome is always detectable in blood and that karyotyping of fibroblasts is not necessary to detect a second abnormal cell line. Only 1 of our 131 patients who are mosaic for Turner syndrome is normal in blood and mosaic in fibroblasts. In all others, the mosaicism was detectable in peripheral lymphocytes. It is probably appropriate to discard the diagnosis of Turner syndrome if none of 50 lymphocytes counted is abnormal, and fibroblast karyotyping is probably unnecessary unless clinical

findings for Turner syndrome are highly suggestive of the diagnosis.

### **Should one search for a normal cell line in patients with Turner syndrome and a 45,X karyotype?**

The answer is "probably not." The presence of a 46,XX cell line in blood or in skin does not accurately predict taller stature, guarantee a better chance of fertility, or promise fewer complications of Turner syndrome. Although some reviews in the literature claim that 45,X/46,XX patients are generally more mildly affected,<sup>4,13</sup> we have not found this to be true in our patients (Tables 1 and 2). Although mosaics are more likely to have spontaneous menses, among our patients they are no more likely to be fertile. They are less likely to have congenital lymphedema and the physical features such as nuchal webbing and nail changes that are secondary to lymphedema. Lymphedema is far more common in infants with 45,X than in any other karyotype abnormalities for Turner syndrome. Thus, patients with 45,X are more likely to be diagnosed at birth than are other Turner syndrome patients, explaining the younger mean age of diagnosis in this group.

It is important to remember that the only persons who will come to medical attention for the diagnosis of Turner syndrome will be those who have some clinical stigmata to suggest the diagnosis. On the basis of current knowledge, it is useful, "if not necessary," to try to find a low level of mosaicism (less than 5%) for a normal 46,XX cell line in patients with Turner syndrome.

### **How important or useful is it to search for an abnormal chromosome complement in females in whom the question of the diagnosis of Turner syndrome is raised, but a cursory study of the karyotype is 46,XX?**

It is reasonable to assume

Table 1

| Characteristics       | 45,X<br>(n=155)   | 45,X/46,XX<br>(n=37)   |
|-----------------------|---|--|
| Mean birth weight     | 2.928 kg  | 2.799 kg   |
| Mean birth length     | 48.34 cm  | 47.82 cm   |
| Prematurity           | 12%   | 10%  |
| Mean age at diagnosis | 8.3 yr  | 12 yr  |
| Reason for diagnosis  | 40% Edema<br>26% Short stature<br>19% Primary amenorrhea<br>15% Other | 3% Edema<br>33% Short stature<br>25% Primary amenorrhea<br>39% Other |
| Final adult height    | 145.9 ± 4.7 cm  | 144 ± 5.6 cm   |

Table 2

| Complications       | 45,X<br>(n=155) | 45,X/46,XX<br>(n=37) |
|---------------------|-----------------|----------------------|
| Mental retardation  | 12%             | 11%                  |
| Psychiatric disease | 4%              | 8%                   |
| Edema               | 70%             | 28%                  |
| Web                 | 51%             | 27%                  |
| Otologic            | 80%             | 80%                  |
| Ophthalmologic      | 54%             | 47%                  |
| Thyroid             | 17%             | 26%                  |
| Cardiac             | 58%             | 56%                  |
| Renal               | 52%             | 31%                  |
| Gastrointestinal    | 33%             | 47%                  |
| Spontaneous menses  | 9%              | 21%                  |
| Orthopedic          | 28%             | 47%                  |

that there may be many women who have minor mosaicism for a 45,X cell line but are phenotypically entirely normal or might have a somewhat early menopause and/or subtle short stature who will not be identified as mosaics because the idea of karyotyping will never be entertained. One of our patients illustrates this point.

*J.S. was initially referred at age 13 for short stature; she has been otherwise entirely healthy and had grown along the 50th percentile until age 9, when she fell below the third percentile. She had no other clinical features of the Turner syndrome. Her mother was 5 ft*

*tall and her father was 6 ft tall. Mother's menarche was at age 14. Karyotyping revealed 45,X/46,XX(6%:94%) in peripheral lymphocytes. Thyroid functions, somatomedin C, and estradiol findings were all normal. She was lost to follow-up until she returned at age 18.5 years. Menarche had occurred at age 16; her menses were regular and she was 157.7 cm tall. A skin biopsy for karyotyping was obtained and revealed a normal 46,XX chromosome count. However, one of the X chromosomes appeared different from its homologue and a repeat blood sample was obtained to allow for better morphology. To our surprise, hav-*

*ing assumed the original 45,X cell line was the result of an in vitro artifact, her repeat lymphocyte study again demonstrated mosaicism for 45,X in 2 of 50 cells (4%). Both X chromosomes were structurally normal. She has recently delivered her first healthy child.*

The discovery of mosaicism in this patient was fortuitous and possibly irrelevant. Had her initial evaluation resulted in the clinical diagnosis of constitutional growth delay and chromosome testing had been deferred, her minor mosaicism would have remained undetected. Had her parents been less concerned, her short stature would not have been evaluated and would have resolved over time.

Among fertile patients with Turner syndrome, there is an increased risk for aneuploidy (both of the X chromosome and of the autosomes) in offspring. Is such a patient at increased risk? Horsman et al<sup>13</sup> karyotyped 100 women with repeated spontaneous loss of pregnancy and found 15 with mosaicism for 45,X (range, 2% to 10%). They found a similar proportion of 45,X mosaicism in women without a history of pregnancy loss and in none of their patients was a 45,X cell line detected in fibroblast culture.

Does a certain level of mosa-

icism for 45,X have to exist before a significant pregnancy risk is posed? Patients with Turner syndrome who have spontaneous menses often have premature ovarian failure or menopause. Is our patient at risk for this? There are no data to address these questions and it is clinically unwarranted to exhaustively search for minor mosaicism for 45,X in a girl with short stature or in a woman with gonadal dysgenesis and no other features of the Turner syndrome. Screening follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels is more likely to accurately measure ovarian function than the presence of minor mosaicism for 45,X and should be performed when appropriate.

#### **How can one interpret mosaicism in older individuals?**

In males and females alike, there is an increasing incidence of monosomy X in tissue culture with aging.<sup>11</sup> No studies have addressed the tolerable upper limits of mosaicism for 45,X in younger individuals. In the absence of clinical features, the diagnosis of Turner syndrome should not be made on the basis of minor mosaicism for 45,X cell line in women over the age of 30. As Horsman et al<sup>13</sup> suggested, the implications of mosaicism for childbearing are unclear and the results of karyotyping do not allow for discriminate predictions or management of patients.

#### **How does mosaicism for a Y or Y-derived chromosome impact the management and treatment of the patient with Turner syndrome?**

Mosaicism for 45,X/46,XY or 45,X/46,X, abnormal Y chromosome constitution is a special situation in Turner syndrome. These patients may be phenotypically classic for Turner syndrome or may be ascertained because of ambiguous genitalia or minor genital anomalies such as hypospadias and

undescended testes. The presence of a Y chromosome confers an increased risk for gonadoblastoma (15% to 30%) in the gonadal streaks.<sup>14-17</sup> This risk increases with age. All patients with Turner syndrome and a Y chromosome should undergo prophylactic gonadectomy.

It has been suggested that those individuals with a nonfluorescent Y and/or those without certain repetitive Y-specific DNA sequences may not be at risk for gonadoblastoma. In at least 1 of our patients this was not true. Thus, until further studies are done or better methods of determining the presence of Y loci that confers the risk for malignancy are available, prophylactic gonadectomy in any patient with even a segment of a Y chromosome remains the appropriate treatment.

There have been a few reports of gonadoblastoma in individuals with 45,X Turner syndrome in whom there was no evidence of mosaicism for the Y chromosome.<sup>18</sup> Interestingly, at least 2 of the reported patients had spontaneous menses and ovulated for a number of years prior to gonadal failure. Katayama et al<sup>18</sup> suggested that spontaneous breast development might be a marker for those patients who were at risk for gonadoblastoma, but who did not have a Y chromosome or segment, because such occurred in his patients with gonadoblastoma and in some of those reported in the literature. This may not be a reliable marker, as we have seen spontaneous breast development (Tanner 2 and 3) in at least 10% of our adult patients with 45,X/46,X,i(Xq) and other "non-risk-bearing" karyotypes, none of whom have developed gonadoblastoma. In addition, no breast development occurred in 5 of 8 cases of gonadoblastoma in non-Y-bearing women with Turner syn-

drome.<sup>19-22</sup> We believe some mild spontaneous breast development is not unusual in Turner syndrome and should not prompt gonadectomy in a non-Y-bearing individual. Gonadoblastoma also has been reported in patients who are mosaic for rings, markers, and fragments. It is often difficult to determine the chromosomal origin of a marker chromosome in patients with Turner syndrome. The assumption has been made that it is only those markers derived from a Y chromosome that confer an increased risk for gonadal malignancy. Until such time when molecular techniques for detecting those Y-specific sequences that confer the risk for gonadoblastoma<sup>23</sup> can be employed routinely and inexpensively in patients with Turner syndrome, management of patients who are mosaic for rings and fragments should be individualized. Routine screening with ultrasound imaging, prophylactic gonadectomy, or judicious non-intervention may all be appropriate.

#### **What is the significance of prenatally detected mosaicism to the patient and the clinician?**

Perhaps the most difficult problem posed by mosaicism for Turner syndrome is its prenatal detection. Mosaicism and pseudomosaicism in amniotic fluid cell cultures are common and may be found in 3.5% of samples.<sup>24</sup> When mosaicism for 45,X/46,XX or 45,X/46,XY is found at prenatal diagnosis, it presents significant problems in interpretation. The mosaicism may result from an *in vitro* artifact, may represent mosaicism limited to the placenta, or may be indicative of true fetal mosaicism. In a survey<sup>25</sup> of 92 cases of 45,X/46,XY mosaicism diagnosed prenatally, 76 were available for clinical examination either after termination or at delivery. Of these, 75 were phenotypic males and 1 was a



phenotypic female. Seventy-two of the 75 males had normal external genitalia. The remaining 3 males had hypospadias ranging from mild to severe. The single phenotypically female infant had clitoromegaly.

Gonadal histology was available for 11 of the fetuses following termination of the pregnancies. Of these, 3 abortuses with normal male external genitalia were found to have gonadal abnormalities. Several other surveys<sup>26,27</sup> also suggest that upwards of 90% of fetuses diagnosed as 45,X/46,XY will have a normal male phenotype at birth. This is in contrast to those patients diagnosed after birth who generally come to medical attention because they are phenotypically abnormal. As Chang et al underscored in their report,<sup>24</sup> dysgenetic gonads can occur in the presence of normal male external genitalia in 45,X/46,XY infants: "Therefore the risk of gonadal pathology is not limited to individuals with hypospadias or ambiguous genitalia." Even those infants who appear phenotypically normal continue to be at risk for gonadoblastoma and should be followed appropriately for that complication.

It will require long-term follow-up of these prenatally diagnosed cases to assess the lifetime risks of infertility, testicular failure, gonadoblastoma, and short stature. It is also clear from published surveys<sup>25-29</sup> that the proportion of karyotypically normal to abnormal cells in amniotic cell tissue culture does not predict the phenotypic outcome in the infant.

The same spectrum of clinically normal to classic dysmorphism of Turner syndrome occurs in mosaicism for 45,X/46,XX detected prenatally. At this time, mosaicism for sex chromosome aneuploidy that is detected prenatally continues to present a difficult counseling situation. Prospective parents need to be informed of the full

range of possibilities. They need an explanation of the possible biases of prospective and retrospective studies and need to understand that there is no single "correct" response (ie, to terminate or to continue). It has been the experience in our clinic that most families consider many factors other than the specific phenotypic risk in arriving at a decision about pregnancy management. In nearly all situations, it has been possible to support the family in the decision they have already made. It is the exception rather than the rule that the information we give to families significantly changes their course of action.

## Conclusions

In summary, the type of chromosomal abnormalities found in patients with Turner syndrome correlates poorly with the clinical findings, the possible exception being that the occurrence of gonadoblastoma in the primitive gonads is more likely to occur in patients with a Y chromosome. However, even this correlation is not absolute since patients with Turner syndrome and no Y chromosome or apparent fragment of a Y chromosome may develop gonadoblastoma. The statistics, however, do not justify routine gonadectomy in patients without a Y chromosome or fragment.

Mosaicism determined by amniocentesis may be misleading, as children without the usual characteristics of Turner syndrome have been reported to have a mosaic karyotype by amniocentesis. Counseling must be judicious when mosaicism of the X chromosome is found in specimens obtained by amniocentesis.

In contrast to beliefs of a year ago, the presence of XO/XY mosaicism does not consistently lead to abnormalities of the external genitalia.

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## Special Report

The Annual Meeting of the American Diabetes Association, Atlanta, Georgia  
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Several abstracts and presentations may be of interest to our readers. These involve the metabolic actions of GH and IGF-I, the relationships between GH, IGF-I, and diabetic complications, and the effect of diabetes on growth.

Fryburg and Barrett (*Diabetes* 1990;39[suppl 1]:115A) describe studies on the effect of GH on protein synthesis in the skeletal muscle of the isolated forearm of normal men. By using an infusion of GH (0.14  $\mu\text{g/kg/min}$ ) and concomitant infusions of  $^3\text{H}$  phenylalanine and  $^{14}\text{C}$  leucine into the brachial artery for 6 hours, the synthesis and degradation of protein were determined at 3 and 6 hours. Systemic insulin, amino acids, glucose, and glucose uptake were unchanged during the study. A net anabolic effect on net forearm balance for both phenylalanine and leucine was observed due to an increase in skeletal muscle protein synthesis. Thus, GH apparently stimulates direct protein synthesis by skeletal muscle in a time-dependent manner in the absence of any change in plasma amino acids, and its action is distinct from that of insulin, which acts on primarily protein degradation.

Elahi et al (*Diabetes* 1990; 39[suppl 1]:88A) reported their studies on the acute effects of IGF-I infusions on glucose kinetics in healthy men. Utilizing the clamp technique, IGF was infused over 4 hours at 2 doses (75 and 112  $\mu\text{g/kg/4h}$ ) and compared to an insulin clamp (15 mU/m $^2$ /min). Glucose production declined equivalently in the 112  $\mu\text{g}$  dose of IGF-I and the insulin clamp studies, but was unchanged when the 75  $\mu\text{g}$  dose of IGF-I was infused by clamp. Glucose disappearance was increased by 165%

by the 112  $\mu\text{g/kg}$  dose and 70% by the 75  $\mu\text{g/kg}$  dose. The larger dose suppressed insulin secretion without affecting GH levels. The authors state that these data indicate that there are different effects of IGF-I and insulin on the production and disappearance of glucose, with a more prominent effect of IGF-I on glucose disappearance. Thus, they speculate that IGF-I may be useful in a variety of insulin resistant states.

Agardh et al (*Diabetes* 1990;39[suppl 1]:31A) measured basal GH levels and TRH (200 mg) stimulated GH levels in 11 patients with type I diabetes who had rapidly progressive severe retinopathy. These were compared to those in a control group matched for age and duration of disease, but without background retinopathy or any retinopathy at all. Basal GH levels were above normal in the severe retinopathy group and higher than in the control group. In addition, the increase in GH levels after TRH was significantly higher in those individuals with severe retinopathy even though IGF-I levels were normal in all patients but one. The authors suggest that the results indicate that abnormal GH, but not IGF-I, may contribute to the development of severe retinopathy in type-I diabetic patients.

Werner et al (*Diabetes* 1990;39[suppl 1]:77A) measured the expression of IGF-I receptor genes in the kidney, brain, and testes of streptozocin-induced diabetic rats by determining the amount of  $^{125}\text{I}$ -IGF-I binding to membrane preparations. The levels of mRNA for IGF-I receptor in the kidneys of diabetics were increased 2- to 3-fold as compared to controls, where no significant changes were detected in the levels of

ligand mRNA for IGF-I binding. Insulin therapy reduced the levels of both receptor mRNA and binding to control values. There were no significant changes observed in the levels of receptor RNA or binding in either brain or testes. The authors suggest that the increased expression of IGF-I receptor in the kidneys of diabetics may explain, at least in part, the proliferation of mesangium in diabetic nephropathy.

A somewhat similar study was reported by Catanese (*Diabetes* 1990;39[suppl 1]:11A), who studied IGF-I mRNA in streptozocin diabetic rats' livers, kidneys, and lungs. Untreated diabetic rats had a 10-fold reduction in hepatic IGF-I mRNA by 24 hours, whereas, a 2- to 3-fold increase of kidney IGF-I RNA was noted at 24 hours. This increase in kidney mRNA was not seen with doses of streptozocin less than 120 mg/kg, suggesting that the severity of the metabolic abnormality may affect this response. Lung IGF-I RNA levels were unchanged. Insulin therapy restored IGF-I mRNA levels toward normal.

The authors state that these data suggest that gene expression for IGF-I is regulated in a tissue-specific manner in diabetes and that factors in addition to GH may modulate the endocrine effects on growth.

Wise et al (*Diabetes* 1990; 39[suppl 1]:29A) reported on a longitudinal study of the growth velocity in 112 children with diabetes. The children were seen at 4-month intervals for a total of 715 visits. Glycemic control was measured by determining glycosylated hemoglobin concentrations (normal, 4% to 8%). Pubertal status was determined by

physical exam, and the height was measured utilizing a stadiometer. Height measurements were normalized for age and sex by converting to Z scores. A linear relationship was seen between glycosolated hemoglobin concentrations and delta Z ( $r = -0.15$ ,  $P < 0.001$ ). Glycosolated hemoglobin values less than 8% were associated with growth acceleration whereas the greatest growth deceleration occurred with HbA<sub>1c</sub> greater than 16%. The level of glycosolated hemoglobin at which growth suppression occurred was dependent on pubertal status: Tanner 1, HbA<sub>1c</sub>  $\geq 10\%$ ; Tanner 2 to 3, HbA<sub>1c</sub>  $\geq 8\%$ ; Tanner 5, HbA<sub>1c</sub>  $\geq 16\%$ . The authors concluded that linear growth velocity is closely related to metabolic control, and that children in early puberty appear to be the most vulnerable to growth suppression when control is poor. Once puberty is well established, growth suppression does not occur until marked hyperglycemia is evident.

William L. Clarke, MD

## In Future Issues

### **Obesity in Childhood and Adolescence, Part I: Genetics, Physiology, and Growth**

by W.H. Dietz, MD, and L.G. Bandini, MD

### **Obesity in Childhood and Adolescence, Part II: Pathophysiology, Associations, and Complications**

by W.H. Dietz, MD

### **Oxandrolone Therapy: 25 Years Experience**

by R.M. Blizzard, MD, P.C. Hindmarsh, MD, and R. Stanhope, MD

### **Update: The Genetics of Insulin-Dependent Diabetes**

by W.E. Winter, MD, and N.K. McLaren, MD

### **Support Groups for Individuals With Growth Problems and Their Families**

by J. Weiss, MSW, LCSW, and J.G. Hall, MD

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## Abstracts From the Literature

### **Effect of Puberty on Initial Kidney Growth and Rise in Kidney IGF-I in Diabetic Rats**

Bach and Jerums studied the development of kidney enlargement and insulin-like growth factor I (IGF-I) levels in kidney tissue in prepubertal and postpubertal male Sprague-Dawley rats, half of whom were made diabetic by streptozocin (STZ) injections. On days 1 through 3 and day 7 after STZ

injection, groups of diabetic and control animals were sacrificed following blood sampling for plasma IGF-I, testosterone, and glucose determinations. Plasma IGF-I and testosterone levels were significantly higher in postpubertal rats. The pattern of kidney enlargement was different despite comparable

blood glucose levels in prepubertal and postpubertal diabetic rats. Kidney weight increased significantly more in postpubertal diabetic rats than in postpubertal controls by day 2 after STZ injection. By day 7, the kidney weight had increased by 36%. In contrast, kidney weights of diabetic

prepubertal rats became significantly greater (by 14%) than that of prepubertal controls on day 7 only. The content of IGF-I in kidneys was significantly greater in postpubertal diabetic rats than in controls, with a peak at days 1 and 2 following STZ injection. In contrast, kidney IGF-I levels in prepubertal diabetic rats were no different from the controls. Because of these findings, the authors suggest that local accumulation of IGF-I may be important in kidney enlargement associated with diabetes.

The authors state that kidney enlargement is a well-described early feature of insulin-dependent diabetes in humans as well in STZ-injected rats. This increase in kidney size in humans is associated with an increase in glomerular filtration and may be associated with an increased risk of developing diabetic nephropathy. Thus, the rise in kidney IGF-I that precedes kidney growth in postpubertal diabetic rats in

this study is consistent with a potential role for local IGF-I accumulation in diabetes-associated kidney enlargement. In addition, the differences observed between the kidney weights of prepubertal and postpubertal diabetic rats are compatible with the hypothesis that nephropathy in human diabetes is a postpubertal event.

Bach LA, Jerums G. Diabetes 1990;39:557-562.

**Editor's Comment:** *Although it is not the policy of Growth, Genetics, and Hormones to abstract manuscripts dealing with animal studies, the present study has such important implications for understanding the relationship between diabetic nephropathy and puberty that it has been included here. It has been suggested that diabetic complications are related to the postpubertal duration of the disease. Several investigators*

*have reported that microalbuminuria, an early sign of nephropathy, never occurs prepubertally; and capillary basement membrane thickening, which is another feature of diabetic microangiopathy, has been shown to be related to the level of glucose control, but only in postpubertal subjects. The data in the present study affirm these clinical findings in that kidney enlargement was observed in postpubertal animals. In addition, the observation that kidney IGF-I levels correlate with kidney weights in these diabetic animals probably is an important observation. Other investigators have demonstrated that the administration of somatostatin analogue can suppress kidney growth in diabetic rats, which suggests a role for IGF-I in the process of kidney growth and in the development of diabetic nephropathy.*

William L. Clarke, MD

## Effect of the Long-Acting Somatostatin Analogue SMS 201-995 on Growth Rate and Reduction of Predicted Adult Height in Ten Tall Adolescents

Tauber et al studied the use of SMS 201-995 as therapy for tall stature in 10 patients (4 boys and 6 girls), aged 11.5 to 17 years, whose mean height deviation was +3.2 standard deviations. Patients were eligible for the study if they were over 11 years old (girls) and 13 years old (boys) and had a predicted adult height of at least 190 cm (boys) and 180 cm (girls) according to Bayley and Pinneau tables. Mean bone age (BA) for boys was 14.6 years and for girls 12.4 years. SMS 201-995 (250 U) was given twice daily (at 0700-0800 hours and 30 minutes prior to bedtime). Six patients were treated for 1 year and 4 patients

were treated for 6 months. Therapy was stopped in boys at a BA of 17 years or greater and in girls at a BA of 15 years. Height and weight measurements were performed every 45 days. Somatomedin C levels were measured at 0, 3, 6, and 12 months of therapy. BA was evaluated at 6 and 12 months. Patients underwent 24-hour growth hormone (GH) evaluation, and integrated concentrations of GH were calculated. Mean growth rates significantly decreased from 7.1 cm/yr to 2.7 and 2.4 cm/yr after 6 and 12 months of therapy, respectively. Mean BA increased from 14.6 years before therapy to 15.8 and 16.8 years, respectively,

after 6 and 12 months of therapy. Delta SDS/BA before and after 6 and 12 months of SMS 201-995 therapy are shown in Table 1. The 24-hour mean integrated concentration of GH decreased from 5.3 to 3.6 ng/mL per minute after 6 months and to 3.9 ng/mL per minute after 12 months of therapy, although individual responses were highly variable.

Somatomedin C values decreased from 1.7 to 0.9 U/mL after 3 months and to 1.1 and 1.0 U/mL after 6 and 12 months, respectively. Mean predicted adult height decreased from 198.7 to 193.7 cm in boys and from 184.5 to 179.7 cm in girls. Final height has not been



**Table 1** — Growth rate SDS/BA before and after 6 and 12 months of SMS 201-995 therapy

|          | Before SMS 201-995<br>SDS/BA | 6 months<br>SDS/BA | 12 months<br>SDS/BA |
|----------|------------------------------|--------------------|---------------------|
| Patients |                              |                    |                     |
| 1        | -0.6                         | -2.2               | -0.4                |
| 2        | +0.2                         | -1.5               | —                   |
| 3        | +0.3                         | 0.6                | -1.7                |
| 4        | +0.4                         | -2.5               | -0.4                |
| 5        | +0.9                         | -2.9               | -1.4                |
| 6        | +0.5                         | -3.4               | -2.2                |
| 7        | +0.7                         | -1.7               | 0                   |
| 8        | +0.2                         | -3.3               | —                   |
| 9        | +0.1                         | 0                  | —                   |
| 10       | -0.6                         | -2.2               | —                   |
| Mean     | +0.4                         | -2.0               | -1.0                |
| SD       | 0.2                          | 1.1                | 0.9                 |

the routine use of ethinyl estradiol in tall girls may be associated with significant hyperlipidemia. SMS was apparently well tolerated during this study, but as the authors point out, the minimum effective daily dose has not been defined. In addition, there were no controls in this study. It would have been useful to have evaluated adolescents or children with similar BAs and similar predicted heights who received no therapy or received traditional estrogen or testosterone therapy. Thus, one cannot conclude that the reduction in growth velocity is different from that which may have occurred with other therapies. In addition, the patients in this study had relatively advanced BAs, and all were either in Tanner stage 3 or 4. It is conceivable, then, that their growth rates may have declined during the year of therapy regardless of the use of SMS. Despite the drawbacks of this study, the information presented suggests that long-acting somatostatin analogues may be useful for the treatment of tall stature.

William L. Clarke, MD

achieved in all cases. No patient discontinued SMS 201-995 because of side effects, although transient diarrhea was noted in all cases during the first 10 days of treatment. Routine blood chemistries; complete blood count; vitamin A, D, and E levels; and glycosolated hemoglobin remained normal in all patients. Other endocrine functions remained within the normal range. Menarche occurred during SMS treatment in 4 girls. One patient developed asymptomatic

gallbladder microlithiasis at 6 months of treatment.

Tauber MT, Tauber JP, Vigoni F, et al. *Acta Paediatr Scand* 1990; 79:176-181.

**Editor's Comment:** SMS 201-995 administration was associated with decreases in growth rate and plasma somatomedin C levels in this study. The authors stated, however, that the response to treatment was variable and unpredictable. This is an important study, as

## Effect of Oral Clonidine Insulin-Induced Hypoglycemia and Exercise on Plasma GHRH Levels in Short Children

The ability to measure radioimmunoassayable growth hormone releasing hormone (GHRH) in the peripheral blood offers some insight into the mechanisms of action of the various stimuli that are used for routine evaluation of the secretion of growth hormone (GH) in children. A previous work (Donnadieu M, Evain-Brion D, Tonon MC, et al. *J Clin Endocrinol Metab* 1985;60:1132-

1134) had shown that L-dopa increases blood GHRH just before the GH peak while ornithine or arginine infusion does not. The current study (Gil-Ad I, Leibowitch N, Josefsberg Z, et al. *Acta Endocrinol* 1990;122:89-95) extended this type of investigation to 3 other GH stimulation tests.

Thirty-one healthy short stature children in whom GH deficiency had been ruled out

underwent 1 of the following tests: oral clonidine, 0.15 mg/m<sup>2</sup> (n = 13); insulin-hypoglycemia, 0.1 U/kg IV (n = 12); or exercise (n = 6). Their GH peaks during these tests were in the normal range. Clonidine induced a significant increase of peripheral GHRH levels from 5.6 ± 1.5 pmol/L at the basal level to 12.2 ± 2.5 pmol/L at 60 minutes. Neither insulin-induced hypoglycemia nor exercise signif-

icantly changed the plasma levels of GHRH. This clearly suggests that clonidine provokes a release of GH through GHRH, whereas stress stimuli such as hypoglycemia and exercise achieve GH release in other ways – possibly inhibition of somatostatin.

Moreover, the current study was extended to determine the effects of clonidine and hypoglycemia upon the thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH). Clonidine did not modify it. Insulin provoked a potentiation of the total response and an anticipation of the TSH peak following TRH injections. Since it is known that somatostatin inhibits the TSH-releasing effect of TRH, these results favor the hypothesis of an inhibition of somatostatin during insulin-induced hypoglycemia.

**Editor's Comment:** *The various ways of evaluating the secretion of GH and/or the releasable pituitary GH stores continue to deserve attention. It could be of physiologic and practical importance to know the sites at which the different stimuli in current clinical use act upon the complex regulatory mechanisms that command the release of GH. Measuring GHRH in peripheral venous blood, although it cannot reflect exactly what happens in the hypothalamo-hypophyseal portal circulation, offers an approach to its study in humans. The parallels between the studies cited here prompt me to point out the similarity between the action of clonidine and that of L-dopa, which both increase not only plasma GH but also GHRH, whereas infusion of amino acids or stress tests do not. This also emphasizes the importance of somatostatin in the regulation*

of GH secretion, which up to now has not been possible to investigate directly in children but could play a major role in various growth disorders and especially in functional or so-called idiopathic GH deficiencies.

Jean-Claude Job, MD

**Editor's Comment:** *Measurements of GHRH in the peripheral circulation are somewhat difficult to interpret, as GHRH may be produced outside the hypothalamus. The importance of these observations depends upon unequivocal certainty that the GHRH measured in the peripheral circulation reflects what takes place in the hypothalamus. Confirmation of this may be difficult. The topic will be followed with much interest by all of us.*

Robert M. Blizzard, MD

## **The Prepubertal Hiatus in Gonadotropin Secretion in the Male Rhesus Monkey (*Macaca mulatta*) Does Not Appear to Involve Endogenous Opioid Peptide Restraint of Hypothalamic Gonadotropin-Releasing Hormone Release**

In higher primates, gonadotropin secretion is elevated in early infancy and again in puberty, but between these times there is a period extending from 6 to 30 months in Rhesus monkeys in which pulsatile gonadotropin-releasing hormone (GnRH) release essentially ceases. Since it has been demonstrated that GnRH neurons of prepubertal monkeys receive innervation from endogenous opioid peptide (EOP) neurons, experiments were done to determine if the restraint of the GnRH secretion was due to EOP secretion.

The EOP antagonist, naloxone, was given to a number of castrated monkeys in 3 doses: as a bolus, as a

continuous infusion, and as an intermittent infusion. Prior to the naloxone, the monkeys had 3 weeks of intermittent GnRH infusion so that the pituitary was appropriately primed to respond. However, the naloxone did not cause any increase in blood luteinizing hormone (LH) although a GnRH injection immediately after the experiment always produced high responses. Thus, the mechanism of the childhood GnRH restraint does not involve EOPs. The authors add that unpublished experiments in female monkeys show the same effect.

Medhamurthy R, Gay VL, Plant TM. *Endocrinology* 1990;126:1036-1042.

**Editor's Comment:** *This characteristically well-designed experiment has a very clear cut result that has been already adumbrated in the human by Mauras, Veldhuis and Rogol (J Clin Endocrinol Metab 1986;62:1256-1263). The mechanism of the GnRH restraint—enormously important from an evolutionary point of view—remains entirely unknown.*

James M. Tanner, MD

## The Predictive Value of Short-Term Growth Using Knemometry

Seventy-eight normal school children aged 3 to 16 years were measured with the knemometer at 1, 2, 3, 6, 9 and 12 months. Height was also measured, in the evening between 1800 and 2100 hours. The error of knemometry was 0.18 mm and of height measurement was 0.70 mm. Month-to-month variability in leg-length velocity averaged 2 mm with a range of 1 to 4 mm among these individuals whose monthly mean growth rate was 1.6 mm. The correlation between growth over 1 month and over 12 months in leg length was virtually zero.

Over 6 months to 12 months, it was .84. The correlation between height measured over 12 months and leg length over 1 month was .3; leg length over 3 months, .66, over 6 months, .85 and over 12 months, .89. The authors conclude that a knemometric rate calculated over less than 6 months is useless for assessing what the annual growth rate will be.

Dean HJ, Schentag CT, Winter JSD. *Acta Paediatr Scand* 1990;79:57-63.

**Editor's Comment:** This is a very welcome independent con-

firmation of the values recently published by Hermanussen and his associates, and confirmed by Wales and Milner in 1987. It is true that the height gain over 12 months was a little better predicted by the leg-length gain over 6 months than by the height gain over 6 months, but this is probably because the error of height measurements is unacceptably high, whereas the error of knemometry is absolutely in line with Hermanussen's values. (It has long been noted by anthropometrists that familiarity breeds contempt.)

James M. Tanner, MD

## Long-Term Treatment With Glucocorticoids/ACTH in Asthmatic Children

Forty children born between 1947 to 1974 with bronchial asthma severe enough to require long-term treatment with glucocorticoids or ACTH have been followed, 31 until adult height was reached. Twenty-three were given prednisolone for an average of 6.5 years beginning at an average age of 6 years, and 17 were given daily ACTH for 3 years, starting at an average of 5.5 years. The prednisolone-treated group had a height SDS of -1.0 at the beginning of treatment, -1.4 after 1 year, -1.8 after 2 years, and -2.4 after 3 years. In contrast the ACTH group, starting at -0.5, after 1 year were -0.1, after 2 years were +0.2, and after 3 years were +0.2. Thus, the height velocity for the ACTH-treated group was at all times above the mean, whereas for the prednisolone-treated group, it was at all times well below the mean. The diminished velocity on prednisolone was not significantly dose-related and was present in doses as small as 0.1 mg/kg

per day.

The adult height of the ACTH-treated group was well within normal limits, as was their age at peak height velocity, whereas the adult height of the prednisolone-treated group was more than 2 SD below the mean in boys and approximately 1.5 SD below the mean in girls. Age at peak height velocity was severely retarded in the boys, by approximately 2 SD, whereas it was not so in the girls, whose age at menarche was within normal limits.

Oberger E, Engstrom I, Karlberg J. *Acta Paediatr Scand* 1990;79:77-83.

**Editor's Comment:** This paper makes a very strong argument for treatment with ACTH rather than with prednisolone. The authors' conjecture is that the retardation in puberty in boys is due to long-term glucocorticoid effect on testosterone levels. Since other series report a normal

adult height in patients despite glucocorticoid treatment, it is perhaps important to terminate glucocorticoids well before the expected time of puberty to allow some degree of catch-up.

James M. Tanner, MD

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## Growth of African Pygmies in Early Childhood

Growth curves are given for the height of Efe pygmy children from 6 months to 5 years of age. The data are mixed longitudinal; all dates of birth were known. At 6 months of age, the mean height standard deviation score, relative to National Center for Health Statistics (NCHS) standards, was -2.7, declining to -4.2 at 5 years of age. The mean score for adults, sexes pooled, was -4.8 SD. Thus, most of the pygmy height deficit is accrued by 5 years of age.

Bailey RC. Letter to the editor. *N Engl J Med* 1990;323:1146.

**Editor's Comment:** This letter to the editor is of importance because Merimee, et al (*N Engl J Med* 1987;316:906-911) suggested that the short stature of adult pygmies is due primarily to a deficient growth spurt during puberty. In the article by Merimee, et al curves for cross-sectional height increment were given for pygmies that appear to show that male pygmies have no pubertal growth spurt, while female pygmies appeared to have a very reduced pubertal growth spurt. Merimee, et al reported that the testosterone values were normal at all ages, while IGF-I levels failed to rise to the same extent in pygmies at adolescence (250 U/mL vs 500 U/mL in American adolescents).

As stated in the abstract of the article by Bailey, most of the pygmy height deficit is determined to accrue by 5 years of age when he studied pygmy infants and children. The question now is why did Merimee, et al conclude that there is little adolescent growth spurt in pygmies. No other human group has such a lack of pubertal growth spurt (see Eveleth and Tanner<sup>1</sup>). The answer to the question probably is that very few adolescents were measured

by Merimee, et al and their conclusion was actually derived from the report of J.M.H. van de Koppel and B.S. Hewlett in a 1986 book called *African Pygmies*.<sup>2</sup> Between 1975 and 1980, the authors of this report measured the heights of 307 Akan pygmies whose ages had been estimated by means of an event calendar — a standard, though imperfect technique, listing major events concerning the tribe back into the past, utilizing the mother's input regarding when a child was born (eg, before or after each of those events). The report contains no tables of value; however, by using the graphs it is possible to estimate that about 50 persons of each sex were probably measured during the pubertal age range. It is quite likely, therefore, that had puberty stages been determined for each, the pattern of mean height increments between those in stages 2 and 3, 3 and 4, and 4 and 5 would have revealed, at least in the boys, whether or not a pubertal growth spurt occurred. Unfortunately, this was not done.

Instead, an exponential curve was fitted to all the data from birth to adulthood. Though the authors suggest the curves explain "more than 99% of the variance," I believe they have confused "within-age variation" with "between-age variation." In fact, there is a great excess of males above the curve at ages 10 to 16 years. The authors also provide plots of approximately year-to-year mean increments calculated cross-sectionally, and these curves permit a reasonable judgment. Female pygmies appear to have a maximum mean increment of about 9.5 cm/yr, which is above the cross-sectional population mean increment for American girls at puberty. Male pygmies

seem to have a maximum growth increment of about 6.5 cm/yr, which is slightly below the Western mean increment value of approximately 7.2 cm/yr. Both values are well within the usual sampling limits, given the small numbers.

The plots of annual mean increments in the *New England Journal of Medicine* article give a very inaccurate impression. The authors have taken the British longitudinal, tempo-conditional mean velocities with their big peaks, and plotted cross-sectional population values (grossly smoothed) upon them, evidently unaware of the differences (see Tanner<sup>3</sup>). The authors' contention regarding the lack of a pubertal growth spurt remains unproven, and, sad to say, this article is yet another example of biochemical expertise combined with auxologic innocence.

Bailey's article, in contrast, is a very clear and unexceptionable statement regarding the early growth of the Efe pygmies. He continues his longitudinal studies there, and the results through puberty will be awaited with interest.

James M. Tanner, MD

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## MEETING CALENDAR

**January 9-12, 1991** 38th Postgraduate Course, American Diabetes Association, Marriott Hotel and Marina, San Diego, CA. Contact: American Diabetes Association, 1660 Duke St., Alexandria, VA 22314 (800-232-3472)

**January 12-16, 1991** 2nd International Symposium on Insulin-like Growth Factors/Somatomedins. The Grand Hyatt, Union Square, San Francisco, CA. Contact: Sarah Burke, Extended Programs in Medical Education, Room C-124, University of California School of Medicine, San Francisco, CA 94143-0742. (Registration information 415-476-5808; program information 415-476-4251; fax 415-476-0318)

**January 27-February 1, 1991** Advances in Gene Technology: The Molecular Biology of Human Genetic Disease. Information: The Miami BioTechnology Winter Symposia, PO Box 016129, Miami, FL 33101-6129. (Tel: 800-642-4363; fax: 305-324-5665)

**February 6-9, 1991** Joint Meeting of the Western Section of the American Federation of Research and the Western Society for Pediatric Research. Various locations in Carmel, CA. Contact: Marilyn Jones, MD, Children's Hospital, 8001 St. San Diego, CA 92123 (619-576-5840)

**February 9-13, 1991** 18th Annual Seminar in Pediatric Nephrology: Current Concepts in Diagnosis and Management. Diplomat Resort and Country Club, Hollywood, FL. Contact: Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, PO Box 016960 Miami,

FL 33101 (305-549-6726)

**March 16-21, 1991** Spring Session, American Academy of Pediatrics, San Diego Convention Center, San Diego, CA. Contact: Department of Education, American Academy of Pediatrics, PO Box 927, Elk Grove Village, IL 60007 (800-433-9016)

**March 16-23, 1991** 3rd International ISGD Course on Update on Diabetes in Childhood. Maega Ciapela, Marmolada, Italy. Information: Dr. L. Pinelli, Servizio di Diabetologia Pediatrica, Policlinico 1-37134 Verona, Italy. (Tel: 39-45-933-667; fax: 39-45-8200-993.)

**March 17-20, 1991** 5th European Workshop on Pituitary Adenomas: New Trends in Basic and Clinical Research, Venice, Italy. Program Information: Dr. C. Faglia, Inst. of Endocrine Sciences, Univ. of Milan, Via F. Sforza 35, I-20122, Milan, Italy. Tel: 39-546-4063. General Information: M. Volpi/A. Cogo, Deltagest, Via E. Toti 9, I-35135 Padova, Italy. (Tel: 39-49-600-288)

**April 10-12, 1991** International Symposium on Growth Disorders: The State of the Art. Bari, Italy. Contact: Sero Symposia, Via Ravenna 8, 00161 Rome, Italy.

**April 26-27, 1991** KABI 11th International Symposium on Growth and Growth Disorders, Stockholm, Sweden. Information: Dr. R. Gunnarsson, Kabi Vitrum Peptide Hormones, S-11287 Stockholm, Sweden. (Tel: 46-8-138-000; fax 46-8-618-2019)

**April 29-May 30, 1991** Annual Meeting of the American Pediatric Society/Society for Pediatric Research/Ambulatory Pediatric

Association, Riverside Hilton, New Orleans, LA. Contact: Society for Pediatric Research, 2650 Yale Blvd SE, Suite 104, Albuquerque, NM 87106 (505-764-9099)

**May 1-3, 1991** Annual Meeting of the LWPES, New Orleans, LA. Information: Dr. G. August, Secretary, LWPES, Children's National Medical Center, 111 Michigan Ave. NW, Washington, DC 20010. (Tel: 202-745-2121; fax: 202-939-4492)

**May 24-26, 1991** International Symposium on Growth and Development: Basic and Clinical Perspectives, Auckland, New Zealand. Information: Prof. P. Gluckman, Dept of Pediatrics, Univ of Auckland, Private Bag, Auckland, New Zealand. (Tel: 64-9-795-780; fax: 64-9-770-956)

**May 12-15, 1991** International Symposium on Epidemiology and Etiology of IDDM in the Young. Chantilly-Gouvieux, France. Contact: Dr. Allen Drash, Children's Hospital, Pittsburgh, PA 15213

**June 19-22, 1990** 17th Annual Meeting of the ISGD, Williamsburg, VA. Information: Dr. W. Clarke, Dept of Pediatrics, Box 386, Univ of Virginia Health Sciences Center, Charlottesville, VA 22908. Tel: 804-924-5897; fax: 804-924-2769. Registration via ADA, 1600 Duke Street, Alexandria, VA 22314. (Tel: 703-836-1500; fax: 703-836-7493)

**June 19-22, 1991** 73rd Annual Meeting of the American Endocrine Society. The Sheraton, Washington DC. Contact: The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 (Tel: 301-571-1802; fax: 301-571-1869)

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# GROWTH

## Genetics & Hormones

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## Oxandrolone Therapy: 25 Years Experience

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### Background

Oxandrolone is a synthetic anabolic steroid having the chemical name 17 $\beta$ -hydroxy-17 $\alpha$ -methyl-2-oxa-5 $\alpha$ -androstan-3-one. Structurally, oxandrolone is a derivative of testosterone but is unique among all other testosterone analogues in that it contains an oxygen atom instead of a methylene group at the 2 position of the phenanthrene nucleus. The structural formula for oxandrolone is shown in Figure 1 (see page 3).

Pharmacologically, oxandrolone possesses both anabolic and androgenic activity at a ratio of approximately 6:1.<sup>1</sup> When taken orally, oxandrolone is rapidly absorbed and excreted primarily in the urine (approximately 25% as the parent compound). Peak plasma levels occur at approximately 45 to 90 minutes after ingestion. The biologic half-life of oxandrolone is approximately 9 hours.<sup>2</sup>

Human experience with oxandrolone is extensive. Although originally marketed for its anabolic activity to

promote weight gain in various medical conditions, review of the published literature indicates that the primary use of oxandrolone over the past quarter century has been for the enhancement of growth velocity in children with various growth disorders (eg, constitutional delay of growth and puberty [CDGP] and Turner syndrome). Toward this end, oxandrolone has been administered to several hundred patients (age 3 to 18 years) in documented clinical trials at a typical dose of 0.1 to 0.125 mg/kg/d for up to several years. Currently, most pediatric endocrinologists recommend a maximum daily dose of 0.1 mg/kg/d or less in the treatment of Turner syndrome and CDGP.

Results from published studies indicate that oxandrolone can be used effectively to increase growth velocity in girls with Turner syndrome and in boys with CDGP and that it can be used safely if the bone age is  $\geq 9$  years.

### Overview of Oxandrolone Use in Turner Syndrome

Clinical management of Turner syndrome in childhood focuses primarily on growth therapy. Of particular importance with regard to growth is the effect a therapeutic agent has on growth velocity during treatment and on final adult height. The ideal treatment should also have a positive effect on the psychosocial status of girls with

### Letter From the Editor

The protein anabolic steroid oxandrolone will again be available in mid-1991. In May of 1989, G.D. Searle & Co made a business decision to halt distribution of oxandrolone (Anavar®). Because this action adversely affected how numerous pediatric endocrinologists treat their patients, the Lawson Wilkins Pediatric Endocrine Society (LWPES) and the American Academy of Pediatrics (AAP) have encouraged Gynex, Inc and the US Food and Drug Administration (FDA) to again make oxandrolone available for the treatment of patients with Turner syndrome and constitutional delay of growth and puberty (CDGP).

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Turner syndrome (presumably by increasing growth), since many of these girls reportedly suffer from self-consciousness, embarrassment, and poor self-esteem.<sup>3-5</sup> Furthermore, improved psychosocial status during adolescence may yield significant long-term

benefits as girls with Turner syndrome reach adulthood. A positive effect of oxandrolone on growth and psychosocial status in girls with Turner syndrome was reported by Rosenbloom and Frias in 1973.<sup>6</sup>

Oxandrolone alone or in combination with growth hor-

mone (GH) has been shown to markedly enhance growth in girls with Turner syndrome. Rosenfeld et al<sup>7</sup> reported the results of a 3-year randomized prospective trial of methionyl human GH alone versus oxandrolone alone versus the combination in Turner syn-

#### Letter From the Editor

Continued from page 1

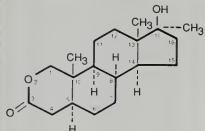
Based on an agreement reached between Gynex, Inc and the FDA, oxandrolone will be made available by 2 separate routes. The first will be under an open protocol that will be available to pediatric and adult endocrinologists with experience in the clinical management of pediatric growth disorders. The patients must meet the specific inclusion criteria for Turner syndrome, ie, having a karyotype that confirms the diagnosis of Turner syndrome, a bone age  $\geq 9$  years, a chronologic age of 10 to 16 years, and have parental informed consent and patient assent. The exclusion criteria for these Turner syndrome patients will include concurrent administration of estrogen, a history of a condition known to adversely affect growth, and/or a medical condition precluding the use of oxandrolone. In addition, the patient cannot be a ward of the state. The inclusion criteria for boys with CDGP will be a chronologic age of 11 to 16 years, a height  $\leq 2$  SD for age, a bone age  $\geq 9$  years, a delayed bone age  $>2$  years from the chronologic age, and pubertal development of G1 or G2 and PH1 or PH2. The exclusion criteria for patients diagnosed as CDGP are GH deficiency, history of a condition known to adversely affect growth, a medical condition that precludes the use of oxandrolone, concurrent therapy with another androgen, and state wardship.

The distribution of oxandrolone will be tightly controlled by: (a) verifying each physician's credentials through a physician registration program, which includes a physician review by a national institutional review board (IRB); (b) confirming that patients are eligible for oxandrolone therapy prior to shipment of drugs; (c) closely monitoring drug use; (d) halting distribution if the patient is noncompliant; and (e) shipping oxandrolone directly to the patient (or physician, if required by state regulations) from a single distribution center. Patients for whom oxandrolone is prescribed under the open-label studies must pay for their prescription. The cost for oxandrolone will be higher than in the past; however, it is important to note that the cost established will be based on a cost recovery program regulated by the FDA. Protocols and informed consent documents for the open-label protocols have been reviewed and approved by a national IRB established by Gynex, Inc in cooperation with the LWPES and the AAP. Members of the IRB are Jose Cara, MD, Wyler Children's Hospital, University of Chicago; Nancy Hopwood, MD, Children's Hospital, University of Michigan; Edward Reiter, MD, Baystate Medical Center, Springfield, Massachusetts; S. Douglas Frasier, MD, Olive View Medical Center, Los Angeles County; Lynn Georgia-Tesch, JD, Turner Syndrome Society; and William D. Stout, lay member. Physicians not constrained by local IRB policies may participate in the open-label use of oxandrolone through the national IRB.

As a part of the agreement between Gynex, Inc and the FDA, the second available route for obtaining oxandrolone involves patient participation in Phase III placebo-controlled clinical trials either in girls with Turner syndrome or in boys with CDGP. Subjects enrolled in either of these control studies will receive oxandrolone free of charge. Those patients who are randomized to the control group will (upon the recommendation of the physician) be eligible to receive up to a 12-month supply of oxandrolone for free following their participation in the clinical trial. The placebo-controlled study in Turner syndrome will be supervised by JoAnne Brasel, MD, Harbor Hospital/UCLA Medical Center, Torrance, California. The placebo-controlled study in boys with CDGP will be supervised by Darrel Wilson, MD, Stanford University School of Medicine, Stanford, California. Forty subjects ( $n=20$ /group) are needed for each 12-month study.

Your support of the studies discussed above will have a *direct impact* on the long-term availability of oxandrolone. **Information about these studies, physician registration, and the distribution programs are available from Gynex, Inc (708-913-7708).**

Robert M. Blizzard, MD — Editor



**Figure 1: Structural Formula Of Oxandrolone**

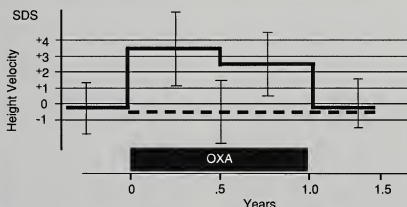
drome. After 12 to 20 months of therapy, the growth velocity of girls treated with 0.125 mg/kg/d oxandrolone alone was significantly greater than controls ( $7.6 \pm 1.5$  cm/yr versus  $3.8 \pm 1.1$  cm/yr) and statistically equivalent to the growth velocity observed in girls treated with GH alone ( $6.6 \pm 1.2$  cm/yr). Of perhaps greater significance, the combination of oxandrolone and GH yielded a growth rate of  $9.8 \pm 1.4$  cm/yr, suggesting a synergistic action of the drugs on growth velocity. These findings are consistent with earlier work<sup>8</sup> and with more recent findings.<sup>9</sup> Although the effect of combination treatment on final adult height is yet to be clearly defined, these results suggest that combination therapy may yield better results than either treatment alone for Turner syndrome.

Oxandrolone alone has been shown to increase growth velocity and, in some cases, final adult height of girls with Turner syndrome. Rosenbloom and Frias<sup>6</sup> treated girls 9 to 18 years of age with approximately 0.1 mg/kg/d oxandrolone for 4 to 36 months. Oxandrolone significantly increased growth velocity from a mean pretreatment rate of 1.8 cm/yr to an average of 5.3 cm/yr over the 4- to 36-month period of treatment. For the 7 girls treated more than 1 year (average, 22 months), the mean bone age advance

was 9 months. Moore et al<sup>10</sup> reported similar results and reported an increase in mean final adult height in 9 of 20 girls treated with oxandrolone. Stahnke et al<sup>11</sup> studied the effects of oxandrolone in girls (mean age of 14 years) treated with 0.1 mg/kg/d for 1.5 to 6 years. Significant increases in growth velocity were observed that tended to decline over time (eg, to 2.1 cm/yr after 5 years). Notably, oxandrolone therapy increased final adult height in many patients, a finding also reported by Heidemann et al.<sup>12</sup> Finally, Joss and Zuppinger<sup>13</sup> conducted the only pair-matched controlled study of oxandrolone in which

the patients were studied to final height. Patients received oxandrolone (0.1 mg/kg/d) for either 1- or 2-year treatment periods (with a 6-month interval off therapy). Oxandrolone led to a marked increase in height velocity from a pretreatment value of 2.9 cm/yr to 5.0 cm/yr during the first year of therapy. Figure 2 provides a graphic example of the positive effect of oxandrolone on growth velocity as expressed in standard deviations (SD) in patients with Turner syndrome.<sup>13</sup>

Fifteen of the 20 patients treated with oxandrolone reached final adult height (n=7 for 1 year of therapy and



**Figure 2: Height velocity (SDS) in patients before, on, and after oxandrolone in a dose of 0.1 mg/kg/day (full line) (n=26) compared to the height velocity of untreated matched controls (broken line) (n=26). Mean  $\pm$  1 SD.**

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n=8 for 2 years). In this study, final adult height was significantly increased in oxandrolone-treated patients compared with untreated matched controls.

A noteworthy aspect of the studies discussed above is the absence of significant side effects in response to oxandrolone therapy. At a dose of 0.1 mg/kg/d, most girls treated with oxandrolone did not experience acne, deepening of the voice, growth of facial hair, or clitoromegaly. Occasional notations have been made in the literature regarding small, transient elevations in liver transaminases in some girls with Turner syndrome treated with oxandrolone. Impaired glucose tolerance (ie, insulin resistance) in some girls treated with oxandrolone (0.125 mg/kg/d) alone or in combination with GH for 12 months has been reported.<sup>14</sup> The clinical importance of this observation could not be determined in light of the fact that both fasting glucose and glycosylated hemoglobin concentrations remained normal. In addition, glucosuria was not observed in oxandrolone-treated girls. Thus, short-term complications from impaired glucose tolerance seem unlikely; and although potential long-term effects of oxandrolone-induced impaired glucose tolerance in girls with Turner syndrome are unknown, it is noteworthy that no serious adverse effects related to glucose metabolism have been reported in girls treated at higher oxandrolone doses for substantially longer periods of time.

Oxandrolone, like all androgens and estrogens, can cause premature skeletal maturation if inappropriately large dosages are administered to young patients. At a daily dosage of 0.1 mg/kg or less, oxandrolone has generally not been associated

with inappropriate aging of bone provided treatment is withheld from girls with a bone age of less than 8 to 9 years.<sup>13,15,16</sup>

Therefore, the collective experience with oxandrolone in the treatment of Turner syndrome indicates that oxandrolone can be safely and effectively used to increase growth velocity. The effect of oxandrolone treatment on final adult height in Turner syndrome is somewhat controversial, but at low doses (ie, 0.1 mg/kg/d or less) and in girls with bone ages of  $\geq 8$  to 9 years at initiation of therapy, there are data to suggest that final adult height can be increased or, at a minimum, not adversely affected. In the latter case, oxandrolone therapy is still likely to be beneficial in that increased growth velocity often is psychologically beneficial for girls with Turner syndrome since oxandrolone therapy will stimulate linear growth at an age when normal peers are undergoing a pubertal growth spurt.

Certainly, optimal treatment of Turner syndrome would require both oxandrolone and GH earlier in childhood and probably low-dose estrogen in the late childhood years, with a suitable increase to induce secondary sexual characteristics after the age of 11 years. It is certain that all 3 therapeutic modalities are synergistic for growth in Turner syndrome but the age of commencement, the dosing regimen, and duration of treatment have not yet been determined. Certainly, oxandrolone has a significant role to play in the modern management of short stature due to Turner syndrome.

#### **Overview of Oxandrolone Use in Constitutional Delay of Growth and Puberty**

CDGP is diagnosed in otherwise healthy adolescents when height is significantly reduced

for chronologic age (eg, 2 or more standard deviations below the 50th percentile) but generally appropriate for pubertal development and bone age — both of which are usually delayed.<sup>17</sup> The condition is often associated with a family history of delayed puberty and is reported more commonly in boys than in girls. Because the pubertal growth spurt in boys occurs when a testicular volume of 10 to 12 cc (G4) is reached, the time before a spontaneous increase in growth velocity occurs in boys with CDGP may be considerable. In addition, the growth acceleration is often blunted, which may result in a slightly lower than predicted adult height.<sup>18</sup> Depending on the emotional stability of the individual and his social setting, CDGP can give rise to extreme distress and may result in severe psychosocial problems.<sup>17-22</sup> Thus, the goal of oxandrolone therapy is to increase growth velocity and thereby improve psychosocial status in boys with CDGP.

Clinical management of CDGP depends largely upon individual patient needs. Although counseling and reassurance that growth and pubertal development will eventually occur may prove sufficient for many boys with CDGP, it may not be sufficient for others, who would thus derive substantial benefit from increased growth velocity in response to drug therapy. The latter is particularly relevant for patients experiencing psychosocial problems related to their delayed development since these may negatively impact future adult behavior. Oxandrolone has been used successfully for many years in the clinical management of CDGP.<sup>23-30</sup> Despite the availability of other therapeutic agents for CDGP (eg, testosterone, fluoxymesterone, GH) Stanhope et al<sup>25</sup> argue that

oxandrolone may represent the treatment of choice based on its (a) long history of use (albeit not yet approved by the FDA for this indication); (b) effectiveness in increasing growth velocity without adversely advancing bone age; (c) low incidence of side effects at doses currently reported in the published literature for clinical management of CDGP; (d) route of administration (oral versus intramuscular injection of testosterone, testosterone analogues, and GH); and (e) cost (compared with, for example, GH).

The use of oxandrolone to treat CDGP has been well documented in the medical literature and spans a 25-year period. In these studies, oxandrolone was administered at typical doses of 0.1 to 0.125 mg/kg/d to patients ( $n > 350$ ) generally between the ages of 8 and 17 years. Although the average duration of treatment was 3 to 12 months, some children were treated with oxandrolone for substantially longer periods (eg, up to 60 months) and at daily doses as high as 2 mg/kg. With rare exceptions, oxandrolone safely and effectively increased growth velocity<sup>10,17,23-38</sup> and when evaluated, improved psychosocial status. The lone exception to these findings was reported by Marti-Henneberg et al in 1975.<sup>39</sup> In their study, oxandrolone was without effect on growth velocity in 9 boys (11.2 to 13.3 years of age) treated with 0.1 mg/kg/d for up to 60 months. A recent (1990) study published by Buyukgebiz et al<sup>27</sup> comparing oxandrolone and rGH therapy showed that while both treatments resulted in significant increases in height velocity, the increment in height velocity induced by oxandrolone at 2.5 mg/d for 3 months, but observed for 1 year, was greater than GH alone at 7.7 mg (20 units)/m<sup>2</sup>/wk for 1

year in increasing growth velocity in boys with CDGP.

A brief review of 3 recent studies with oxandrolone in boys with CDGP provides further insight into the clinical usefulness of oxandrolone. Joss et al<sup>26</sup> treated 27 boys (10.6 to 11.5 years old) with 0.12 to 0.22 mg/kg/d oxandrolone for 12 months and followed them until they reached adult height. While receiving oxandrolone, the mean height velocity of the boys increased 107% to 115% (ie, approximately 4.1 to 8.7 cm/yr). The mean ratios of change in bone age to change in chronologic age were 2.0 and 2.3, respectively, in boys treated with 0.12 and 0.22 mg/kg/d oxandrolone compared with a ratio of 0.9 for untreated controls. Joss et al<sup>26</sup> concluded that although oxandrolone did not improve final adult height, it has therapeutic value in the treatment of CDGP by increasing growth velocity and stimulating an earlier onset of puberty — the occurrence of which may benefit boys suffering from psychologic problems due to delay of growth and development. Also of significant importance was Joss's finding that use of oxandrolone did not compromise final adult stature.

Stanhope et al<sup>25</sup> conducted a double-blind, placebo-controlled trial of low-dose oxandrolone in CDGP. Nineteen boys with CDGP (mean age, 14.4 years) were randomized to a control or treatment (0.072 mg/kg/d oxandrolone) group. Treatment duration was 3 months. Oxandrolone-treated boys exhibited a significant increase in growth velocity (4.5 to 9.6 cm/yr). Despite cessation of treatment, growth velocity in oxandrolone-treated boys was sustained at a mean of 8.6 cm/yr over an additional 3-month period.

Tse et al<sup>30</sup> treated 40 boys (median age of 14.2 years) with oxandrolone at the low-dose of 1.25 mg or 2.5 mg per day. Twenty-six subjects received treatment for 3 months, 12 for 6 months, and 1 each for 9 and 12 months. There was a significant increase in growth velocity, and all final heights were within the 95% confidence limits of predicted heights by the Tanner-Whitehouse II (TW2) method. The mean final height was 167.3 ± 6.6 cm versus 165.8 ± 5.9 cm predicted height ( $P = 0.03$ ), indicating there was no compromise in final height.

The method of treatment, as given in various reports by British investigators, differs from that used in the United States. In the latter, oxandrolone has been used on a continuing basis in boys with CDGP until testosterone and/or sexual maturity (testicular size of  $> 10$  to 12 cc volume) occurs. The British groups frequently have used only 3 months of treatment and noted that the increased growth velocity induced by oxandrolone persists with discontinuation of the agent if the testes are  $> 4$  cc in volume.<sup>23-25,30</sup> They report that in boys who are prepubertal (testes  $< 4$  cc) there is a growth spurt while the patient receives oxandrolone, but there is no sustained growth with discontinuation of therapy.

The mechanism of increased growth remains controversial. The studies of Link et al<sup>31</sup> indicated no significant increase in growth hormone production in 10 boys with CDGP with 3 months of oxandrolone therapy (approximately 0.1 mg/kg/d). Also, there was no significant increase in insulin-like growth factor I (IGF-I) levels. IGF-I levels were found to increase modestly in several reports. For example, in 1 report,<sup>26</sup> the mean serum IGF-I concentration increased from 1.01 U/mL to 1.23 U/mL ( $P < 0.05$ ). Clayton et al<sup>24</sup> reported

that GH concentrations during sleep did not change in prepubertal boys receiving 2.5 mg/kg/d of oxandrolone although in pubertal boys an increase was reported. Loche et al<sup>29</sup> and Stanhope et al<sup>28</sup> observed that oxandrolone increased GH secretion. Ulloa-Aquirre et al<sup>30</sup> reported that the mean GH production increased in 5 boys treated with oxandrolone 1.25 mg tiw. However, the increased production occurred primarily in 1 of the 5, increasing 250%, while the increase in the other 4

was marginal and, therefore, difficult to interpret. The differences in ages, total doses, time intervals between doses, and length of time between last dose and measurement of integrated GH concentration may account for the controversial reports. Further studies are indicated, such as dose response curves, to evaluate the extent to which oxandrolone stimulates growth directly and the extent to which it results in increased GH secretion.

Thus, based on the pub-

lished literature, collective experience with oxandrolone indicates that it can be safely and effectively used to increase growth velocity and, although less well documented, improve psychosocial status in boys with CDGP. It should be noted that treatment of CDGP with oxandrolone advances the timing of the growth spurt with little or no interference in the rate of sexual maturation.<sup>25,30</sup>

*References available upon request.*

# Obesity in Childhood and Adolescence

## Part 1: Physiology, Genetics, and Growth

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### Introduction

Childhood obesity is a multifactorial disease resulting from an imbalance of energy intake and expenditure. Environmental and hereditary factors play a role in the development of obesity. Environmental factors particularly contribute to increased food (energy) intake and to expenditure of energy through activity. In this review, we will consider the physiology of obesity, the evidence that genetic factors operate to produce obesity, how these factors may be expressed, and how growth may be affected in obesity.

### Physiology

An individual is in energy balance when energy intake equals energy expenditure. When energy intake exceeds expenditure, the storage of body fat increases. Conversely, when energy intake is lower

than expenditure, the depots of body fat decrease. Relatively small excesses in energy intake that are maintained for long periods produce significant increases in body fat. For example, an excess energy intake of 100 cal/d for a year results in 10 pounds of accumulated fat.

Food (or energy) intakes have been reported to be comparable among obese and nonobese adults,<sup>1,2</sup> thus suggesting that obese individuals have a reduced energy expenditure. However, others have reported that in obese individuals energy intake is significantly lower than energy expenditure, which casts doubt on the reliability of dietary records to provide a valid measure of energy expenditure.<sup>3</sup>

Daily total energy expenditure (TEE) is calculated based on 4 components: (1) the basal metabolic rate (BMR); (2) the thermic effect of food (TEF); (3) the energy spent in physical activity ( $E_A$ ); and (4) the energy required for growth ( $E_G$ ). Under normal circumstances in adolescence, the BMR accounts for 55% to 60% of TEE, TEF for approximately 10% of TEE, and

$E_A$  for approximately 25% of TEE;  $E_G$  is extremely variable according to growth velocity and/or replacement of tissue. Heredity may produce obesity by decreasing TEE through decreased BMR and/or decreased TEF and, possibly, through the energy necessary for activity ( $E_A$ ) and growth ( $E_G$ ).

TEE can be determined by the doubly labeled water method ( $^2\text{H}_2^{18}\text{O}$ ) described by Schoeller.<sup>4</sup> Because  $^{18}\text{O}$  is lost as both water and carbon dioxide ( $\text{CO}_2$ ) and  $^2\text{H}$  is lost as water, the differential loss of the 2 isotopes from body water over time is a measure of the rate of  $\text{CO}_2$  production. With the knowledge of the food quotient of the diet, TEE can be measured within 5% of that determined by respiratory gas exchange. This method is ideal for children and adolescents because no equipment or confinement is necessary.

BMR can be measured continuously using indirect calorimetry with a ventilated hood.<sup>5</sup> Calculations are made from measures of oxygen consumption and  $\text{CO}_2$  production according to the modified Weir's formula.<sup>6</sup> In

adolescents, BMR accounts for 55% to 60% of TEE. The BMR is affected by fat-free mass (FFM), fat mass, age, stage of sexual development, and familial characteristics. However, the *principal determinant* of the BMR is FFM. Increase in Tanner staging and in age improve the correlation between the BMR and the FFM ( $r = 0.93$ ) in adolescents.<sup>7</sup>

A different relationship between FFM and BMR has been shown for males and females and obese and nonobese adolescents, which suggests that *both* sex and fat mass contribute to the variability in the BMR.<sup>7</sup> Bogardus et al reported that familial characteristics also contributed significantly to the BMR in a group of Southwestern Indians.<sup>8</sup> Presumably, the same could occur in other family groups.

Because the BMR contributes significantly to the total metabolic rate, decreases in BMR will reduce total energy needs. In a study of obese and nonobese adolescents,<sup>7</sup> the BMR adjusted for differences in body composition was increased in the obese group (Table 1). These findings suggest that the normal obese adolescent does not have a reduction in

metabolic rate. Bogardus et al<sup>8</sup> found no significant differences in fat mass in individuals from families with high and low metabolic rates.

Although a reduction in BMR does not seem to be a factor in the *maintenance* of adolescent obesity, it theoretically could contribute to the *development* of obesity.

Prospective studies by Ravussin et al<sup>9</sup> in adults suggest a significant relationship between TEE and weight gain. Specifically, Ravussin et al demonstrated greater weight gains in those Pima Indians who had low adjusted BMRs and TEEs. Following weight gain the metabolic rates increased. These data suggest that individuals with a low metabolic rate may gain weight as a compensatory mechanism to normalize the BMR and increase energy expenditure.

The TEF is reflected in the rise in metabolic rate after eating. This increase in energy expenditure is the energy necessary to process the food. The TEF has a genetic component<sup>5,10</sup> and contributes approximately 10% to the TEE. Small decreases in the TEF over a prolonged period of time could lead to a significant energy imbalance and an increase in body fat stores.

Therefore, significant attention has recently focused on the TEF. However, these studies, which were performed primarily in adults, are inconclusive. Some studies reported a reduced TEF in the obese while others did not. However, there were significant differences in study designs, nutrients ingested, caloric content, criteria for obesity, heterogeneity of the subjects, and duration of the studies. For example, some investigators fed similar amounts of calories to obese and nonobese subjects, while others based the caloric intake on body weight, FFM, or a percent of BMR. Some of the differences in outcomes can be attributed to the altered body composition in obese subjects. Segal et al<sup>11</sup> controlled many of these variables by matching obese and nonobese subjects for FFM. Their results indicate that there is a blunted TEF in the obese. However, we were unable to demonstrate significant differences in the TEF in obese and nonobese adolescents, although FFM was similar in the 2 groups.<sup>5</sup>

$E_A$  is the most variable component of energy expenditure.  $E_A$  can be calculated if TEE, BMR, and TEF are known by using the formula  $E_A$

**Table 1**  
Fat-Free Mass and Energy Expenditure in Obese and Nonobese Adolescents<sup>\*</sup>

|                      | NONOBESE |       | OBESE   |       | SIGNIFICANCE <sup>*</sup> |       |
|----------------------|----------|-------|---------|-------|---------------------------|-------|
|                      | Females  | Males | Females | Males | Females                   | Males |
| FFM (kg)             | 40.9     | 47.1  | 52.6    | 55.9  | -                         | -     |
| BMR (kcal/d)         | 1,441    | 1,742 | 1,918   | 2,253 | yes                       | yes   |
| TEE (kcal/d)         | 2,385    | 3,109 | 3,282   | 3,612 | yes                       | yes   |
| TEE-BMR <sup>+</sup> | 944      | 1,367 | 1,364   | 1,359 | yes                       | no    |
| TEE/BMR <sup>-</sup> | 1.69     | 1.79  | 1.68    | 1.68  | no                        | no    |

+ Nonbasal energy expenditure (direct calculation)

- Nonbasal energy expenditure (relative or indirect calculation)

\* Obese vs nonobese

† Table modified from reference number 7

FFM, fat-free mass; BMR, basal metabolic rate; TEE, total energy expenditure.



= TEE - ( $E_{\text{BMR}}$  +  $E_{\text{TEF}}$ ). The energy costs of growth ( $E_{\text{G}}$ ) are very small and are considered negligible in this calculation. Another calculation that reflects  $E_{\text{A}}$  is the ratio TEE:BMR, which reflects the amount of energy spent above the BMR. The ratio of TEE:BMR did not differ significantly between obese and nonobese adolescents in our study,<sup>7</sup> although TEE was greater in the obese group (See Table 1, page 7). These results indicated that the proportion of  $E_{\text{A}}$  and TEF was not reduced in the obese groups. However, a significant reciprocal relationship existed between nonbasal energy expenditure and body fat,<sup>7</sup> suggesting that the amount of energy spent above basal level decreases with increased body fat and that obese and nonobese individuals are not equally active. Because an increase in body size requires an increased amount of energy be spent in performing the same physical activity, the overall or total physical activity level of obese individuals may be lower than that of comparable nonobese individuals. This finding supports the previously reported work by Bullen et al<sup>12</sup> who found obese girls to be less active than nonobese girls. Together, these data suggest that the obese adolescent is less active overall, although the energy spent in performing similar activities may be relatively equivalent. These observations are supported by studies of infants which demonstrate excess weight gain despite unaltered metabolic rates, when a lower TEE exists, ie, the infants who gained the most weight had decreased levels of physical activity.<sup>13</sup>

There is a theory that some individuals are able to overeat but burn the excess calories as heat, while others are more energy efficient and store the excess calories as fat. This

concept has been termed *facultative thermogenesis* or *luxus consumption*. In obese adolescents in whom BMR, TEF, and TEE were measured during a maintenance period and after 2 weeks of overfeeding, the thermogenic response to overeating was not reduced.<sup>5</sup> Additionally, the majority of overfeeding studies in which energy expenditure was measured do not support a role for facultative thermogenesis in the maintenance of body weight.<sup>14-17</sup>

### Genetics

Obesity occurs with a greater prevalence among children with 2 obese parents than among those with 1 obese parent or no obese parents.<sup>18</sup> Although studies of the resemblance in fatness between pets and their owners suggest a strong environmental component,<sup>20</sup> genetic factors play an important role. This has been demonstrated in studies of subcutaneous fatness, as determined from measures of skin-fold thickness of twins. Bouchard et al<sup>21</sup> examined subcutaneous skin-folds in adopted and biologic siblings, cousins, and monozygotic (MZ) and dizygotic (DZ) twins. The intraclass pair correlations were highest for MZ twins ( $r = 0.76$  to  $0.87$ ), followed by DZ twins ( $0.30$  to  $0.49$ ), biologic siblings ( $0.18$  to  $0.43$ ), and cousins ( $0.21$  to  $0.29$ ). The intraclass correlations for pairs of adopted siblings or unrelated siblings were essentially zero.

Because a similar environment existed for these twins, it is difficult to determine the genetic contribution in fat accumulation. More recently, definitive studies to determine the heritability of fatness and obesity have focused on MZ and DZ twins living in similar or dissimilar environments or on adoptees separated from biologic parents. For these studies, body mass index

(BMI) (weight in kilograms/body surface area in square meters), which is strongly correlated with body fatness in adults, was used as the parameter to determine the heritability of fatness. In both Stunkard et al's twin study<sup>22</sup> and adoption study,<sup>23</sup> heredity appeared to be a major determinant of BMI. In the adoption study, Stunkard et al found a strong relationship between the BMIs of Danish adoptees with the BMIs of their biologic parents, but not with the BMIs of their adoptive parents.

These findings were interpreted to suggest that childhood family environment alone has little or no effect on the development of obesity. Careful inspection of the data, however, suggested that the significance of the BMI relationship between adoptees and their biologic parents resulted from the resemblance of the BMIs of lean adoptees and their biologic parents.<sup>24</sup> No significant difference in the prevalence of obesity existed between obese and overweight adoptees and either their adoptive or biologic parents.

In a more recent (1990) study, Stunkard et al<sup>25</sup> compared the BMIs of Swedish adult MZ and DZ twins reared together or apart. The mean age of the population was 58 years but few members of the study were obese. Intrapair BMI correlations were  $0.66$  to  $0.77$  in MZ twins reared apart and were comparable to the correlation observed in twins reared together ( $0.66$  to  $0.74$ ). Although the authors concluded that the childhood environment has little or no influence on BMI, these findings offer limited insight into the heritability of obesity. As stated previously, BMI is an indirect measure of body fatness. Therefore, similarities in members of a nonobese population may reflect that the

size of the body frame rather than obesity is inherited. Moreover, this study was limited to Scandinavia, where similar lifestyles may have minimized the environmental contribution to fatness in twins reared apart.

Recently, Bouchard and coworkers overfed 6 pairs of MZ twins for 100 days to elucidate the role of the genetic component on the storage of energy.<sup>18</sup> Although weight gain and body fat distribution were more similar within twin pairs than between twins, the intrapair correlation coefficient was approximately 0.5. This indicated that a significant portion of the variance in weight gain and fat distribution was unexplained by genetics. Since TEE was not measured, it is unclear whether the energy cost of fat accretion differed more between than within twin pairs. Some twin pairs apparently were more energy efficient and unmeasured differences in  $E_A$  may have contributed to the variability.

We conclude that the relative contributions of genetic and environmental factors to the energy imbalance that produces childhood obesity are as yet unclear. Obesity is clearly related to genetic factors, but published studies have been confounded by environmental factors and failure to distinguish frame size from fatness. Lack of differences in energy expenditure between obese and nonobese adolescents does not exclude the possibility that before becoming obese, the obese child had a reduced energy requirement. The next major challenge for research in obesity is to demonstrate to what extent reductions in BMR, TEF, activity, and/or daily energy expenditure are genetically mediated and, therefore, increase the susceptibility to obesity.

### Growth

Clinically obese children tend to be taller and to demonstrate

greater maturational advancement than their nonobese counterparts. Fatter children are both larger in body size and advanced in skeletal maturation, as reported in a review of the literature by Garn et al in 1973.<sup>26</sup> Lean (<15th percentile for triceps skin-fold measurements) and obese (>15th percentile) children were separated out of a group, and their heights were analyzed. The obese children were significantly taller (by as much as 6 cm or more) than the lean children. The lean boys and girls averaged -0.21 Z scores or 0.2 SD below stature expectancy while the obese children averaged 0.48 Z scores or 0.48 SD above height expectancy, as calculated on the basis of the total 4,888 children studied. By the ages of 11 and 12 years, the lean children were nearly 0.4 SD below the median and the obese children were nearly 0.6 SD above the median, with a difference of nearly 1 SD between the groups. The lean boys and girls were below the median at all ages considered, and the obese children were above the median at all ages. Appropriately, the authors did not conclude whether obesity was prone to produce accelerated growth or whether children with accelerated growth were more prone to be obese.

Forbes<sup>27</sup> reported that obese children who became obese during infancy tended on average to have a greater relative height than those who became obese in childhood. Subsequently, using data collected in a longitudinal growth study, Forbes reported that children who developed obesity during childhood reveal a distinct tendency for height to accelerate coincident with or after the onset of excessive weight gain.

The magnitude of the relative height increment is related to

the degree of overweight. "Over-nutrition accelerates growth just as undernutrition retards it."<sup>27</sup>

Recently, Vignolo et al reported a study on growth and development in obesity in 303 subjects.<sup>29</sup> Obesity was defined as a weight >20% than that expected for height and sex. Adiposity strongly correlated with BMI and skin-fold measurements. Twenty-five percent of boys and 29% of girls were above the 90th percentile for height when first seen. As they approached adolescence, they moved closer to, and then below, average stature.

Thus, although prepubertal children who are obese are taller than their peers, the data are conflicting as to whether these children remain taller by adolescence.

Growth velocities (GVs) decrease during weight reduction. We demonstrated that even mildly restrictive diets may be associated with a reduction in linear growth velocity.<sup>28</sup> In 19 children studied, the mean SD score for GV was  $2.3 \pm 2.4$  prior to weight reduction, which is in accord with the relationship between obesity and increased height. For the 11 patients with GVs >2 Z scores above the mean, the mean Z score decreased significantly to  $0.62 \pm 2.37$  on a restrictive diet. The data did little to identify the cause of the reduction in GV, and further research in this field is very much indicated. Regardless, the data emphasize the need for careful monitoring of GV's of obese children during weight reduction.

The hormonal and nutritional biochemistry that produces a correlation of increased growth and maturation in obesity remains to be unraveled, but offers a fertile field for investigation.

*References available upon request.*

## Final Height in Turner's Syndrome and Effect of Oxandrolone

Two separate papers published simultaneously by a group from Denmark provide new data for growth studies and therapeutic trials in Turner syndrome. The first paper compares the results of different methods for predicting final height in 20 Turner girls aged 9.5 to 18 years, also incorporating data from 78 adult Turner women in the same geographic area. This allowed the researchers to calculate an index of potential height (IPH) appropriate to Turner syndrome that yielded more accurate adult height predictions than those, usually overestimated, obtained using either Bayley-Pinneau or Tanner-Whitehouse II methods. Combination of the IPH with 1 of these 2 bone age-based methods yielded the greatest accuracy.

The second paper reports the effects of oxandrolone (0.125 mg/kg/d for 2 years) in 32 Turner girls aged 11.5 to 16.7 years at the onset of treatment. The results were calculated by comparison with the Danish Turner standards mentioned above, and showed a significant increase in growth velocity in the patients aged <13 years. Twenty-two patients have reached their final height and a significant ( $P<0.001$ ) improvement of 3 to 4 cm over the height predicted by the IPH-bone age method was obtained in those with an initial bone age below 13 years, but not in the others. At this dosage level, oxandrolone produced mild androgenic side effects in 5 patients that were reversible in all cases (except in 1 girl with slight deepening of the voice).

Naeraa RW, Eiken M, Legarth EG, et al. Prediction of final height in Turner's syndrome: a comparative study. *Acta Paediatr Scand* 1990;79:776-783.

Naeraa RW, Nielsen J,

Pedersen IL, et al. Effect of oxandrolone on growth and final height in Turner's syndrome. *Acta Paediatr Scand* 1990;79:784-789.

**Editor's comment:** *Once again, these papers show how difficult it is to make height predictions in patients with Turner syndrome. The IPH calculation proposed has been, in the authors' hands, better than the usual Bayley-Pinneau, Tanner-Whitehouse II or Roche-Wainer-Thissen methods. However, it is complicated and depends on data from untreated Turner adults in the same locale, so that it is probably more appropriate for large-scale scientific studies than for daily clinical use. The application of the IPH method here to evaluate the end results obtained with oxandrolone is a good example. Regarding treatment with oxandrolone, it must be stressed that good results were limited to girls with a bone age of 10.0 to 12.9 years treated over 2 years with a relatively high dose of oxandrolone, which resulted in androgenic side effects in 16% of the girls treated. Thus, only in this sense does it seem that the data will contribute to a better evaluation of the end results to be obtained in Turner patients with growth hormone alone or with the growth combined with low-dose androgens.*

Jean-Claude Job, MD

**2nd Editor's comment:** *The primary importance of these studies is that oxandrolone as used did not decrease ultimate height, which is consistent with previous reports. The data strongly suggest that there may*

*be an increase of 3 to 4 cm in the final adult stature of girls receiving treatment initially at 10 to 12 years of age. The mild androgenicity reported at a dose of .125 mg/kg/d has been observed by others, but seldom occurs if the dose is kept to <0.1 mg/kg/d. Further, these androgenic effects usually resolve when treatment is discontinued.*

Robert M. Blizzard, MD

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## Mutation in the Gene Encoding the Stimulatory G Protein of Adenylate Cyclase in Albright's Hereditary Osteodystrophy (Pseudohypoparathyroidism)

G proteins are a large family of guanine nucleotide-binding proteins that facilitate signal transduction across cell membranes. Of the 3 subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) that comprise each G protein, the  $\alpha$  subunit is thought to confer functional specificity to the molecule. One such protein,  $G_s\alpha$ , has been found to be defective in most cases of Albright's hereditary osteodystrophy (pseudohypoparathyroidism type Ia).  $G_s\alpha$  is responsible for stimulating the hormone-sensitive adenylate cyclase system that generates the intracellular second messenger cyclic adenosine monophosphate (AMP). Albright's hereditary osteodystrophy (AHO) is an autosomal dominant disorder characterized by resistance of target organs to parathyroid hormone (PTH) and other hormones that utilize this signal transduction pathway.

Patten et al studied a family with AHO. They first showed that erythrocyte membranes from the affected mother and son (but not from an unaffected son) contained reduced  $G_s\alpha$  bioactivity. They next demonstrated 2 populations of  $G_s\alpha$  protein in the patients: 1 of normal and 1 of abnormal size. Analysis of genomic DNA subsequently showed the heterozygous loss of a restriction enzyme cleavage site in the 5' part of the  $G_s\alpha$  gene; and sequencing of the region revealed a point mutation (ATG-to-GTG) in the initiator codon of 1 of the 2 alleles. The mutation was not present in normal individuals, nor was it detected in 7 other patients with  $G_s\alpha$  deficiency.

An interesting aspect of this investigation was that although both the mother and son carried the same mutation, the son presented

with the classic clinical and biochemical features of pseudohypoparathyroidism, including elevated levels of PTH. His mother had normal levels of calcium, phosphorus, and PTH and a normal urinary cyclic AMP response to bovine PTH. The authors speculated about why this should be but were unable to offer any firm explanations.

Patten JL, Hohns DR, Valle D, et al. *N Engl J Med* 1990;322:1412-1419.

*reported. However, in an accompanying editorial, Spiegel (N Engl J Med 1990;322:1461-1462) noted that 3 other mutations have been identified but not published. Thus, Albright's hereditary osteodystrophy is clearly a genetically heterogeneous disorder at the molecular level. Spiegel also addressed the variable expression issue, concluding with the suggestion that a 50%  $G_s\alpha$  deficiency is necessary but insufficient by itself to produce full expression of the disease phenotype.*

**Editor's comment:** Although G protein abnormalities have been recognized for some time in this disorder, this is the first mutation of the  $G_s\alpha$  gene to be

William A. Horton, MD

### In Future Issues

**Obesity in Childhood and Adolescence, Part II: Pathophysiology, Association, and Complications**  
by W.H. Dietz, MD

**Update: The Genetics of Insulin-Dependent Diabetes**  
by W.E. Winter, MD, and M.K. McLaren, MD

**Support Groups for Individuals with Growth Problems and Their Families**  
by J. Weiss, MSW, LCSW, and J.G. Hall, MD

**Abstracted from the work of:**  
Joseph D. Schulman, MD  
**Preimplantation Genetics**  
and  
David L. Rimoin, MD  
**Limb Lengthening: Past, Present, and Future**



## Marfan Syndrome: The Basic Defect May Be in Sight

Marfan syndrome is the prototypical inherited disorder of connective tissue. Its manifestations, which involve the ocular, cardiovascular, and skeletal systems in particular, are familiar to most clinicians. Despite intense interest over the last several decades, its etiology and pathogenesis have remained elusive; however, 1990 has seen remarkable progress.

Godfrey and colleagues<sup>1</sup> demonstrated an apparent deficiency of elastin-associated microfibrils in skin and fibroblast cultures from affected members of 9 families. These fibrils constitute a fibrillar system that is widely distributed throughout the body, including tissues affected in Marfan syndrome. The fibrils are thought to serve as scaffolding for the deposition of elastin during elastogenesis. The samples were examined by immunofluorescence using monoclonal antibodies to fibrillin, a major structural protein of the microfibrils. The analysis was done "blindly," and deficient immunostaining cosegregated with the disorder when specimens from affected family members were compared with those from unaffected members, which stained normally. In an accompanying article, this group found similar microfibrillar abnormalities in skin biopsies and fibroblast cultures from the affected side but not from the unaffected side in a patient with asymmetric involvement of the syndrome.<sup>2</sup>

Hollier et al<sup>3</sup> next assessed fibrillin immunostaining in another group of patients with Marfan syndrome, as well as in patients with other connective tissue disorders. They confirmed the staining abnormalities, which included decreased numbers of fibers and abnormal staining patterns,

in skin biopsies and fibroblast cultures from most Marfan patients (16 of 23 and 16 of 18, respectively). Seven of 25 patients with non-Marfan connective tissue disorders also showed abnormalities. These disorders included pseudoxanthoma elasticum, homocystinuria, ectodermal dysplasia, coronary artery dissection, cutis laxa, and epidermolysis bullosa-like syndrome. Interestingly, 3 Marfan patients had normal staining of skin, fibroblast cultures, or both.

Thus, microfibrils appear to be abnormal in most patients with Marfan syndrome. The abnormalities involve both the number and organization of the fibers, and are not completely restricted to Marfan syndrome. These observations led several groups to search for biochemical abnormalities of fibrillin in the syndrome. Indeed, McGookey et al<sup>4</sup> identified 3 types of abnormalities in fibrillin synthesis and secretion in fibroblasts from 21 patients. In 1 group of 7 probands, half the normal amount of fibrillin was synthesized, although it was secreted normally. Reduced synthesis and defective secretion were detected in the second group of 7 patients. Four patients in the third group of 7 patients showed normal synthesis and secretion, but defective incorporation of fibrillin molecules into the extracellular matrix. These types of abnormalities were consistent within families and were not found in unaffected members.

Coincident with these microscopic and biochemical studies have been attempts to map the gene locus of Marfan syndrome by reverse genetics. An international consortium was formed to pool linkage data from families with Marfan syndrome. Data from 25 three-generation families led to an exclusion map

in which three fourths of the genome was excluded.<sup>5</sup> Searching for linkage in nonexcluded areas subsequently led Kainulainen and coworkers<sup>6</sup> to the long arm of chromosome 15. They studied DNA polymorphisms in 8 three-generation Finnish families with multiple affected members using anonymous chromosome 15q gene probes. Positive line of descent (LOD) scores were determined in 5 families, yielding a total score of 3.92. Since a score of >3.00 is considered strong evidence for linkage, these data provisionally map the Marfan locus to chromosome 15q. Confirmatory studies are now underway.

**Editor's comment:** *These reports demonstrate that the elusive basic defect in Marfan syndrome is falling victim to medical progress much as the molecular defects in many other genetic diseases have in recent months, eg, cystic fibrosis and neurofibromatosis. Despite several loose ends, such as failing to detect microfibrillar abnormalities in all Marfan patients while detecting abnormalities in some patients with other conditions, fibrillin has been established as a strong candidate for the mutant protein in Marfan syndrome. If the fibrillin gene locus can be mapped to chromosome 15q where the syndrome maps, although much work will still remain, the elucidation of the defect may not be far behind.*

William A. Horton, MD

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- abnormalities with the Marfan phenotype in families. *Am J Hum Genet* 1990;46:652-660.
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## Insulin-like Growth Factor II in Antenatal Growth

While considerable circumstantial evidence suggests that insulin-like growth factor II (IGF-II) plays an important role in antenatal growth, there is little direct evidence for this. DeChiara, Efstratiadis, and Robertson have provided such evidence through studies of mice in which the IGF-II gene was disrupted. Their strategy was to substitute by homologous recombination a mutated IGF-II gene for the endogenous (normal, wild-type) gene in embryonic stem (ES) cells. Essentially, 2 mutations were introduced into the gene that allowed for selection of cells expressing the mutant gene and also abolished the function of the gene product. Injection of ES cells, selected for the inactivated gene, into mouse blastocysts produced chimeric mice, which could be distinguished because the coat color of the host mice differed from that of the mice from which donor ES cells were obtained. Mating of male chimeric mice with germ cells thought to be derived from the injected ES cells to normal female mice generated offspring that were heterozygous for the inactivated IGF-II gene.

Comparison of newborn mice carrying a single dose of the functional IGF-II gene to newborn control mice carrying 2 doses of the gene revealed that the former were much smaller. Their weight was about half that of controls. Their absolute postnatal growth rate was also

less than that of controls; however, when plotted as a function of birth weight, it was the same. Although the mice carrying the inactivated gene were not extensively evaluated other than for growth, they appeared to be otherwise normal, and they were fertile.

One of the most interesting observations was that mRNA transcriptase levels from the functioning IGF-II allele were approximately 10-fold less in heterozygote embryos compared with control mice embryos. Much less of a reduction had been predicted based on gene dosage, which was half, and weight, which was also about half. IGF-II peptide levels were not measured in the embryos. The authors were unable to explain this discrepancy but pointed out that the situation may be complex and needs further study. Breeding experiments to produce mice homozygous for the inactivated IGF-II gene are in progress.

DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* 1990;345:78-80.

**Editor's comment:** This study provides direct evidence for the growth-promoting effects of IGF-II during in utero growth. The "apparent" normality of the heterozygous mice in non-

*growth-related characteristics raises the possibility that IGF-II, at least in the usual amounts, may not be essential for organogenesis in the early embryo. Breeding of mice in which both IGF-II genes have been inactivated should shed light on this matter and provide further insight into the role of this peptide in prenatal and postnatal growth in general.*

*These studies were performed with mice for a variety of reasons. However, since IGF-II and its mRNA are widely distributed in human fetal tissues, the results presumably apply to humans as well.*

William A. Horton, MD

### Special Announcement

The 8th International Congress of Human Genetics, sponsored by the American Society of Human Genetics, will be held October 6-11, 1991 at the Washington Convention Center in Washington, DC. The deadline for receipt of abstracts is April 1, 1991. For abstract and registration forms or additional information, contact: M. Ryan, Meetings Manager, ICHG, 9650 Rockville Pike, Bethesda, MD 20814 USA (Telephone 301-571-1825; Fax 301-530-7079).

## Treatment of Constitutional Delay of Growth and Puberty With Oxandrolone Compared With Growth Hormone

Twenty-six boys (12.1 to 15.9 years of age) with constitutional delay of growth and puberty (CDGP) were given either 20 U/m<sup>2</sup>/wk (approximately 0.3 mg/kg/body weight) of growth hormone (GH) or 2.5 mg of oxandrolone per day. The former group received GH for 12 months and the latter received oxandrolone for the first 3 of 12 months. At the end of 12 months, the boys in both groups grew significantly and advanced toward puberty at comparable rates. These data are presented in the table.

Bone age advancement was comparable, as was the increase in testicular volume. Only 3 of the oxandrolone-treated group and 2 of the GH-treated group had testes greater than 15 cc at the end of the treatment year.

The authors conclude that GH is not indicated for the management of delay in the

| Mean                                  | Growth Hormone |          | Oxandrolone |          |
|---------------------------------------|----------------|----------|-------------|----------|
|                                       | Before Rx      | After Rx | Before Rx   | After Rx |
| Chronologic age (yr)                  | 13.9           | 15.0     | 13.8        | 14.8     |
| Testicular volume (cc)                | 4.8            | 10.7     | 5.8         | 10.0     |
| Growth velocity (cm/yr)               | 3.8            | 6.8      | 3.9         | 8.3      |
| Bone age delay (yr)                   | -2.3           | -3.0     | -2.1        | -2.2     |
| Standard deviation score for bone age | -0.6           | -0.1     | -0.8        | -0.2     |

pubertal growth spurt. Oxandrolone can be very beneficial.

Buyukgebiz A, Hindmarsh PC, Brook CGD. *Arch Dis Child* 1990;65:448.

**Editor's comment:** Oxandrolone is used by both the British and US endocrinologists for treatment of CDGP but is used differently.

*The British induce puberty over a 3-month period in boys who have testicular volume of at least 4 cc. The Americans provide the drug over an extended period until the patient is secreting significant testosterone (>100 ng/mL). Possibly the Americans may wish to evaluate the length of treatment after considering the data of Buyukgebiz et al.*

Robert M. Blizzard, MD

## Final Height in Boys With Untreated Constitutional Delay in Growth and Puberty

Short stature due to constitutional delay in growth and puberty (CDGP) is the most frequent cause of short stature referred to the pediatric endocrinologist. Although an extreme of normal development rather than a clinical disorder, it can still pose clinical concerns for patients, parents, and physicians. These patients are believed to grow to a normal height and, therefore, treatment with growth-promoting agents is primarily for psychological reasons. Crowne et al undertook a retrospective study (1976-1986) of patients with CDGP to determine the natural history of growth patterns and psychological impact of these growth patterns in 118 boys

with CDGP. Forty-three were followed to final height.

At presentation, the mean chronologic age (CA) was 14.0 years  $\pm$  1.9 SD, the mean bone age (BA) delay, 2.7  $\pm$  1.0 years, and height SD score (SDS), -3.4  $\pm$  0.6. The predicted adult height SDs by the Tanner-Whitehouse II method was -1.3  $\pm$  0.7 years. The "final" adult height SDS measured when all patients were more than 21 years of age or growing less than 2 cm per year was reported at -1.6  $\pm$  0.9 SD.

Comparison of final adult height and midparental height revealed a significant difference of -6.5  $\pm$  6.0 cm. In 14 of the 20 sets of parents who were measured and who

reported their heights, the measured heights were less than 1 cm different than those reported. In 6 sets of parents, the difference was greater than 2.5 cm, with the reported height being greater. Regardless, when the measured height was used, the difference between midparental height and final height was significant ( $P = 0.003$ ).

In respect to self esteem, there was no significant difference between the boys with CDGP and a normal control group, as measured by the Coopersmith self-esteem inventory. Further, there was no significant difference between the groups in recorded social activity, number either married or in

stable relationships, or in the number of unemployed. Less than 10% think of their height currently or on an occasional basis only.

Seventy-nine percent of the CDGP group are satisfied with their height versus 99% of the control group. Fifty percent would have liked to have had treatment to bring on their growth spurt, and 55% would access treatment for their children should they be faced with a similar growth problem.

Crowne EC, Shalet SM,

Wallace WHB, et al. *Arch Dis Child* 1990;65:1109-1112.

**Editor's comment:** *The authors conclude that the results concerning the difference in midparental heights and final predicted heights deserve further study to determine if boys with CDGP are shorter than expected for their midparental heights and to determine whether growth-promoting agents enhance the heights of boys with CDGP. They also*

*conclude that the principal reason for treating boys with CDGP with growth-promoting agents is to alleviate short-term stress and distress. The fact that 55% of those responding wanted their children treated in order to avoid the stresses they had endured as adolescents justifies treatment consideration in certain patients with CDGP.*

Robert M. Blizzard, MD

## Growth of Males With Idiopathic Hypopituitarism Without Growth Hormone Treatment

Twenty-three males with idiopathic hypopituitarism who were not treated with growth hormone (GH) were evaluated with respect to their ultimate adult height. A majority of these individuals were born by breech delivery, which accounted for the hypopituitarism. A majority also had gonadotropin deficiency in addition to GH deficiency. Treatment with androgen in those with gonadotropin deficiency was started at a mean age of 17.4 years. At that time, all patients had heights -3 SDs below the mean. Bone maturation was greatly retarded, with bone ages (BAs) more than 3 years below the chronologic age (CAs). Patients whose puberty developed spontaneously had comparable BAs and heights when puberty began.

Patients with "induced" puberty reached a mean final height of 157.0 cm at a mean age of 26.1 years. The mean adult height was -3.9 SD for 4 patients with spontaneous puberty and -3.1 SD for the 19 with "induced" puberty. The pubertal period had a mean duration of 8.7 years in these 19 patients, during which

height increased by a mean of 20.4 cm. The mean difference between the predicted adult height at the onset of "induced" pubertal growth and the attained final height was -7.1 cm, ranging from -24 cm to +4 cm. Adult heights were positively correlated with heights at the onset of pubertal growth. The total mean height gained during "induced" puberty (20.4 cm) compares favorably with the height gained during spontaneous puberty by normal late maturing boys (approximately 18 cm).

The authors conclude that physicians should make every effort possible to increase the heights of GH deficient patients to within the normal range before puberty begins. If this is not done, significant short stature will persist in adulthood.

Van der Werff ten Bosch JJ, Bot A. *Clin Endocrinol* 1990; 32:707-717.

**Editor's comment:** *The authors document the ultimate heights of patients with hypopituitarism seen in their clinic. There are few tabulations of final adult heights of*

*hypopituitary patients not treated with growth hormone, and this paper is a significant contribution in this respect. It is surprising that the mean growth attained following initiation of testosterone treatment was 20.4 cm. This was over a protracted treatment period, and probably results from relatively low doses of replacement therapy. It is to be noted that the vast majority of the patients did not reach their predicted heights.*

*The role of GH is important in the treatment of the GH deficient patient. Early diagnosis and adequate treatment are necessary for individuals with GH deficiency to reach their predicted heights or a height in accord with the target range based on midparental height. These are the emphatic points made by the authors and this editor.*

Robert M. Blizzard, MD



**Volume 6, Number 1**

"Growth, Thyroid Function, and Sexual Maturation in Down Syndrome"

Siegfried M. Pueschel, MD, PhD

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# GROWTH

## Genetics & Hormones

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## Heritable Origins of Type I (Insulin-Dependent) Diabetes Mellitus: Immunogenetic Update

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### Introduction

The etiology of type I (insulin-dependent) diabetes mellitus (IDDM) is multifactorial. Studies of identical twins reveal maximum concordance rates of 50%, suggesting that environment, in addition to heredity, influences the development of pancreatic beta-cell autoimmunity. Susceptibility to IDDM is not inherited as a simple mendelian trait since only 5% to 10% of patients with IDDM have a parent or other first-degree relative affected with IDDM.

Genetic predisposition to IDDM is most strongly associated with specific HLA-DR and HLA-DQ alleles of the major histocompatibility complex (MHC) located on the short arm of chromosome 6. Other candidate genetic loci—including the insulin gene

(chromosome 11),  $\kappa$  light-chain gene (chromosome 6), immunoglobulin heavy-chain gene (chromosome 12), and Kidd blood group—have little or no influence on inherited susceptibility to IDDM. Although unmapped, autosomal dominant thyrogastric autoimmunity provides increased proclivity to IDDM. Gender has a modest influence on predisposition to IDDM early in life as males are in definite excess by ~20% in IDDM onset at 5 years or less. By adolescence, males and

females are affected equally frequently.

Class II MHC molecules, antigen-derived peptide fragments, and T-cell receptors are necessary for the generation of immune responses. Once nominal pancreatic beta-cell antigen is available, variations in antigen structure between controls and IDDM patients can be sought. The present search for genetic susceptibility to IDDM has included studies of HLA class II and T-cell receptor genes.

### Letter From the Editor

Although a section (Letters to the Editor) has been dedicated to receiving comments, few have been forthcoming the past 2 years. You as a colleague and reader are encouraged to challenge our editors, bring new information to the attention of our readers and ourselves, and probe for different or additional information. This section is too important for it to lapse because of disuse. Please let us hear from you.

From the Editorial Board  
Robert M. Blizzard, MD

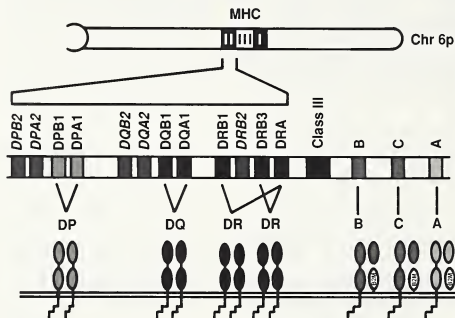
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Figure 1



The human MHC is located on the short arm of chromosome 6 (Chr 6p). Class I MHC genes code for a 44-kD glycoprotein present on all nucleated cells that noncovalently associates with the non-MHC protein  $\beta 2M$  (open ellipses). Class II MHC molecules, which are normally restricted to antigen-presenting cells and B lymphocytes, are coded for by separate  $\alpha$  and  $\beta$  genes coding, respectively, for 34-kD  $\alpha$  chains and 29-kD  $\beta$  chains. The class III MHC region includes loci for the adrenal cortical enzyme 21-hydroxylase gene, the complement protein C4 gene, TNF- $\alpha$  gene, and TNF- $\beta$  gene. In this diagram, the class II MHC genes are named using the 1989 histocompatibility workshop nomenclature.

**Table 1**  
Class II MHC Gene Terminology  
(Nonexpressed genes are in *italics*.)

| Nomenclature                  |             |
|-------------------------------|-------------|
| Pre-1989                      | 1989        |
| <i>DPB2</i>                   | <i>DPB2</i> |
| <i>DP<math>\alpha</math>2</i> | <i>DPA2</i> |
| <i>DPB1</i>                   | <i>DPB1</i> |
| <i>DP<math>\alpha</math>1</i> | <i>DPA1</i> |
| <i>DX<math>\beta</math></i>   | <i>DQB2</i> |
| <i>DX<math>\alpha</math></i>  | <i>DQA2</i> |
| <i>DQB1</i>                   | <i>DQB1</i> |
| <i>DQ<math>\alpha</math></i>  | <i>DQA1</i> |
| <i>DRB1</i>                   | <i>DRB1</i> |
| <i>DRB1I</i>                  | <i>DRB2</i> |
| <i>DRB1II</i>                 | <i>DRB3</i> |
| <i>DR<math>\alpha</math></i>  | <i>DRA</i>  |

An illustrated key to the new taxonomy. The DRA chain can pair with either of 2 DRB chains to produce 2 possible DR molecules. The DQA1 and DQB1 chains pair to form the DQ molecule, while DPA1 and DPB1 chains pair making the DP molecule. *DRB2*, *DQA2*, *DQB2*, *DPA2*, and *DPB2* are pseudogenes and as such are not expressed.

### MHC Genes in IDDM: Studies in Humans

The MHC (Figure 1 and Table 1) controls which antigens an individual responds to and the degree of the response. Class I molecules are glycoproteins coded for by single genes within the MHC (HLA-A, HLA-B, and HLA-C) located on the exterior of all nucleated cells. They survey the intracellular milieu in order to present endogenous or invading antigens as possible targets of CD8 cytotoxic lymphocytes. Class II molecules (HLA-DR, -DQ, and -DP) are heterodimeric cell surface glycoproteins composed of an  $\alpha$  and a  $\beta$  chain coded for by 2 separate genes within the MHC. Macrophages digest phagocytized matter to release antigen-derived peptides. These fragments, which are initially of extracellular origin, bind to class II MHC

molecules to be presented to CD4 T lymphocytes, which in turn orchestrate the immune response.

In the 1970s, increased frequencies of the class I MHC alleles HLA-B8 and -B15 were noted in patients with IDDM compared with controls. With the developments of Dw

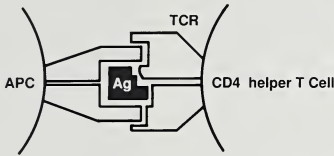
(primed lymphocyte) cellular typing and HLA-DR serotyping somewhat later, IDDM was shown to be more closely associated with certain class II MHC alleles than class I MHC alleles. Empiric risks for IDDM based on HLA-DR phenotype have been previously reviewed in *GGH*, Volume 2,

### Special Announcement

We have recently undertaken the reproduction of back issues of *GROWTH, Genetics, & Hormones* as a service to our readership. In the event that you have become a recent subscriber or perhaps may be missing copies of previous issues of *GGH*, this material is now available through written request, free of charge. To receive copies of back issues of *GGH*, Volumes 1 through 6, Numbers 1 to 4, please write to: Ms. J. Christopher, c/o SynerMed, Route 513 & Trimmer Road, PO Box 458, Califon, NJ 07830.

This material is provided through an educational grant from Genentech, Inc.

Figure 2



Class II MHC molecules of antigen-presenting cells (APC) display antigen-derived peptide fragments (Ag) to the T-cell receptors (TCR) of CD4 helper T cells. APCs such as macrophages consume cellular debris and exogenous antigens, and degrade the ingested proteins into peptides. These peptides in turn are displayed on the APC cell surface cradled in the peptide binding cleft or groove of the class II MHC molecule. Theoretically, if a TCR recognizes the MHC beta-cell autoantigen peptide assembly, an immune response against the pancreatic beta cell is initiated and the eventual clinical consequences are insulinopenia and IDDM.

chains) does not appear to contribute to susceptibility to IDDM as DP and DQ are separated by  $\alpha$  and  $\beta$  chains of the class II molecule (Figure 2). Each chain contributes an  $\alpha$  helical "wall" and 4  $\beta$  pleated sheets of the cleft "floor" to form a peptide binding pocket (2  $\alpha$  helical "walls" and a "floor" of 8  $\beta$  pleated sheets). Polymorphic amino acids, which produce allelic variability, generally face into the cleft to influence peptide binding. DQ $\beta$   $\beta$  1 exon amino acid 57 is located at the beginning of an  $\alpha$  helix. Theoretically, negatively charged aspartic acid at this position can alter peptide binding by facing into the cleft, or by forming a salt bridge with a conserved DQ $\alpha$  chain arginine at position 79. Also, as helical amino acids can interact with T-cell receptors, the strength of MHC-T-receptor interactions thus could be modified, further influencing the immune response.

Several evaluations of DQ $\beta$  position 57 have been performed in various populations. When 39 IDDM patients were genotyped by Todd et al for the presence or absence of the putative critical amino acid, 90% were found to be non-aspartic acid homozygotes while only 10% were heterozygotes and none were aspartic acid homozygotes. The association of non-aspartic acid residues at DQ $\beta$  position 57 with IDDM has been recognized in population studies from the United States, Denmark, Finland, Norway, and France. However, from these studies, HLA susceptibility to IDDM was not strictly recessive, since some individuals heterozygous at position 57 (aspartic acid/non-aspartic acid) or homozygous for aspartic acid had IDDM. Furthermore, if HLA susceptibility was strictly recessive, 100% of affected sib pairs in multiplex families would be HLA identical. This is not the case, as only 60% of cases are HLA identical while 35% are haploidentical (ie, share 1 HLA haplotype) and a minority ( $\leq 5\%$ ) of sibships are HLA

No. 1 (1986). Serologic studies in whites demonstrated that HLA-DR3, -DR4, and -DR1 were positively associated with IDDM while HLA-DR2 and -DR5 were negatively associated with IDDM. The other DR serotypes have a neutral effect on predilection to IDDM. Of interest, HLA-DR1 was a risk allele only when associated with DR3 or DR4, while the protective effect of DR2 was greater than that of DR5. Blacks exhibited a consistent association of HLA-DR4 and a weaker relationship of DR3 with IDDM. In contrast, Japanese IDDM patients displayed increased frequencies of HLA-DR4 and -DRw9.

Since no HLA-DR associations with IDDM proved to be absolute and since specific HLA-DQ and HLA-DR alleles are in tight linkage disequilibrium, recent investigations have examined the possible relationship of HLA-DQ to IDDM. Variability in HLA-DR alleles is strictly due to the genetic diversity of DR $\beta$ 1 (DRB1) or DR $\beta$ III (DRB3) since DR $\alpha$  (DRA) is essentially nonpolymorphic. In contrast, diversity in DQ $\alpha$  (DQA1) and DQ $\beta$  (DQB1) chains can both

contribute to DQ variability. Researchers have examined the *hypothesis* that serologically defined HLA-DR types might be further partitioned into diabetes-prone and diabetes-resistant alleles using DQ $\alpha$  or DQ $\beta$  typing. These studies were initiated in 1983 by David Owerbach, who used restriction fragment length polymorphism (RFLP) analysis of DR4-associated DQ $\beta$  gene alleles. He found that a DQ $\beta$  *Bam*HI 3.7-kb fragment was associated with resistance to IDDM. In addition to the DR4-DQ $\beta$  subtypes, many studies followed that delineated RFLP subtypes of DQ $\beta$  chains of DR2 and DR6 haplotypes that defined susceptibility or resistance to IDDM.

DQ $\alpha$  (DQA2) and DQ $\beta$  (DQB2) are located between DQ and DP. DQ $\alpha$  has been implicated as a modifier of genetic susceptibility; however, a DQ molecule is not expressed. Associations of DQ $\alpha$  alleles and IDDM appear solely to reflect linkage disequilibrium of DX with DR and DQ alleles in extended HLA haplotypes that display resistance or proneness to IDDM. As well, HLA-DP (DPA1 and DP $\beta$  [DPB1]



nonidentical (ie, share no HLA haplotypes).

From the above analysis it is also apparent that DQ $\beta$  alleles containing aspartic acid at position 57 are not uniformly protective of the development of IDDM. The only allele to provide dominant protection is the DR2-linked DQ $\beta$ 1.2. Even in association with DR4-DQ $\beta$ 3.2, DR2-DQ $\beta$ 1.2 prevents IDDM. Rarely, DR2 alleles are seen in IDDM patients; however, in such cases, the DR2-linked DQ $\beta$  (DQ $\beta$ 1.AZH) allele lacks aspartic acid, which is consistent with the association of IDDM and non-aspartic acid residues at DQ $\beta$  position 57. While DR5, like DR4, can be linked to either DQ $\beta$ 3.2 or DQ $\beta$ 3.1, DR5 is most commonly linked to DQ $\beta$ 3.1, a nonsusceptibility allele, which explains the protective effects of most DR5 alleles.

Non-aspartic acid 57 alleles do not provide equal disease susceptibility (strength of susceptibility: DQ $\beta$ 3.2 > DQ $\beta$ 2 > DQ $\beta$ 1.1 > DR $\beta$ 1.AZH), nor are all aspartic acid 57 alleles equally neutral or protective (strength of protection: DQ $\beta$ 1.2 > DQ $\beta$ 3.1). In one study, DQ $\beta$ 3.2 was more strongly associated with IDDM than DQ $\beta$ 2. This implies that amino acids other than those at DQ $\beta$  position 57 modify susceptibility or resistance. DR $\beta$ 1 and DQ $\alpha$  genes may also temper DQ $\beta$  genetic susceptibility to IDDM. For example, Sheehy et al simultaneously studied DQ $\beta$  alleles and DR4-DR $\beta$ 1 alleles (Dw4,Dw10, etc) to demonstrate their interaction. DR4-Dw4,DQ $\beta$ 3.2 or DR4-Dw10,DQ $\beta$ 3.2 displayed a relative risk for IDDM of 38, which was several times higher than risk assessments based on DR or DQ $\beta$  allele typing alone.

In some researchers' hands, DQ $\beta$  assessment has increased the predictability of IDDM. In a US population, Morel et al reported that individuals homozygous for non-aspartic acid 57 DQ $\beta$  alleles had a relative risk for IDDM of 107. In France, individuals heterozy-

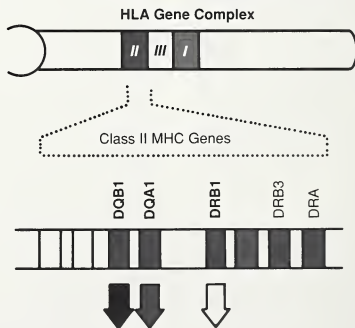
gous for DQw2,DQw3.2 (both alleles lack aspartic acid at position 57) had a relative risk for IDDM of 52.9. Such estimates, approaching 1 in 5, compare favorably with empiric IDDM risks in identical twins (~1 in 3 to ~1 in 2). In siblings who are HLA identical to a diabetic proband, the absolute risk for IDDM is ~1 in 7 and rises to ~1 in 4 if HLA-DR3 and -DR4 are shared. However, in the French studies, non-aspartic acid 57 homozygosity alone was less predictive of IDDM, with a relative risk of 13.2, comparable with that of the 17.5 in Finns and 12.2 among Norwegians. Recently published risk estimates from Texas are even more conservative, with a relative risk for IDDM of 4.5 in aspartic acid-negative homozygotes.

Important exceptions have arisen to the correlation of diabetes susceptibility with specific charged or neutral

amino acids at DQ $\beta$  position 57. As noted above, it has become apparent that a small percentage of IDDM patients are, in fact, homozygous for aspartic acid-positive DQ $\beta$  alleles, and that in DR1,DR4 heterozygosity, DQ $\beta$ 3.2 is not increased in frequency. Thus, in DR1,DR4 heterozygous IDDM patients, DR1 (DQ $\beta$ 1.1, aspartic acid negative) appears to supply the diabetes predilection.

A particular problem for the DQ $\beta$  position 57 theory is DR7. Although DR7 is linked to DQ $\beta$ 2, as is DR3, DR7 is not associated with IDDM in whites. One possibility to consider in this regard was the influence of the respective DQ $\alpha$  alleles, as the DQ molecule is a heterodimer. While studies on the influence of the DQ $\alpha$  to date have been limited, Todd and colleagues have pointed out that the DR7 in whites is linked to the DQ $\alpha$  A2 allele, in

**Figure 3**  
**Genetic Susceptibility To**  
**Type I Diabetes (IDDM)**



Alleles at the DQB1, DQA1, and DRB1 loci influence susceptibility to type I diabetes (IDDM). The darker the arrow, the relatively greater influence the locus has on predilection to IDDM. Only the expressed loci are labeled and the DP loci are not depicted (see Figure 1 for comparison).

contrast to the DR3 in whites which is linked to the DQ $\alpha$  A3 allele. In blacks, however, DR7 can be linked to DQ $\alpha$  A3 and DQ $\beta$ 2, similar to white DR3, and thus can be associated with IDDM like DR3 in whites. Thus, the DQ $\alpha$  A3 allele may be required in addition to DQ $\beta$ 2 to confer diabetogenicity (Figure 3, page 4).

Another challenge to the DQ $\beta$  position 57 theory comes from studies of IDDM patients in Japan. Japanese patients with IDDM do not demonstrate an increased frequency of aspartic acid-negative DQ $\beta$  alleles. Whereas the low number of non-aspartic acid alleles is consistent with the low prevalence of IDDM in the Japanese (~1:10,000 vs ~1:500 for the US), those with IDDM may carry a DQ $\alpha$  A3 susceptibility allele to account for HLA susceptibility in Japan. Of interest, in black patients with IDDM from Zimbabwe, only DQ $\alpha$  RFLPs correlated with susceptibility to IDDM. The weak relationship of DR3 to IDDM in blacks may reflect the linkage of DQW4 (IDDM nonassociated) to DR3 in this ethnic group. In whites, DR3 is exclusively linked to DQ $\beta$ 2.

Aspartic acid position 57-diabetes susceptibility correlations also do not explain why DR3, DR4 heterozygosity produces the highest relative risk for IDDM. Nepom et al demonstrated that the DR3-linked DQW2  $\alpha$  chain can pair with the DR4-linked DQW3  $\beta$  chain, producing a novel class II MHC product. However, such transcomplementation occurs in controls as well as in patients with IDDM and thus is not unique to patients with IDDM.

#### **MHC Genes in IDDM: Studies in Nonobese Diabetic Mice**

The nonobese diabetic (NOD) mouse experiences spontaneous autoimmune destruction of pancreatic beta cells and is thus an excellent model for human IDDM. As in humans, IDDM in NOD mice is multifactorial, and up to 6 autosomal recessive genes are postulated. According to RFLP studies and DNA sequencing,

the NOD class II molecule IA (homologous to DQ) is unique. Similar to several other strains of mice, NOD mice do not express a class II IE molecule (homologous to DR). Predilection to insulinitis and IDDM is strongly associated with IA<sup>nod</sup>. In outbreeding experiments, diabetic mice are homozygous for IA<sup>nod</sup> with only rare exceptions.

Similar to DQ $\beta$  susceptibility alleles in humans, A $\beta$ <sup>nod</sup> at the  $\beta$  1 domain position 57 lacks aspartic acid, as A $\beta$ <sup>nod</sup> is positive for serine. However, we now recognize that other strains of mice also lack aspartic acid at A $\beta$  position 57 and do not develop IDDM. Boehme and coworkers have identified at least 7 A $\beta$  alleles in various *Mus* species (my1, Eccles, W250, STC90, W253, stf, and zbn) that carry a neutral amino acid at A $\beta$  position 57.

Recent experiments in transgenic NOD mice have directly addressed the issue of aspartic acid 57 in A $\beta$ <sup>nod</sup>. When NOD mice were made transgenic for IA\* (aspartic acid 57 positive), insulinitis was prevented. This is consistent with the hypothesis that the A $\beta$  gene on chromosome 17 is the locus of diabetes susceptibility or resistance. Other investigators mutated the transgenic A $\beta$ \* position 57 to serine but did not restore diabetogenicity to the allele. Furthermore, in a third study, amino acid 56 of A $\beta$ <sup>nod</sup> was mutated from histidine to proline, preventing diabetes in the model.

These studies demonstrate that position 57 of A $\beta$ <sup>nod</sup> is not exclusively responsible for susceptibility or resistance and other class II MHC amino acid motifs may impact extensively on diabetogenicity. The transgenic expression of IE, either through breeding or direct microinjection, is also able to prevent pancreatic beta-cell autoimmunity in NOD mice. Thus, 2 elements of the NOD MHC are required for diabetogenicity: a novel IA molecule and the absence of IE expression.

#### **T-Cell Receptor Genes in IDDM: Studies in Humans and NOD Mice**

Concurrent with the above analyses,  $\alpha/\beta$  T-cell receptor inheritance has been studied. While there was no consensus of opinion among early investigations concerning T-cell receptor  $\beta$  inheritance and IDDM, Concannon et al recently demonstrated in human sib pair analysis that T-cell receptor  $\beta$  polymorphisms were not associated with IDDM. The data are consistent with our own published work in the NOD mouse in which we were able to demonstrate that T-cell receptor  $\beta$ <sup>nod</sup> did not function as an autosomal recessive autoimmune gene in influencing the development of insulinitis in (INOD x NZW)F1 x NOD) backcross mice. In a limited population of such backcross mice, we also demonstrated that neither T-cell receptor  $\alpha$ <sup>nod</sup> nor  $\gamma$ <sup>nod</sup> modified genetic susceptibility to pancreatic beta-cell autoimmunity, excluding a genetic influence derived from genes coding for  $\gamma/\delta$  T-cell receptors. A similar conclusion for T-cell receptor  $\alpha$  was drawn in NOD, NON outcross, backcross experiments. Unresolved is the question of whether specific V $\beta$  or V $\alpha$  segments are utilized in the immune response to pancreatic beta-cell autoantigen. This issue is a postfertilization concern and does not relate to inherited proclivity for IDDM.

#### **Chromosome 9 Genes in IDDM: Studies in NOD Mice**

Studies in a NOD x NON outcross-NOD backcross by Prochazka and colleagues and by Hattori and colleagues suggested that murine chromosome 9 influenced predilection to IDDM. Initial findings indicated that the chromosome 9 IDDM susceptibility gene mapped centromeric to Thy-1. However, markers centromeric of Thy-1 showed weaker associations with IDDM than Thy-1. Thus, any pancreatic beta-cell autoimmunity gene on chromosome 9 would not function as an absolute

autosomal recessive. Chromosome 11 in humans, which is homologous to murine chromosome 9, does not appear to be associated with IDDM.

### Summary

As human IDDM is clearly polygenic in etiology, future genetic analysis might best be undertaken in the NOD mouse model. When regions of the murine genome show potential association with pancreatic beta-cell autoimmunity, the studies can then be undertaken in humans. Reverse genetics can be employed using random, regularly spaced DNA probes in NOD outcrosses to identify previously unknown immunoregulatory genes of importance to the inheritance of IDDM. The creation of transgenic mice will allow the direct testing of molecular theories of IDDM *in vivo*. In the near future, we expect to be able to predict genetic susceptibility to IDDM with considerable certainty. In humans, future studies should simultaneously assess DQ $\beta$ , DQ $\alpha$ , and DR $\beta$  gene alleles in large numbers of subjects of diverse ethnic origin to further unravel the class II MHC relationships with IDDM. The

long-term goals of those studies would be to develop accurate estimates of susceptibility based on the entire HLA haplotype of an individual.

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**Additional references available upon request.**

## Assisted Reproductive Technologies and Preimplantation Genetics

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Abstracted by  
Robert M. Blizzard, MD

### Introduction

In his presentation of this topic at the Genentech National Cooperative Growth Study held in Fort Lauderdale, Florida, on November 10 - 13, 1990, Dr. Schulman discussed techniques of *in vitro* fertilization, the technology of

preimplantation genetics, and current ethical considerations for embryo implantation. He completed his discussion with some considerations for future research. The presentation was exceedingly informative and lucid. To the extent possible in a short abstract of the presentation, the Editorial Board wishes to share this information with the readers.

### In Vitro Fertilization

Historically, the first birth from *in vitro* fertilization (IVF), the

famous Louise Brown, occurred in 1978 in England as a result of the efforts of Drs. Patrick Steptoe and Robert Edwards. Today, over 5,000 births have occurred as a result of *in vitro* fertilization. The incidence of malformations has been similar to those observed in infants conceived *in vivo* and born to similar maternal age groups. The common indications for doing IVF are given in Table 1, page 7.

The methodology is geared to obtain multiple embryos in

**Table 1**  
Indications For In Vitro Fertilization

|                            |   |
|----------------------------|---|
| <b>Tubal Factors</b>       | Tubal obstruction (when surgery has failed)   |
| <b>Peritoneal Factors</b>  | Peritonitis encasing ovaries or reproductive organ with distortion of ovary and fallopian tubes |
| <b>Male Infertility</b>    | Mild to moderate sperm defect that requires treatment or separation of sperm                    |
| <b>Endometriosis</b>       | Patients failing to respond to usual surgical or pharmacologic treatment                        |
| <b>Ovarian Failure</b>     | Premature menopause; requires ovum donor  |
| <b>Immunologic Factors</b> | Patients with antisperm antibodies; requires special handling of sperm                          |

order to maximize success. Therefore, Pergonal®, a gonadotropin mixture from the urine of postmenopausal women, is frequently used to induce maturation and ovulation of multiple oocytes. The retrieval of ova by laparoscopy has been replaced by transvaginal ultrasound techniques. The advantages of this approach are its speed, reduced risks to the patient, lower costs, the ability to circumvent extensive pelvic disease, and the use of local rather than general anesthesia.

A needle, pushed about 1 to 2 cm through the top of the vagina, can readily enter the ovary, and ova are then aspirated. The process takes about 15 minutes and if there has been a good response to gonadotropins, an average of 5 to 7 eggs can be recovered. The eggs are transferred into special culture dishes containing a solution, highly controlled for pH ion concentration, that permits effective production of embryos from insemination with a tiny amount of washed sperm.

Sixteen or 18 hours post-fertilization, the male and female pronuclei become clearly visible within the zygote, providing conclusive evidence that fertilization has occurred. The zygote undergoes

cleavage and by 40 hours embryos of 2 cells are formed. These are then transferred into the uterus after 2 to 3 days when the morula reaches the 2- to 8-cell stage. More fully developed blastocytes can also be transferred.

A large proportion of the IVF pregnancies (~50%) in the United States occur in the 9 or 10 largest centers where 500 to 700 IVF cycles per year are performed. Unfortunately, only a small number of centers offer high-quality embryo freezing programs. This facilitates the establishment of pregnancy safely when many embryos are produced, and also can facilitate the diagnosis of normalcy of embryos subjected to preimplantation genetic testing.

Major determinants of IVF success include the woman's age; the response to ovarian stimulation, which depends upon the oocyte number and quality; the sperm quality; the fertilization rate or embryo number; and appropriate hormone levels. Maternal age has a marked effect on the efficiency of assisted reproductive technologies. A woman 25 years of age has a 25% chance of pregnancy with each IVF cycle. The woman who is 42 years old will have about a 5% pregnancy rate. In the

younger age group, 20% to 25% become pregnant with a simple embryo transfer, 30% to 40% with 2 transfers, 45% or more with 3, etc. There is also a significant difference in the miscarriage rates depending on age. The IVF miscarriage rate is probably similar to that in natural pregnancies. In women in their 20s, the miscarriage rate is about 10%; for those in their 30s, about 20% to 25%, and about 35% to 40% in women in their 40s. A woman in her 20s will have approximately a 23% live birth rate, while a woman in her 40s will have about a 3% rate.

### Implantation Genetics

Genetic considerations for the implanted embryo are the same as those in most pregnancies. However, amniocentesis, chorionic villus sampling (CVS), and intravaginal ultrasound studies are more frequently performed.

### Preimplantation Genetics

Embryo biopsy before implantation and analysis of oocytes and sperm before conception are the most recent developments in this rapidly advancing field. The major X-linked defects may be approached utilizing DNA-based techniques. A not remarkable scenario is one in which the embryos are sexed, and only female embryos are replaced into the uterus. This avoids the birth of a child with X-linked diseases such as Duchenne type muscular dystrophy, hemophilia, fragile X syndrome, etc.

Preimplantation testing is an exciting alternative to CVS. The early morula is biopsied, usually at the 8- to 16-cell stage. If necessary, the embryo is frozen to permit time for the genetic analysis. DNA from a single cell or a pair of cells is amplified utilizing PCR. However, this analysis is difficult, and it is uncertain whether or not single copy genes can be reliably amplified starting with 1 or 2 molecules of DNA. It is very important in these studies to avoid sperm contamination of the biopsied



material. Since 100,000 sperm are in the dish with 1 egg, this is difficult. Sperm DNA is extremely difficult to amplify because it is highly concentrated unless it is treated with dithiothreitol or certain other agents. It is recommended that female laboratory technicians preferentially perform these studies in order to reduce the risk of false results caused by contamination of Y chromosome material from male skin cells.

Fortunately, preimplantation testing is not limited to PCR-based techniques. It soon may be possible to perform biochemical analysis on individual blastomeres such as has been done in mouse embryos. It also may be possible to look at the possibilities for in situ hybridization or actual karyotyping of single blastomeres from embryos. In performing 1-cell biopsies, the embryo is held against the tip of a pipette, and a fine glass needle or acid Tyrode's solution is then used to make a defect in the zona

pellucida, and a blastomere is removed by gentle suction.

Currently, reported pregnancies following preimplantation testing are limited to the Hammersmith Hospital team in London. They have produced 5 or 6 pregnancies by this method. In all cases, these were families with a high risk for X-linked disorders of various types. The investigators did not do specific testing for Duchenne type muscular dystrophy or other diseases, but biopsied the embryos in the 6- to 8-cell stages and then did PCR analysis using probes for Y-specific repeat sequences. These studies are still very investigational.

The diseases that, hopefully, can be prevented by preimplantation genetics and that are currently under investigation are listed in Table 2. As mentioned previously, it may be possible to do enzyme assays on 1 or 2 cells from embryos. This has been possible in a mouse test system where the enzyme associated with Lesch-Nyhan syndrome has been successfully measured in individual blastomeres. It is important to be certain that one is measuring a true embryo enzyme level, since the oocyte has its own cytoplasmic enzyme. The first few divisions of a human embryo are reduction divisions and, unfortunately, new enzyme synthesis may not occur until about the 8-cell stage. This is in contrast to the mouse, where the embryo begins making its own enzyme around the 2-cell stage. Consequently, there is concern that biopsying and measuring enzyme levels in 6- or 8-cell human embryos may give incorrect conclusions about whether or not one has an affected embryo. However, it is certainly possible to do enzyme studies on later embryonic stages (late morulas or blastocysts), and this is something that will probably occur in the future. Embryos at the late morula or early blastocyst stage can be replaced into the uterus and a certain number of pregnancies will occur.

In situ hybridization involves using chromosome-specific probes that are usually fluorescent labeled, will hybridize on a slide to metaphase preparations, and can even be used to examine interphase nuclei. Bright spots occur, which presumably are representative of the region of the chromosome to which they are bound. A number of chromosome-specific probes are available now for in situ study. Such studies should open the door to embryonic chromosome analysis in certain high-risk situations.

Uterine lavage is another technique that has potential to alleviate some of the problems of preimplantation manipulation. Following insemination, a special catheter is placed into the uterus through the cervix; the uterus is then lavaged and embryos are recovered. The risks involved are embryo retention and failure to recover the embryo. Embryo retention can be dealt with pharmacologically while failure of recovery can hopefully be addressed with improved catheters. In this technique, one looks for recovery of blastocysts because the embryo does not enter the uterine cavity until just before the blastocyst stage — at 16 to 32 cells. Since blastocysts have many cells, they are good candidates for preimplantation diagnosis. However, one might not always get the information reflective of the genotype of the embryo inside if one biopsies the trophoblastic outside of a blastocyst.

#### Separation of X-Bearing and Y-Bearing Sperm

The separation of X-bearing and Y-bearing sperm is potentially possible. Theoretically, this technique has the advantage of permitting insemination with only X-bearing sperm, thus eliminating X-linked diseases. Techniques have been developed that use albumin or other gradients to separate the lighter from heavier sperm (Y from X), but it does not appear to be reliable. Recently,

**Table 2**  
Inherited Defects

#### Dominant Inherited Conditions

- Huntington's disease
- Neurofibromatosis
- Marfan syndrome
- Achondroplasia
- Charcot-Marie-Tooth disease
- Familial hyperthermia
- Numerous sublethal diseases

#### Recessive Inherited Conditions

- Cystic fibrosis
- Sickle cell anemia
- Thalassemias ( $\alpha$ ,  $\beta$ )
- Tay-Sachs disease
- Gaucher's disease
- Familial spinal muscular atrophy (Werdnig-Hoffman paralysis)
- $\alpha_1$  antitrypsin deficiency
- Numerous other lethal conditions

workers at the United States Department of Agriculture (USDA) in Beltsville, Maryland, have developed an approach where sperm are exposed to a DNA dye and then fluorescence-activated sorting is used. The instrument to separate the sperm can be adjusted in such a way that the Y sperm, which have about 3% less DNA than the X-bearing sperm, may be sorted with some degree of reliability. With as much as 5% or 6% difference in DNA content of the X and Y sperm, extremely reliable separation of the X and Y embryos has been done in nonhuman species. Studies are being conducted in collaboration with the investigators in Beltsville to see whether this technique can be accomplished with human sperm. Unfortunately, human sperm and those of some of the higher primates tend to be polymorphic, which makes the technique more difficult to implement. However, it should be possible in the future to accomplish this, and this will be a very powerful approach to eliminating X-linked disorders.

### **Preconception Analysis of Oocytes**

Preconceptive testing of oocytes would obviously cause cell death. However, biopsy of the polar body, which is presumably the genetic mirror image of what is left in the egg, is feasible. For example, if the DNA of the polar body had the cystic fibrosis gene, then one could say by implication that the egg itself would not. Then the egg could be inseminated, and, theoretically, the embryos resulting would be free of cystic fibrosis. While this is technically feasible, meiotic recombination reduces the number of eggs that are useful for fertilization so that actually only about 1 egg in 4 will be appropriate to use. Because the polar body biopsy is only one third as efficient as direct embryo biopsy, this technique will probably have only very special applications, for example, in rare couples willing to undergo highly artificial

technologies to avoid a conceptus with a genetic disease. Parenthetically, polar body biopsy is useless for determining the sex since all the polar bodies are female.

### **Comparison of Preimplantation Testing to CVS**

Preimplantation testing associated with IVF is costly, approximately \$10,000 with a 20% to 25% chance of pregnancy resulting in the young healthy couple. With CVS, there is 100% chance of pregnancy because pregnancy has already been achieved, and CVS, including the analytical methods, costs only 10% to 15% as much. Consequently, preimplantation testing is going to be limited to a few specialized centers and carefully selected patients.

### **Ethical Considerations**

Preimplantation testing obviates abortion, which may be ethically more acceptable to some potential parents. It is possible that when couples are at high risk of having a child affected with disorders like cystic fibrosis, sickle cell anemia, and fragile X syndrome, which are not rapidly lethal, the family will prefer preimplantation testing to CVS.

### **Research for the Future**

All of the preimplantation genetic procedures discussed here are currently research techniques. They are very new and will not be generally available for some time. The technique used to simply sex embryos by PCR is the only fairly reliable procedure at present.

PCR in single cells is technically demanding and of very limited success, especially with single copy genes. Use of a multicopy Y chromosome-specific probe is usually successful, since it is much easier to start out with 100 or so of the gene to conduct a successful analysis by PCR than to start with 1 or 2.

The development of enzyme assays to be utilized during later embryonic stages is

receiving significant attention. Much more accurate data than that currently obtained are needed.

A technique that still has to be taken beyond the thinking stage is the recovery of fetal cells from maternal blood. The development of reliable methods for recovering fetal cells among large numbers of circulating maternal cells would open the way to noninvasive testing of the fetus.

### **Question-and-Answer Period**

**Q:** Please discuss briefly ovum donation, fertilization, and implantation in patients with Turner syndrome.

**A:** That is now standard technology. It is very easy to obtain donor oocytes using nonsurgical techniques. Coordination is done of the cycles between the recipient and the donor either by exogenous hormonal control, natural synchronization, or freezing and thawing of the embryos. A number of pregnancies have been effected in this manner in patients with Turner syndrome.

**Q:** How long can one maintain an egg in the frozen state?

**A:** There is a big difference between freezing eggs and freezing embryos. Embryos can be frozen for many years, probably at least 5 or 10. Egg freezing is much less effective, much less reliable, and there have only been 3 or 4 pregnancies around the world from eggs that have been frozen. Our egg freezing attempts and those of most others have been disappointing, even though we have achieved many pregnancies with frozen embryos. There is some characteristic about the egg that makes it less suitable for freezing.

### **Special Announcement**

**See Page 2 for ordering back issues of GGH, Volumes 1 through 6, Numbers 1 to 4.**

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### **Rickets and Growth**

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by Barbara MacGillivray, MD

### **Fetal Growth Factors**

by Joseph D'Ercole, MD

### **Growth Hormone Resistant Syndromes**

by William Daughaday, MD

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by Alan Rogol, MD, PhD

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## Abstracts From the Literature

### **Human Growth Hormone Prevents the Protein Catabolic Side Effects of Prednisone in Humans**

Four groups of normal adult males ( $n = 8$ , each group) were studied for the effects of glucocorticoid therapy on protein catabolism, using traditional  $N_2$ -balance and isotope dilution techniques. Administration of either prednisone (0.8 mg/kg/d), or recombinant human growth hormone (rhGH; 0.1 mg/kg/d), or both were studied for 7 days and compared with a control group.

Prednisone induced protein wasting, as determined by both methods, whereas rhGH alone resulted in positive protein balance, as compared with controls. Prednisone produced

proteolysis, whereas rhGH increased whole body protein synthesis. rhGH plus prednisone at the doses used yielded results that were identical to the controls, implying that protein catabolic effects of glucocorticoids were prevented by the concomitant administration of rhGH.

Carbohydrate intolerance was observed only with the combined therapy, although hyperinsulinemia was noted in those receiving rhGH alone or prednisone alone.

The authors conclude that rhGH may have a distinct role in preventing the protein losses associated with administration

of pharmacologic doses of glucocorticoids in humans. However, several potential drawbacks may exist to combined rhGH and prednisone therapy: (1) carbohydrate intolerance evolves within 8 days; (2) rhGH increases glomerular filtration rates, and recent attention has been drawn to the progression of renal failure associated with glomerular hyperfiltration; and (3) acromegaly may occur with long-term administration of rhGH. The authors caution that further studies using smaller pharmacologic doses are indicated before any attempts at therapy are made.

Horber FF, Marsh HM, Haymond MW.  
*J Clin Invest* 1990;86:265.

**Editor's comment:** This study will be quoted and referred to as a classic study for many years. The authors identify the physiologic effects of prednisone and/or rhGH on protein, fat, and carbohydrate metabolism, and show how the concomitant

administration of rhGH can counter the effect of prednisone on protein metabolism and multiply the effects of both on carbohydrate intolerance. Every reader who has any interest in endocrinology, metabolism, or nutrition should read this lengthy but superb article.

Robert M. Blizzard, MD

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## Differential Effects of Prednisone and Growth Hormone on Fuel Metabolism and Insulin Antagonism in Humans

Horber et al have previously reported (*J Clin Invest* 1990; 86:265) that recombinant human growth hormone (rhGH) therapy may have a role in preventing the protein losses associated with the administration of glucocorticoids in humans. This study is also reviewed in this issue of *GGH*. The present paper reports additional data regarding fat and carbohydrate metabolism obtained during the original study. Glucose and fat oxidation were determined utilizing isotopic dilution studies and indirect calorimetry. Four groups of normal adult males (N=8, each group) treated with (1) prednisone alone (0.8 mg/kg/d); (2) rhGH alone (0.1 mg/kg/d); (3) prednisone and rhGH; or (4) placebo were studied.

Fasting plasma glucose concentrations increased in the groups treated with prednisone and prednisone plus rhGH but fasting plasma insulin levels were higher only during combined treatment. Protein oxidation was decreased in the postabsorptive state in subjects receiving rhGH alone and increased in subjects receiving prednisone alone, but there was no difference in protein oxidation observed between the placebo-treated subjects and subjects treated with combined prednisone and rhGH. However, fasting fat oxidation was decreased in subjects treated with prednisone but not significantly increased in subjects treated with rhGH alone. The ratio of protein to fat

oxidation was increased in subjects on prednisone alone, decreased in subjects treated with rhGH alone, and unchanged in subjects given the combined treatment as compared with controls. No differences in carbohydrate oxidation were observed among the different groups. The prednisone-treated subjects oxidized more protein but less fat than the controls, whereas the subjects treated with rhGH alone oxidized more fat but less protein than the controls. Subjects treated with both rhGH and prednisone oxidized more fat and less protein than controls.

The authors state that this study suggests that rhGH and prednisone induce insulin antagonism by independent mechanisms. Their conclusions are based on the observations that the increases in concentrations of glucose, insulin, and C peptide with combined rhGH and prednisone were synergistic. Secondly, prednisone alone decreased the plasma concentrations of free fatty acids and ketone bodies in the postabsorptive state but decreased fat oxidation and increased protein oxidation and plasma lactate and pyruvate in both the fed and fasted states. In contrast, therapy with rhGH alone increased fat oxidation, decreased protein oxidation, and had no effects on plasma concentrations of free fatty acids, ketone bodies, lactate, and pyruvate. Finally, although the combined treatment normalized protein and fat oxidation, the

plasma concentrations of free fatty acids, lactate, and pyruvate remained elevated in the fed state. The authors further suggest that the mechanism for carbohydrate intolerance in subjects treated with prednisone alone appears to be a decrease in glucose clearance possibly related to a post-receptor defect. The mechanism for the inverse relationship between fatty acids and protein oxidation observed in this study remains unclear, but may be a result of reciprocal effects of the 2 drugs on enzymes that regulate the mobilization and oxidation of fatty acids and amino acids.

Horber F, Marsh H, Haymond M.  
*Diabetes* 1991;40:141-149.

**Editor's comment:** This is an extremely detailed study that extends a previous report on the effects of rhGH and/or prednisone on protein homeostasis in normal adults. The authors have presented data suggesting that the insulin antagonism of rhGH and prednisone is probably caused by independent mechanisms, since it would appear that rhGH and prednisone reciprocally regulate the oxidation of protein and fat while decreasing the efficiency of glucose disposal. This paper along with the previously reported article should be read and studied together.

William L. Clarke, MD



## Growth Rate Reduction During Energy Restriction in Obese Adolescents

Amador et al studied the effects of energy restriction on growth and sexual development in a group of obese children who were participating in a multidisciplinary weight-loss program. Ninety-four children whose relative fat weight was determined to be above 25% but less than 40% in males and above 30% but less than 45% in females were studied. These children, aged 10.6 to 12.9 years, were all in Tanner stage II puberty and their body weight for stature was above the 97th percentile. The children were randomly classified into 2 groups: a control group in which energy intake was maintained (0.25 mJ/kg of expected body weight for height) and an experimental group in which energy intake was restricted to 30% of energy requirements (0.17 mJ/kg of expected body weight for height). All children participated in a program of physical activity, nutritional education, and behavioral modification. All subjects were measured and examined for height and stage of sexual development at the end of 6 months and again at 6 months

following the intervention. Seventy-eight children completed the 1-year study.

No differences between the 2 groups were found with respect to Tanner stage, body weight, lean body weight, or fat body weight at the initiation of the study. However, after 6 months of therapy, puberty progressed at a significantly slower rate in the group with the lower energy intake. In addition, there was a significantly greater reduction in body weight in this group, with significantly greater loss of fat body weight than in the control group. The height velocity was also significantly slower in the energy-restricted group. During the subsequent 6 months, catch-up growth was evident in the energy-restricted group, but pubertal development continued to lag behind the group with less restriction of energy intake.

The authors suggest that the restriction of energy intake in early adolescence should be avoided in the dietary management of overweight early adolescent children. They suggest that a nonrestrictive diet with the addition of physical activities, nutritional education,

and behavioral modification is a more appropriate method for achieving weight loss in this group.

Amador M, Ramos L, Morono M, et al. *Exp Clin Endocrinol* 1990;96:73-82.

**Editor's comment:** This very interesting paper demonstrates once again the need for monitoring linear growth during weight-reduction therapy in children. Dietz et al (*AJCD* 1985;139:75) demonstrated previously that diets with even a mild restriction of energy may be associated with a reduction in height velocity. The present study confirms this finding and, in addition, demonstrates a reduction in the tempo of pubertal development in children whose energy intake is restricted. They have demonstrated that a multidisciplinary program of exercise, education, and behavioral modification is exceedingly important to weight reduction programs in children.

William L. Clarke, MD

## Identification of the 64K Autoantigen in IDDM as Glutamic Acid Decarboxylase (GAD)

An antigen previously found only in the beta cells of the islet cells here is reported also to be present in certain neurons that secrete gamma-aminobutyric acid (GABA) in the central nervous system (CNS). This antigen is identified as glutamic acid decarboxylase (GAD), the biosynthesizing enzyme of the inhibitory neurotransmitter GABA.

Individuals with stiff-man syndrome (SMS) frequently have associated insulin-dependent diabetes mellitus (IDDM). Individuals with SMS have autoantibodies to 64K antigen at much greater titers than do most patients with IDDM. The authors found that the GAD antibodies in SMS were 10 to 200 times higher than those found usually

in IDDM patients. A reference by Solimena et al in the *New England Journal of Medicine* in 1990 is quoted.

The authors used acceptable immunologic and microbiologic techniques to demonstrate that the 64K antigen in the beta cells and in the GABA-secreting neuron cells is the same. This finding is expected to motivate the creation of studies to elucidate the pathogenesis of IDDM and SMS, and to determine the mechanisms of generation of self-tolerance by the immune system and its failures.

The authors suggest there are components other than the GAD antibodies responsible for these diseases. For example, beta cells express major histocompatibility complex class I

molecules whereas CNS neurons normally do not.

Baekkeskov S, Aanstoot HJ, Cristgau S, et al. *Nature* 1990;347:151-156.

**Editor's comment:** The pancreatic 64K beta-cell autoantigen is a major target of autoantibodies associated with IDDM. The finding that this antigen is identical to that found in GABA-secreting neurons is a significant contribution. The details as presented in the abstract above are convincing. Those readers who are the least bit interested in the possible role of autoimmunity as a cause of IDDM can benefit by reading the entire article.

Robert M. Blizzard, MD

## Transplacental Passage of Insulin in Pregnant Women With Insulin-Dependent Diabetes Mellitus: Its Role in Fetal Macrosomia

Menon et al demonstrated that there is a cause other than the placental transfer of glucose from mother to fetus in the pregnant diabetic to account for the macrosomia that is frequently seen in the offspring. Specifically, placental transfer from the mother to fetus of animal insulin antibodies, which carry with them insulin, may account for some cases of macrosomia seen in the offspring of diabetic women.

The authors demonstrated a direct correlation between the insulin levels in umbilical cord blood and macrosomia. The 12 infants reported with macrosomia had significantly higher cord-serum concentrations of animal, human, and total insulin than did those infants who did not have macrosomia.

There were also significant correlations between birth weight and cord-serum concentrations of animal, human, and total insulin. There was no significant difference between the infants with macrosomia and those without in cord-serum insulin antibody

|                       | Insulin in Cord-Serum* |             |             |
|-----------------------|------------------------|-------------|-------------|
|                       | Animal                 | Human       | Total       |
| Macrosomic infants    | 1,113 ± 32             | 2,726 ± 599 | 3,839 ± 840 |
| Nonmacrosomic infants | 402 ± 110              | 908 ± 163   | 1,309 ± 259 |
| P                     | <0.05                  | <0.02       | <0.02       |
| *pmol/L               |                        |             |             |

activity, years of maternal diabetes before conception, maternal insulin dosage, maternal HbA<sub>1c</sub>, incidence of respiratory distress syndrome, or low blood glucose concentration in the infant during the first 4 hours of life.

Menon RK, Cohen RM, Sperling MA, et al. *N Engl J Med* 1990;323:309.

**Editor's comment:** Previously, the teaching has been that insulin does not pass from the mother to the fetus. The authors have shown the fallacy of that teaching. When antibodies to insulin are present, insulin crosses the placenta. The data implicate transplacental insulin as one of the accountable causes for macrosomia in infants of

diabetic mothers. This is an important contribution to our understanding of fetal growth in the offspring of diabetic women. However, Dr. Robert Schwartz of Brown University commented in an editorial in the same issue of the New England Journal of Medicine that, usually, hyperinsulinemia is primarily fetal in origin in the fetus of the diabetic woman. Dr. Schwartz does not discount the role of insulin transferred via antibodies from mother to fetus in the production of macrosomia but does not readily accept it as the major mechanism in the production of macrosomia.

Robert M. Blizzard, MD

## Treatment of Short Stature in Renal Disease With Recombinant Human Growth Hormone

Rees et al report on their experience with the use of recombinant human growth hormone (rhGH) in 18 children with renal disease. These children were selected for study because they had the lowest height standard deviation scores (SDS) among children attending the chronic renal failure clinic and were failing to show catch-up growth despite theoretically optimum medical management. Three groups of 6 children each were studied: group 1 — prepubertal children with chronic renal failure (CRF); group 2 — prepubertal children with renal transplants; and group 3 — pubertal patients with renal transplants. All patients had attended the clinic for at least 18 months. Their height SDS and/or height velocities were >2 SD below the mean. All patients with transplants were receiving alternate-day prednisolone therapy. Height,

weight, and pubertal status were assessed every 3 months. Bone age (BA) was assessed at the beginning of the study and after 12 months. Blood samples were evaluated every 3 months for electrolytes, BUN, creatinine, calcium, phosphate, bilirubin, albumin, hemoglobin, glucose, and glycosylated hemoglobin. Glomerular filtration rate was calculated at each visit. GH pulsatility was assessed by blood sampling (q15min) from 2000 to 0700 hours (8 PM to 7 AM) in all patients. Insulin-like growth factor I (IGF-I) levels, thyroid function, and parathyroid hormone concentrations were determined at the beginning and end of the study.

Each patient received rhGH at 30 U/M<sup>2</sup> per week (divided into daily doses) for up to 1 year (a median of 0.98 years [range, 0.25 to 0.99]). Two patients stopped

GH therapy after 3 months (1 due to patient fear of the hypodermic needles and 1 due to noncompliance), and were not included in the analysis. As shown in Table 1 (page 14), mean height SDS increased significantly in group 1 and mean height velocity SDS increased significantly in all groups. BA, which was delayed in all subjects prior to treatment, remained delayed, and changes in BA and height SDS/BA were not significant. There were no correlations between the response to treatment and the severity of the CRF in group 1, or between the dosage of prednisolone and the growth responses in groups 2 and 3. In addition, there were no correlations between GH pulsatility and growth response in any groups since GH pulsatility was normal in all children prior to rhGH. Initial IGF-I levels were below the reference range for the prepubertal

**Table 1**  
Treatment of Short Stature With Recombinant  
Human Growth Hormone

|                          | <b>Group 1</b><br>Prepubertal<br>With Chronic<br>Renal Failure<br>(n=6) | <b>Group 2</b><br>Prepubertal<br>With Renal<br>Transplants<br>(n=6) | <b>Group 3</b><br>Pubertal<br>With Renal<br>Transplants<br>(n=6) |
|--------------------------|---|---|--|
| <b>Prior to rhGH Rx</b>  |   |   |  |
| Mean age (years)         | 7.7   | 12.1  | 15.6   |
| Mean height SDS          | -2.9  | -3.3  | -3.4   |
| Mean height velocity SDS | -1.3  | -2.0  | -1.0   |
| Mean BA delay (years)    | -2.1  | -2.5  | -2.7   |
| <b>End of rhGH Rx</b>    |   |   |  |
| Mean height SDS          | -2.1*   | -3.1  | -3.2   |
| Mean height velocity SDS | 6.0*  | 0.6*  | 3.5*   |
| Mean BA delay (years)    | -1.6  | -2.5  | -2.9   |

\*  $P$  at least  $< 0.05$  compared with scores prior to rhGH Rx.  
SDS, Standard deviation score; BA, Bone age.

children (group 1), but were within the reference range in groups 2 and 3. No significant changes in renal function were noted in any group. There was a significant increase in the mean glycosylated hemoglobin level during treatment; however, this increase did not result in values outside the normal range.

The authors point out that catch-up growth can occur in some children with CRF with correction of fluid, electrolytes, and acid-base balance, attention to energy and protein intake, and prevention or treatment of renal osteodystrophy. However, many children do not

experience catch-up growth. With careful renal management, growth velocity may return to a normal rate, but catch-up growth is rare in the child over 2 years of age.

After the age of 2 years, normal growth rate is usual, but catch-up growth is rare. Catch-up growth can occur in prepubertal children after transplantation, although corticosteroids can interfere with the onset and progression of puberty and the pubertal growth spurt. Thus, the rates of growth observed by the authors far exceeded those previously achieved in their clinic by other means.

Rees L, Rigden S, Ward G, et al.  
*Arch Dis Child* 1990;65:856-860.

**Editor's comment:** This paper presents data that can be added to the growing body of information concerning the effects of GH therapy in children with chronic renal disease (see also GGH Vol. 4, No. 3). As more data accumulates, it becomes clear that GH administration may be of clinical usefulness in some of these children. The failure of rhGH to increase height SDS in patients with renal transplants may be attributable to the effects of prednisolone or to the older ages (>12 years) of the children in these groups at the initiation of rhGH therapy. It is unfortunate that the authors were unable to match their patients to a control group. They have stated that their reason for not doing so was the number of variables involved (diagnosis, age of onset, severity of renal failure, etc). Thus, it would appear that a large-scale matched trial is needed before one can document the usefulness of GH therapy in CRF. In addition, such a trial may identify any adverse effects that might occur and the relative frequency of occurrence associated with rhGH therapy in chronic renal disease (ie, renal function deterioration, hypercalciuria, enhanced immune function, abnormal carbohydrate metabolism, mitogenic activity).

William L. Clarke, MD

## Pubertal Growth in Chronic Renal Failure

This paper analyzes the height growth of 15 boys and 14 girls with end-stage renal failure first studied before puberty and followed at 3- to 6-month intervals until growth ceased or nearly ceased. The height data were smoothed by the kernel estimation method, which is a form of moving average. The records were from Heidelberg, and the curves were compared with those from the Zurich Longitudinal Growth Study. This made possible a comparison with late normal maturers as well as with the average maturers in a normal growth study.

The start of the pubertal growth spurt was delayed by 2.5 years in both the girls and boys, and its duration and intensity were also very significantly reduced, with the mean height gain at around 50% of that observed in the late-maturing control group. However, mean height at the onset of the spurt was approximately the same as that in the late-maturing control group. The data indicate that most patients with end-stage renal failure occurring before or during puberty irreversibly lose growth potential. Renal trans-

plantation did not consistently improve pubertal growth.

Schaefer F, Seidel C, Binding A, et al. *Pediatr Res* 1990;28:5.

**Editor's comment:** This paper is particularly striking because of the use of the kernel estimation method, which, in my opinion, is currently the most advanced technique for analyzing growth curves. Since it is nonparametric, it is particularly applicable in cases of growth disorder, and this paper constitutes a real model for

other research workers studying growth in chronic disease. It is interesting that in the patients with renal failure, puberty did not start until their height had reached virtually that of the controls when they started puberty; however, by this time height velocity was far below normal and the subsequent pubertal spurt was very much

reduced. Such a fine analysis does require many measurements of height to be made during the growth period but results in a much better understanding of the dynamics associated with the disorder than has previously been possible.

James M. Tanner, MD

## Hypersomatotropism in the Dysmature Infant at Term and at Preterm Birth

de Zegher et al report umbilical cord-serum growth hormone (GH) levels in a large group of small (<2.4 kg), appropriate (3.4 ± 0.1 kg), and large (>4.4 kg) infants born at term and in the cord-serum of prematurely born twins (28 to 36 weeks) in which both twins were appropriate for gestational age or 1 twin was appropriate and the other small for gestational age. The results demonstrate that appropriate and large infants have similar cord-serum GH concentrations (16.7 ± 1.0 ng/mL versus 16.5 ± 1.2 ng/mL respectively), but small infants have significantly elevated cord-serum

GH levels (24.2 ± 1.8 ng/mL) when compared with either of the other 2 groups ( $P < 0.001$ ). Serum GH concentrations in twins concordant for weight and appropriate for gestational age were similar, while GH levels were significantly higher ( $P = 0.007$ ) in the smaller of twins discordant for weight.

The authors point out that the mechanisms underlying the elevations in cord-serum GH levels at birth are unclear, but that increased cord-serum levels of GH have been documented in both the human and ovine fetus when acidotic or hypoxic, in fetuses of undernourished ewes, in ovine

fetuses undergoing surgery, and in ovine fetuses in conditions associated with growth retardation. Although GH is not known to influence fetal circulating insulin-like growth factor (IGF)-1 levels and is not essential for fetal growth, the authors state that the elevations in GH in the small-for-gestational age infant are likely to be related to insulin antagonizing actions or lipid metabolism. These data support the hypothesis that GH plays a homeostatic role in the late-gestational fetus in particular, and possibly in the metabolic adaptations to conditions associated with subnormal intrauterine growth.

de Zegher F, Kimpfen J, Raus J, et al. *Biol Neonate* 1990;58:188-191.

**Editor's comments:** This is a well-conducted study with appropriate controls that suggests that GH, although not necessarily involved in stimulating fetal growth, may play a very important role during the last trimester of pregnancy. Obviously, more research is needed to establish the significance of these findings.

William L. Clarke, MD

## 30-Second Sampling of Plasma GH in Man: Correlation With Sleep Stages

The authors used a refined technique to draw 2 drops of blood every 30 seconds over an 8-hour period in 6 young male adults, following 24 hours of fasting. Growth hormone (GH) was measured on each sample. The accuracy was verified by comparing the GH concentrations in plasma and in whole blood. EEG recordings were used to correlate GH pulsatility with stages of sleep. GH pulses were analyzed by cluster analysis; GH secretion rates were determined by deconvolution analysis. Data analysis revealed the nocturnal pulse frequency to be 1.2 pulses per hour. If analysis had been done on blood samples drawn every 20 minutes, the number of identifiable peaks would have been 61% less, or 0.5 pulses per hour. Mean GH concentrations

and secretory rates were significantly higher during stages 3 and 4 of sleep as compared with stages 1 and 2 and REM sleep. There was a close correlation of EEG-identifiable sleep and initiation of the GH secretory peaks (4.5 minute time delay). The authors suggest that normally there are major episodes of GH release (secretory episodes) that consist of multiple small pulses within each major episode.

Holl RW, Hartman ML, Veldhuis JD, et al. *J Clin Endocrinol Metab* 1991;72:854-861.

**Editor's comment:** Working with these authors at the University of Virginia through the years has been both a pleasure and an enlightening experience. The method described for measuring GH in 2 drops of

whole blood is remarkable — and it works! It is a research, not a diagnostic, tool. From the data, we can conclude that the number of GH pulses increases phenomenally based on the frequency with which the investigator analyzes GH. The reader needs to realize, however (as pointed out by Evans et al *Am J Physiol* 1987;252:E459-E556), there are major secretory episodes, each comprised of multiple pulses. Sampling every 20 minutes permits identification of the majority of GH secretory episodes, but not pulses. For many physiologic and diagnostic studies, sampling blood and measuring GH in blood drawn every 20 minutes over 12 to 24 hours is adequate.

Robert M. Blizzard, MD



## Meeting Calendar

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**August 25-29, 1991** 30th Annual Meeting of the ESPE, Berlin, Federal Republic of Germany. Information: Dr. V. Hesse, ESPE Meeting 1991, Children's Hospital, Lindenhof, Goltlindenstrasse 2-20, 1130 Berlin, FRG.

**September 15-19, 1991** 6th International Congress of Auxology, Madrid, Spain. Scientific Information: Dr. M. Hernandez, Univ Autonoma de Madrid, Dept de Pediatria, Hospital del Nino Jesus, Ayda Menendez Pelaya 65, 28009 Madrid, Spain. Fax: 34-1-574-4669. General Information: Compania Hispanoamericana de Turismo, Edificio Espana, Gran Via 88, 28013 Madrid, Spain. Tel: 34-1-247-5717. Fax: 341-541-2037.

**September 23-27, 1991** Kabi Advanced Postgraduate Course on Growth and Growth Disorders, Stockholm, Sweden. Deadline for registration is July 1, 1991. Scientific information: Dr. P. Wilton. General information: Dr. S. Dahlskold/Dr. S. Renstad, Kabi Vitrum Peptide Hormones, S-11287 Stockholm, Sweden. Tel: 46-8-138-000. Tlx: 16338 Kupert S. Fax: 47-8-618-2019.

**October 6-11, 1991** 8th International Congress of Human Genetics, Washington, DC. Information: M. Ryan, ICHG, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1825. Fax: 301-530-7079.

**October 20-25, 1991** 8th International Beilinson Symposium on Prediabetes, Jerusalem, Israel. Scientific Information: Prof Z. Laron, Institute of Pediatric and Adolescent Endocrinology, Beilinson Medical Center, Petah Tikva 49100, Israel. Tel: 972-3-9225108. Fax: 972-3-9229685. Information: Kenes, PO Box 50006, Tel Aviv 61500, Israel. Tel: 972-3-654571. Fax: 972-3-655674.

**June 18-23, 1992** 52nd Annual Meeting of the ADA, San Antonio, TX. Information: Meetings Department, ADA, 1660 Duke Street, Alexandria, VA 22314 USA. Tel: 703-549-1500, ext 330. Fax: 703-836-7439.

**June 24-27, 1992** 74th Annual Meeting of The Endocrine Society, San Antonio, TX. Information: Ann Singer, Meetings Manager, The

Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1802. Fax: 301-571-1869.

**August 30 - September 5, 1992** 9th International Congress of Endocrinology, Nice, France. Information: N.I.C.E. 92, c/o SOCFI, 14 rue Mandar, 75002 Paris, France.

**September 7-10, 1992** 31st Annual Meeting of the ESPE, Zaragoza, Spain. Information: Dr. A. Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hospital, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

**June 3-7, 1993** 4th Joint Meeting of the ESPE/LWPES, San Francisco, CA, USA. Information: Prof M. Grumbach, Dept of Pediatrics, Univ of California School of Medicine, San Francisco, CA 94143 USA. Tel: 415-476-2244. Fax: 415-476-4009.

**June 9-12, 1993** 75th Annual Meeting of The Endocrine Society, Las Vegas, NV, USA. Information: Scott Hunt, Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1802. Fax: 301-571-1869.

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# GROWTH

## Genetics & Hormones

Vol. 7 No. 3

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AN UPDATE:

## Growth Hormone Physiology and Pathophysiology

Alan D. Rogol, MD, PhD

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Robert M. Blizzard, MD

This subject was presented in the premiere issue of *GROWTH, Genetics, & Hormones (GGH)* in 1985. Since then, much new information has become available concerning factors that stimulate the synthesis and release of growth hormone (GH), GH transport in serum by GH binding proteins (GHBP), the structure of GH and related receptors, insulin-like growth factors (IGF-1 and IGF-2), IGF-binding proteins (IGFBPs), and the actions of GH. Placental GH (GH-V) has been identified in 2 distinct forms and preliminary information concerning its structure and action has been identified. The current presentation emphasizes information obtained regarding these phenomena since the previous review was written. Readers are encouraged to review the previous article (*GGH* 1985;1:1) to supplement the information presented here.

### GH Synthesis and Release

As known for some time, GH synthesis by and its release from the pituitary somatotrope are under the regulation of GH-releasing hormone (GHRH) and somatostatin, or somatotropin release-inhibiting hormone

(SRIH).<sup>1,2</sup> A pulse of GH is generated by the simultaneous rise of GHRH and decline in SRIH. The amount of GHRH released is believed to determine the amplitude of the GH peak, and the frequency and duration of the GH secretory event is primarily under SRIH control.<sup>1,2</sup> These rhythmic patterns may be intrinsic to the hypothalamus or may be under control of higher neural oscillatory mechanisms.

The hypothalamic hormones, GHRH and SRIH, are regulated by neuromodulatory biogenic monoamines and other neuropeptides (eg, galanin), although some of these factors such as dopamine, norepinephrine, and epinephrine may also act directly on the somatotrope. Cholinergic agonists and cholinesterase inhibitors generally enhance GH secretion, particularly in response to GHRH and other stimuli, while cholinergic antagonists inhibit GH secretion by GHRH and other stimuli. These data suggest that somatostatin secretion is primarily regulated by cholinergic mechanisms: cholinergic antago-

nists stimulate SRIH production or action and agonists inhibit SRIH production or action.

Alpha-adrenergic agonists stimulate GH release via GHRH and beta-adrenergic agonists inhibit GH secretion in vivo, presumably through increased release of SRIH. In contrast, atenolol, a beta-adrenergic blocking agent, enhances GH release when pharmacologic stimuli for GH release are given. Dopamine agonists both stimulate and inhibit GH release depending upon specific conditions. For example, L-dopa (which crosses the blood-brain barrier) will cause the release of GH in normal individuals, but may inhibit GH release in acromegalic patients.

These in vivo effects may relate to conversion of dopamine to norepinephrine, since dopamine inhibits GH release following arginine administration or the induction of hypoglycemia in some acromegalic patients. This is a logical assumption since norepinephrine blocks the release of GH to arginine or insulin-induced hypoglycemia.

### In This Issue

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Serotonin and its precursors, tryptophan and 5-hydroxytryptophan, also induce the release of GH *in vivo*, but the mechanism is uncertain.

Since 1985, 2 other stimuli have been described. A GH-releasing peptide (GHRP), a hexapeptide (his-D-trp-ala-trp-D-phe-lys-NH<sub>2</sub>),<sup>3,4</sup> has been developed synthetically but is not homologous to the GHRH-related peptides of 40 or 44 amino acids. Its activity in humans is not diminished by SRIH and its ability to cause the release of GH when submaximal amounts of GHRH are given is at least additive and possibly synergistic. The role of this peptide or its native homologue, if such exists, has not yet been defined.

Galanin<sup>5,6</sup> is a naturally occurring neuropeptide present in considerable amounts in the median eminence of the hypothalamus. It causes GH release under appropriate conditions; however, its physiologic role is poorly understood. In humans, the administration of porcine galanin is followed by GH release, and it enhances by 3-fold the GH release in response to GHRH.<sup>5</sup> Its effect is thought to be indirectly mediated by epinephrine acting on GHRH neurons. Galanin is considered to control pulsatile GH secretion by decreasing somatostatin inhibitory tone.<sup>6,7</sup> Neither GHRP nor galanin appears at this time to be as important as GHRH in producing GH release, although the presence of galanin in the median eminence suggests an innate biologic function.

Other aspects of GH secretion that have been clarified in the past 6 years include the effects of estrogen and testosterone. At even very low doses of gonadal steroid hormones, the quantity of GH secreted over a 24-hour period is increased by 2 to 3 times the amount secreted in their absence. These increases occur as a result of an increase in the pulse amplitude of the GH secretory episodes and not by

increasing the frequency of peaks (approximately 7 or 8 per day) in both the prepubertal and pubertal periods.<sup>8</sup> Also included in new information is the observation reported recently by Martha and coworkers<sup>9</sup> and Veldhuis and colleagues<sup>10</sup> that GH secretion is inversely related to body mass index (wt/ht<sup>3</sup>). This observation may provide one explanation for extremes of GH secretion among normal individuals who, except for differences in body mass index, are indistinguishable.

### GH Transport in Serum

GH is transported in serum attached to binding proteins, the major one of which is closely related to the GH receptor.<sup>11</sup> This binding protein is identical to the extracellular domain of the receptor, and appears to be generated either by alternative splicing of the receptor mRNA with direct extrusion of the protein into serum or by a trypsin-like cleavage of the extracellular component of the receptor *in vivo*. Approximately 45% of circulating GH is bound to this high affinity protein, and 5% is bound to a low affinity GHBP of 100 kd molecular weight. Approximately 50% of the GH remains unbound, or "free." Circulating levels of GHBP are low in the fetus and newborn, but increase over time, especially during the pubertal growth spurt. GHBP<sup>11</sup> and the GH receptors<sup>12</sup> are absent as a result of partial gene deletion<sup>13</sup> in children with GH insensitivity (Laron type dwarfism) who have a GHD-like phenotype and fail to respond to GH administration. The exact roles of these binding proteins are unknown, but they may decrease the rate of degradation of GH or modify the availability of GH in some other way.

### GH Receptor and Related Receptors

The GH receptor is a member of a family of 3 related straight-chain polypeptides with specificity

toward GH (somatogenic), prolactin (lactogenic), and chorionic somatomammotropin or placental lactogen. The clinical significance of the ability of GH to affect the prolactin receptor and prolactin to act at the somatogenic receptor is not known. Some patients with acromegaly (approximately 25% to 50%) will have mild hyperprolactinemia, but only a small percentage will have galactorrhea. Whether this biologic effect is due to GH acting at the lactogenic receptor, due to prolactin itself, or due to some other mechanism has not yet been determined. Other members of the superfamily include receptors for some of the interleukins, granulocyte-macrophage colony-stimulating factor, and erythropoietin. All are single-chain glycoproteins with homology in a 210 amino-acid sequence with the extracellular, ligand-binding region of the amino terminus. This segment is attached to a variable length intracellular component of the receptor by a highly homologous transmembrane segment of 22 amino acids.

### IGF and IGFBPs

IGF and IGFBPs are closely associated. Both IGF-1 (somatomedin C) and IGFBP-3, the major BP for IGF-1, increase with increasing GH production and fall with decreasing GH production. Recent data<sup>14</sup> indicate that IGFBP-3 controls the bioavailability of IGF-1 and IGF-2. Therefore, IGFBP-3 appears to control the action of IGF-1 as well as GH secretion.

These BPs, which now number at least 6, may be classified on the basis of (1) the amino-acid sequences, (2) molecular weight, or (3) immunoreactivity. Three of these are shown in Table 1. IGFBP-3, the acid-stable component of the 150 kd complex, probably is a major factor in growth regulation and GH secretion. It is glycosylated and consists of a 53 kd and a 47 kd component. Serum levels are high in the fetus, but drop shortly

after birth and then increase slowly until late prepuberty or very early puberty, at which time the values subsequently increase approximately 3-fold. IGF-1 levels increase concomitantly, as does the quantity of GH secreted with each secretory episode in adolescent boys at Tanner stage III or IV of pubertal development.<sup>8</sup> Peak values occur earlier in pubertal females. The levels of IGF-1 and IGFBP-3 are high in GH hypersecretory states, eg, acromegaly. The role of IGFBP-3 in respect to IGF-1 remains to be elucidated. From the clinical aspect, however, it is now abundantly clear that acid extraction of IGF-1 from its binding protein and measurement of the resultant IGF-1 more clearly reflects the growth-promoting effect than measurement of the IGF-1 concentrations using the nonextracted procedure.

IGFBP-1 is generally independent of GH, is found normally in amniotic fluid (placental source), is high in fetal blood, and declines immediately after birth and falls further throughout infancy, childhood, and adolescence. Since IGFBP-1 and IGFBP-2 are GH-independent, and because their function may be minimal in respect to growth, they are not considered further.

### GH and IGF-1 Actions

GH, IGF-1, and other growth factors work together to promote cartilage and bone growth, as described separately in *GGH* 1990;6:2 by Horton and by Mohan. Many factors have been shown to influence 1 or more aspects of this scheme through endocrine, paracrine, and possibly autocrine mechanisms. GH has a dual effect of epiphyseal cartilage growth and differentiation of cartilage cells as well as generation of IGF-1.<sup>15,16</sup> The proximal zone, close to the bony epiphysis, consists of a narrow band of germinal or stem cell chondrocytes. GH

preferentially stimulates differentiation of these prechondrocytes while IGF-1 stimulates the clonal expansion of the more differentiated cells in the distal proliferative zones.

The demonstration by Walker et al<sup>17</sup> that IGF-1 produces both positive nitrogen balance and hypercalciuria in patients with absent GH receptors indicates that GH itself is not necessary for the metabolic actions of GH except to generate IGF-1, but does not exclude the need for GH to promote chondrogenesis.

New data also have accumulated concerning the complex pathway of direct GH action to generate protein synthesis and growth. Three messengers or messenger systems appear to be involved in at least certain cell systems. GH is the first messenger, and by binding to its receptor, activates the second messenger system, diacylglycerol and protein kinase C. The oncogene, *C-fos*,<sup>18</sup> may play a role as a nuclear switch or as a third messenger in some signal transducing systems to activate transcription of appropriate genes to influence the biologic pathway of GH action.

### Placental GH (GH-V)

Since 1985, several investigators have determined the structure and role of "placental" GH, the natural product of the GH-V (GH variant) gene. This protein is expressed primarily by the placenta.<sup>19</sup> Daughaday and colleagues<sup>20</sup> have demonstrated large quantities of this GH variant (20 to 30 times the mean GH level of nonpregnant women) late in pregnancy at a time when essentially no pituitary GH was demonstrable. IGF-1 levels do not increase into the acromegalic level during pregnancy, reflecting either inhibitors of IGF-1 generation by the large amounts of estrogen or failure of the GH-V to activate the second messenger system(s), despite the known effect of GH-V binding to the somatogenic receptor.

Two distinct species (hGH-V and hGH-V2) are synthesized by the placenta.<sup>21</sup> The first is a 22 kd and the second a 26 kd protein. The hGH-V2 protein differs from the hGH-V protein in the location of its intramolecular disulfide bonds. The hGH-V2 protein is secreted by the syncytiotrophoblast. The configuration of residues suggests that this hGH variant may be an integral membrane protein. hGH-V2 constitutes one third or more of total hGH-V mRNA. Interestingly, the predominant hGH-V or 22 kd placental protein differs by only 13 amino acids from the 22 kd pituitary hGH-N gene product.

MacLeod et al<sup>22</sup> demonstrated that the hGH-V variant is a biologically active somatogen and lactogen. hGH-V binds efficiently to both somatogenic and lactogenic receptors, but with a 7.4-fold greater specificity for the somatogenic receptor than does hGH-N. Both hGH-V and hGH-N produced similar weight gain at similar doses in hypophysectomized rats, but the mitotic response of the lactogen-inducible Mb2 cells was significantly less for hGH-V. The comparable somatogenic, but lower lactogenic, bioactivity of hGH-V relative to hGH-N parallels the receptor binding profiles of the 2 hormones and suggests hGH-V has the potential to perform a unique role during human gestation.<sup>23</sup>

### Summary

GH appears to be even more important and more complex in its physiologic actions than was imagined in 1984 when the first review of this topic was written for the premiere issue of *GGH*. GHRH as a potential therapeutic agent has been temporarily shelved awaiting a depot formulation. GHRP and galanin have been recognized and continue to be studied although their direct therapeutic role can only be speculated. The binding proteins for GH and



the IGFs are now recognized as potent players in the physiologic arena of GH and IGF action. IGF-1 is an effective therapeutic agent in patients who have absent receptors for GH. Possibly of equal importance, a new GH regulatory system of the placental-fetal unit has been recognized and is being studied.

We never anticipated in 1984 when the previous article was written that so much would be learned in the short period of 7 years. We anxiously await the passage of another 7 years when, hopefully, we will have the opportunity to update you regarding GH physiology and pathophysiology in volume 14 of *GROWTH, Genetics, & Hormones*.

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# Limb Lengthening: Past, Present, and Future

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Los Angeles, California

Abstracted by  
Robert M. Blizzard, MD

## Introduction

In November 1990 at the National Cooperative Investigators Meeting of Genentech, Inc, Dr. David Rimoin gave an exciting presentation regarding the current and prospective status of leg lengthening to increase the height of individuals with short stature of skeletal etiology. This manuscript is an abstract of his presentation.

Limb lengthening has been a topic of increasingly developing interest over the past 2 years when reports first came out of Europe that it is possible to lengthen the limbs of chondroplastic children. Previously, the technique was used to correct limb asymmetry due to

polio, neurologic disease, and some congenital anomalies.

Wagner in Germany established one of the early techniques, which entailed breaking the bone surgically, performing an open osteotomy, cutting the periosteum, and using an external fixator and telescoping rod to stretch the tissues, which pulled the fracture site apart. Subsequently, he put in a metal plate, filled the area with chips, and it would heal. He operated upon achondroplastic individuals. He claimed that some needed up to 50 operations and that the complications were numerous. This technique has been abandoned for dwarfed individuals.

Ilizarov, an orthopedic surgeon working in a small institute in Siberia, was also one of the first to apply limb-lengthening techniques to dwarfs. In contrast to Wagner, Ilizarov utilized

"bloodless surgery," as all the incisions were small percutaneous cuts. Utilizing little scalpels and chisels, he broke the bone percutaneously, put on circular fixators, and then gradually stretched the extremities. This limb-lengthening procedure involved slow, controlled distraction of the callus during its formation. Interestingly, histologic examination revealed that the new bone was very organized, longitudinally oriented, and that the organic matrix was capable of mineralization, which began to occur in the first few days. The new bone that started in the medullary canal ossified rapidly and underwent corticalization after stretching was stopped. Perfectly normal appearing bone was present once the procedure was completed. Complications of extended limb lengthening were significant, however, and only a

rare patient did not have at least 1 complication; and most had numerous complications. The real question was pointed out by Dr. Rimoin, who asked, "Are these complications worth what one gains?" Muscle contractures, neurologic compromise because of the stretch of the nerves, vascular complications, joint stiffness, problems at the pin site, and a variety of psychologic problems may occur.

A variety of techniques have been developed subsequently by Villarubias in Barcelona and a number of Italian investigators who use external distraction with only 1 bar rather than the circular fixator developed by Ilizarov. Dr. Rimoin stated that he now is convinced that these techniques are worth trying—particularly the Spanish technique. Villarubias lengthens both tibias initially, and both femurs are subsequently lengthened. This is in contrast to the techniques used by the Soviets and Italians, in which a femur and a tibia may be lengthened on the same or opposite sides. Villarubias's technique also differs in that distraction is started within a few days after the initial surgery rather than waiting a longer period. His technique also differs in that he does not permit weight bearing during stretching, as opposed to the weight bearing required by the Soviet and Italian procedures. Rimoin's observation is that there is much less discomfort and little pain in patients who utilize the Spanish technique. Villarubias also does prophylactic tenotomies while he is doing the initial surgery. This consists of splitting the edges of the tendons, thus preventing the contractures that have occurred with the other techniques. By his technique, the limbs are stretched approximately 1.2 mm per day. Patients are kept in the hospital for only a brief period (3 to 5 days) and are then allowed to go to school in a wheelchair.

It is of great importance not to disturb the blood supply, which

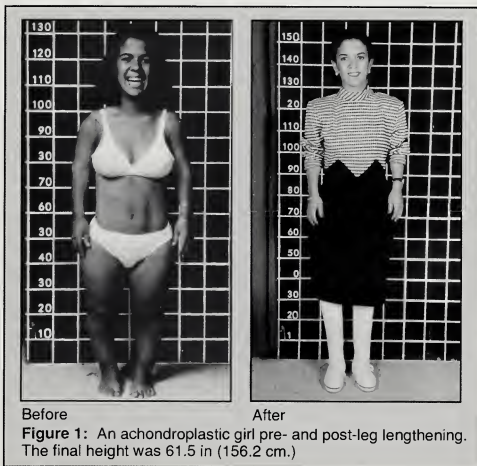
maintains the callus and allows it to heal rapidly. A few patients will have as much as a 30% reduction of ankle mobility and/or premature consolidation of the fibula, which may give obvious deviations of the extremity; about 2% have had to have repeat tenotomies. These complications are mild compared with those incurred with the other techniques.

Once the tibias have been lengthened, attention is focused on the femurs. Percutaneous tenotomy of the adductors is done early, and screws are put in the bones asymmetrically so that a rotational osteotomy is accomplished. A percutaneous tenotomy of the internal rectorus also is done, which relieves the tight muscles and tendons. No flexion of the knees is allowed during this time because the knee may be dislocated during femur stretching if it is placed in a fixed position. Patients are confined to wheelchairs.

Before initiating studies in Los Angeles, Dr. Rimoin visited Barcelona to observe the

technique of Villarubias. Subsequently, Rimoin sent 2 orthopedic surgeons, Drs. J. Isoaesón and W. Oppenheim, who are his collaborators, to learn the technique in Barcelona. As of the time of Rimoin's report in November 1991, 5 patients had been treated in Los Angeles. Two essentially had completed their leg lengthening. In Figure 1, the end result of a patient with achondroplasia who had such leg lengthening of both the tibias and femurs is demonstrated. This patient had a final height of 61.5 in. Utilizing this procedure, up to 12 in of increased growth can be anticipated. Dwarfs can be made into normal-sized individuals. Amazing to all observers is that the achondroplastic patients who have severe lordosis end up with essentially no lordosis when the Villarubias technique is applied. The coccyx is pulled down vertically, which reduces the lordosis markedly (Figure 2, page 6).

Dr. Rimoin states that the technique is still difficult to perform but it can achieve results. It must



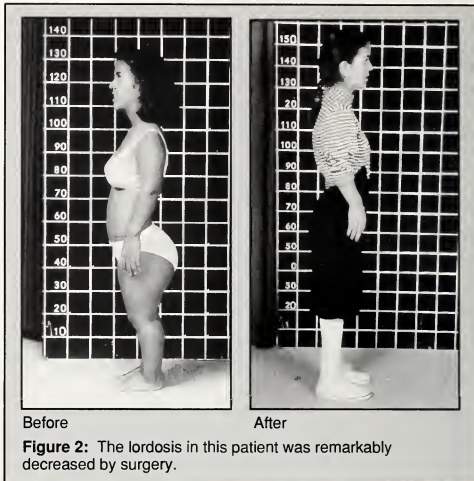
Before After  
**Figure 1:** An achondroplastic girl pre- and post-leg lengthening. The final height was 61.5 in (156.2 cm.)

be done with a team whose participants are comfortable dealing with little people, that has broad surgical expertise, and comprises neurologists, geneticists, physical therapists, and psychiatrists or psychologists, all of whom are willing to be involved extensively.

Rimoin emphasized that one of the reasons that achondroplastic patients are such ideal candidates is that they have excess soft tissue in association with their short limbs. Because the soft tissues are in excess, and the blood vessels are long and tortuous, the soft tissues stretch readily and lengthening is primarily of the bone and not of the soft tissues.

What is the best time to perform the procedure? Ilizarov does it any time after 6 years of age. Villarubias does it any time after 10 years of age. Rimoin and his collaborators have decided upon 14 to 20 years. The emotional maturity that develops during the teenage period is important in helping the patients cope.

Rimoin thinks that in most instances it may be unwise to do the procedure before age 14 or 15. He emphasizes that it should be the child who makes the decision and not the parents, and



he emphasizes very strongly that the technique should be done in centers with extensive capabilities and experience by the team members. Dr. Rimoin pointed out that leg lengthening was less expensive in treating achondroplasia on an inch-for-inch basis than growth hormone

treatment in growth hormone-deficient patients. He emphasized that we all should be looking with great interest upon this technique, but that only a few should be trying it at this time.

## Support Groups for Individuals With Growth Problems and Their Families

**Joan O. Weiss, MSW, LCSW**  
*Coordinator, Alliance of Genetic Support Groups*  
**Judith G. Hall, MD**

Genetic support organizations for families of children with growth disorders have increased steadily since the 1960s. Support groups help individuals with genetic disorders and their families discover that they are not alone and can be helped by others affected. Ideally, a strong

partnership between the support group members and interested health professionals enables the organization to meet its goals and sensitizes health care providers to the needs of those they are serving. Genetic support groups are gaining recognition as an important component of ongoing health care for individuals and families with genetic disorders. To enhance the effectiveness of these efforts, it is essential that

physicians providing care to children with growth problems be familiar with these voluntary organizations.

Group members share information on effective ways to cope, often working with health professionals to stimulate and fund research and to educate themselves and the public on a specific genetic disorder. Members also cooperate to effect changes in discriminatory laws and to obtain federal/state

funding for people with similar genetic disorders.

Below you will find several of these support groups listed for your reference.

Two national "umbrella" organizations have been formed to encourage greater public and professional awareness of these resource groups and to help extend group services when appropriate. The NATIONAL ORGANIZATION FOR RARE DISORDERS (NORD) was created in response to the unavailability of orphan drugs from the pharmaceutical industry for the treatment of rare disorders due to the economic infeasibility of producing these agents. NORD fosters communication among agencies and governmental and scientific communities, promotes scientific research on rare disorders, and represents people with rare disorders (when an appropriate organization does not exist), by putting them in touch with others through NORD's computer data base. The mailing address for NORD is: PO Box 8923, New Fairfield, CT 06812; (Telephone 1(800)999-6673).

Another national network of voluntary organizations is the ALLIANCE OF GENETIC SUPPORT GROUPS, formed to unify efforts of genetic support groups in educating the public about genetic disorders and to strengthen relationships between consumers and professionals. A recent survey of genetic support groups confirmed the value of professional services, such as genetic counseling, and recognized support groups as an important extension of health-care provider services. The ALLIANCE is trying to improve the availability and appropriateness of genetic services by developing model programs to meet needs identified by the membership. The mailing address is: ALLIANCE OF GENETIC SUPPORT GROUPS; 1001 22nd St, Suite 800; Washington, DC 20037; (Telephone 1(800)336-GENE).

LITTLE PEOPLE OF AMERICA (LPA) was founded in 1957 by Billy Barty, a little person himself and a Hollywood actor. LPA was established to assist members in acquiring the skills needed to become participating members of society, with an emphasis on education and employment. It is a unique organization administered by affected individuals, rather than their parents. While one important aspect of LPA is to facilitate social interaction, recently the support group has incorporated educational/therapeutic workshops and panels into its annual convention format.

LPA has 12 regional, districts, with local, regional, and national meetings occurring regularly. Today LPA has more than 4,000 members, each meeting the membership criterion of being 4 ft 10 in or less. Active subgroups have been formed for parents, teens, and young adults, with committees and workshops on subjects including careers, nutrition, adoption, social attitudes, and exercise and fitness. (The DWARFS' ATHLETIC ASSOCIATION has participated in the Special Olympics.) The LPA Foundation obtains and distributes funds for vocational training, scholarships, and medical/scientific research. The Medical Advisory Board serves as a resource for medical care and advice, and the review of research projects for ethical and scientific merit. LPA publishes some excellent reading material (eg, *The Idea Machine* providing tips for easing daily living and *My Child Is a Dwarf*, a booklet for parents) and a newsletter which is distributed nationally.

Physicians, allied health professionals, and families are encouraged to write directly to LPA National Headquarters; PO Box 9897; Washington, DC 20016 or to its Canadian counterpart, Little People of Canada; PO Box 453; Abbotsford, British Columbia V2X 275, Canada for additional information.

THE HUMAN GROWTH FOUNDATION (HGF) was

organized in 1965 by parents of children with severe growth problems. Largely through the efforts of the HGF, growth hormone therapy became available. From its inception, the HGF has worked to support basic clinical research pertaining to growth disorders. The 3 main goals of HGF are to disseminate information about growth disorders, to encourage the development of parent support groups at the local level, and to oversee a grants program to support growth disorders research. HGF publishes a monthly bulletin, *Fourth Friday*, in addition to a periodic newsletter.

HGF has also produced excellent informational booklets on growth problems including achondroplasia, Turner syndrome, intrauterine growth retardation, short stature, and dwarfism. These booklets are an important resource to parents and affected children. Inquiries may be addressed to Deborah Swansburg, Esq; Executive Director; HUMAN GROWTH FOUNDATION; 7777 Leesburg Pike, Suite 202 S; Falls Church, VA 22043; (Telephone 1(800)451-6434).

### Societies for Specific Disorders

Several voluntary organizations have been established for families and individuals with short stature due to a specific disorder. Among these are the TURNER'S SYNDROME SOCIETY OF THE UNITED STATES, the OSTEOPENESIS IMPERFECTA FOUNDATION, the MUCOPOLYSACCHARIDOSIS SOCIETY (MPS, Inc), the ASSOCIATION OF CHILDREN WITH RUSSELL-SILVER SYNDROME (ACRS, Inc), and the PRADER-WILLI SYNDROME ASSOCIATION (PWSA).

Individuals with Turner syndrome have a special set of concerns, in addition to those associated with short stature. The TURNER'S SYNDROME SOCIETY OF THE UNITED STATES addresses these



concerns. The office address is: 768-214 Twelve Oaks; 15500 Wayzata Boulevard; Minnetonka, MN 55391; (Telephone 1(612)475-9944). The US group has educational booklets for patients/families and physicians available.

1. *Turner Syndrome: A Guide for Families* (P. Reisner, RN, and L. Underwood, MD).

2. *Turner Syndrome: A Guide for Physicians* (R. Rosenfeld, MD).

The CANADIAN TURNER'S SYNDROME SOCIETY has produced an excellent videotape and publishes an informative newsletter every few months. A booklet prepared by the Society, *The X's and O's of Turner's Syndrome*, can be obtained by writing to: TURNER'S SYNDROME SOCIETY; York University; Administrative Studies Building, No.006; 4600 Keele St; Downsview, Ontario M3J 1P3, Canada.

The OSTEOGENESIS IMPERFECTA FOUNDATION distributes information about osteogenesis imperfecta, provides moral support, and funds research. The Foundation also publishes a quarterly newsletter, *Breakthrough*. Although not all individuals with osteogenesis imperfecta are short statured, many do have medical and social problems. All types of osteogenesis imperfecta appear to be linked to genetic collagen abnormalities. Complications include frequent bone fractures, dental anomalies, and deafness. For further information about this disorder or the support group, contact: OSTEOGENESIS IMPERFECTA FOUNDATION; 12807 W. Hillsborough Avenue, Suite G-10, Tampa, FL 33635; (Telephone 1(813)855-7077).

The mucopolysaccharidoses and mucopolipidoses are rare hereditary disorders with enzyme deficiencies in which abnormal compounds collect in the cells of various body tissues. Most of these disorders result in short stature and are associated with a variety of other problems. The NATIONAL MUCOPOLYSAC-

CHARIDOSES (MPS) SOCIETY, INC is dedicated to serving parents through support, networking, physician referrals, professional and public education, and fund raising to support MPS research. The MPS SOCIETY is located at 17 Kraemer St; Hicksville, NY 11801; (Telephone 1(516)931-6338) and the CANADIAN SOCIETY for MPS is located at 382 Parkway Blvd; Flen Flon, Manitoba R8A 0K4, Canada.

THE ASSOCIATION OF CHILDREN WITH RUSSELL-SILVER SYNDROME (ACRSS) has recently been formed for families of children with Russell-Silver intrauterine growth retardation. Contact can be made through ACRSS, Inc, c/o Jodie Swain; 22 Hoyt St; Madison, NJ 07940; (Telephone 1(201)377-4531).

The PRADER-WILLI SYNDROME ASSOCIATION (PWSA), established in 1975, provides educational materials and supportive services to parents and professionals. It publishes a bimonthly newsletter, with a catalogue and audiovisuals available upon request. For additional information contact: Marge A. Wett; Executive Director; PRADER-WILLI SYNDROME ASSOCIATION; 6490 Excelsior Blvd, E-102; St Louis Park, MN 55426; (Telephone 1(612)926-1947).

### International Support Groups

Recently, support groups for short statured persons and their families have developed in several countries. One of the early groups, ASSOCIATION FOR RESEARCH INTO RESTRICTED GROWTH (ARRG), founded in 1970 in Great Britain, is now called the RESTRICTED GROWTH ASSOCIATION. (For information contact: Miss Pam Rutt; 61 Lady Walk; Maple Cross, Rickmansworth; Herts, WD3 2YZ England). Other active support groups exist throughout Europe. Recently, the INTERNATIONAL GROWTH FEDERATION emerged to

represent individuals from a variety of countries interested in growth disorders. For additional information about the INTERNATIONAL GROWTH FEDERATION, contact the HUMAN GROWTH FOUNDATION office in Virginia. (See above.)

This list of short stature support organizations is not all inclusive, but is intended to alert physicians to the availability of these resources for patients and families. It is important for patients and their families to be aware of the availability of reliable educational information and support groups, and for physicians to participate in this educational process. Lay groups also need the support of the medical profession to work effectively in dealing with the problems associated with short stature.

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## Review and Editor's Comment: X Inactivation of the X Chromosome Is Not Complete

A series of papers recently published present concurring data indicating that the inactivated X chromosome is not completely inactivated. There are now known at least 4 areas of Xp and 2 areas of Xq of the inactivated X chromosome that are active. Those genes known to be subject to X inactivation, and those known to escape X inactivation on the X chromosome, are depicted in Figure 1. One of the 2 areas on Xq encompasses the inactivation center, which is believed to be responsible for inactivation of 50% of the X chromosomes. Intriguingly, current thinking regarding this center is that this area is active on the inactive X chromosome but inactive on the active X chromosome. This area on the inactive X chromosome, known as the X inactive specific transcript (XIST), produces a transcript, whereas the same area on the active X does not. This area is an area for a candidate gene that could be involved in influencing the process of X inactivation.

The importance of this phenomena is considered further in the references listed below. The readers are encouraged to

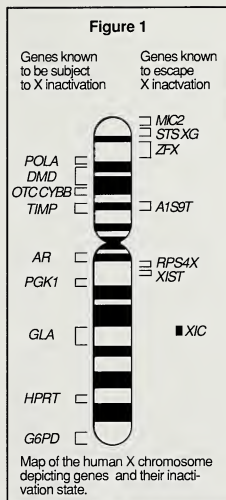
review these articles in detail, as Ohno's law of the constancy of the genetic material of the mammalian X chromosome is now being challenged by Watson et al after having been accepted for almost 25 years. The field is also intriguing as rare families manifesting female-to-female transmission of X-linked traits such as hemophilia B may have a co-inherited defect in the X inactivation center (XIC) resulting in the exclusive inactivation of the normal chromosome. XIST also may be involved in the phenotype of X-chromosome disorders such as Klinefelter and Turner syndromes. Deletion mapping in 46,XY Turner females has refined 1 probable Y chromosome localization to a 90 kb stretch between the possible sex determining gene, SRY, and the more proximal ZFY gene.

Judith G. Hall, MD

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## Review: Magnetic Resonance Imaging in Patients With Hypothalamic and/or Pituitary Disorders

In recent years, magnetic resonance imaging (MRI) has developed as an extremely useful tool to evaluate hypopituitarism, sexual precocity, diabetes insipidus, and other endocrine entities occurring in children. Pellini et al report the results of MRI in 30 growth hormone deficient (GHD) children, aged  $10.1 \pm 3.5$  years, who were considered after routine investigation to have idiopathic GHD, and compared these with data from 15 healthy

age-matched controls. Eighteen patients had isolated GHD and 12 had multiple deficiencies. The sellar and/or the hypophyseal volumes were significantly reduced in the GHD patients, without correlation with auxologic or endocrinologic findings. Twenty patients (all 12 with multiple deficiencies and 8 of the 18 with isolated GHD) had an abnormality of the pituitary stalk. In 18 cases, the bright spot indicating the neurohypophysis was dislocated to the distal part

of the stalk, but there was no change of water balance.

Cacciari et al report a study done in 70 patients with GHD and short stature, and in 6 with hypogonadotropic hypogonadism, 4 with isolated diabetes insipidus, and 21 with true central precocious puberty. Of the 70 patients, 23 had multiple anterior pituitary deficiencies and 42 had isolated GHD. The remaining 5 had anterior and posterior pituitary hormone deficiencies. The patients with multiple pituitary deficiencies had morphologic findings consistently involving the stalk and posterior lobe. Only 5 of 42 (12%) patients with isolated GHD had abnormalities of the sella.

In contrast, Pellini et al report abnormalities in 8 of 20 patients with isolated GHD. This group suggests that if a defect is found by MRI in a patient with isolated GHD, the patient very probably is at increased risk to develop multiple hormone deficiencies of the anterior pituitary. Stanhope et al previously reported in *Acta Paediatr Scand* 1986;75:799 that

64 of 77 patients affected by either isolated GHD or GHD with other tropic hormone deficiencies had pituitary hypoplasia.

Of significance also was the finding by Pellini et al that substitution therapy with GH does not induce shrinkage of the pituitary as determined by MRI, thus suggesting that a hypoplastic pituitary in a patient treated with GH probably can be interpreted as having been present before GH treatment was given.

Cacciari et al reported that patients with diabetes insipidus frequently had an absent or ectopic posterior gland and the bright spot was absent. They also reported that among 21 patients with precocious puberty, the MRI studies in 17 were normal, 1 was questionably abnormal, and hamartomas were observed in 3. All the latter were in children less than 2 years of age. No lesions were found in the 6 patients with hypogonadotropic hypogonadism.

Pellini C, et al. *Eur J Pediatr* 1990;149:536-541.

Cacciari E, et al. *Arch Dis Child* 1990;65:1199-1202.

Stanhope R, et al. *Acta Paediatr Scand* 1986;75:779.

**Editor's comment:** I concur with the authors that the probability of a pathologic finding in a MRI study is high in multiple pituitary deficiencies, in diabetes insipidus, and in precocious puberty of very early onset. In most patients with hypogonadotropic hypogonadism, with or without anosmia, and in patients with precocious puberty occurring after 3 or 4 years of age, a functional etiology of the pathologic process is probable. It is unclear at this time whether all patients with isolated GHD should have an MRI. However, if an abnormality is detected by MRI in a patient with isolated GHD, the patient should be followed closely for the possible development of multiple pituitary hormone deficiencies.

Jean-Claude Job, MD

## Adult Panhypopituitarism Presenting as Idiopathic Growth Hormone Deficiency in Childhood

The goal of this short clinical report is to suggest that protracted follow-up is needed after treatment of growth hormone deficiency (GHD), and that reassessment of pituitary function in adult life may be useful.

The first patient reported was a girl whose GHD was diagnosed at age 11 years. At 11 years, no other pituitary functions were involved, and the CT scan of the pituitary was considered normal. She was treated with GH for 7 years and grew rapidly. However, at 19 years, she had thyrotropin, corticotropin, and follicle-stimulating hormone releasing hormone deficiencies. A repeat CT scan showed an empty sella with a thin pituitary stalk and no glandular tissue.

The second patient was a boy, referred at 9 years of age with a height deficiency of -3.6 standard deviations (SD). Like in the first patient, no deficiency other than that of GH was evident. The pituitary seemed normal on a CT scan. He was then treated with GH for 10 years. Hypothyroidism was discovered at age 16 years. Corticotropin and gonadotropin deficiency became obvious at 18 years. A repeat CT scan showed a small sella containing apparently normal pituitary tissue.

The authors discuss the possibility of progressive loss of anterior pituitary function, irrespective of a radiologically demonstrable lesion. And they stress the importance of

continuous attention to patients with so-called isolated GHD.

Crowne EC, et al. *Acta Paediatr Scand* 1991;80:255-258.

**Editor's comment:** This article augments those cases reported in "Review: Magnetic Resonance Imaging in Patients With Hypothalamic and/or Pituitary Disorders," which is in this issue of GGH (see page 9). Evidence is rapidly accumulating that a diagnosis of isolated GHD today does not mean that deficiencies of other tropic hormones will not appear subsequently even if initial CT scans reveal no pathology.

Jean-Claude Job, MD

## A New Syndrome of Congenital Hypoparathyroidism, Severe Growth Failure, and Dysmorphic Features

Sanjad et al describe the identification of 12 patients with an unusual syndrome of congenital hypoparathyroidism associated with growth failure and dysmorphic features distinctive from those of the DiGeorge syndrome. The onset

of symptoms of hypocalcemia occurred between 1 and 30 days of life. Ten of the 12 patients had parents who were first cousins. All but one patient had severe intrauterine growth retardation, with birth weights ranging from 1,500 to 2,150 g.

All had moderate to severe hypocalcemia and hyperphosphatemia but no chromosomal abnormalities detectable by karyotyping. Physical features of this syndrome include: deep set eyes, microcephaly, thin lips, beak nose tip, external ear anomalies, micrognathia, depressed nasal bridge (Figure 1). Mental retardation was found in all patients and all had severe postnatal growth retardation despite treatment with vitamin D and calcium supplements, which normalized serum calcium levels.

Sanjad SA, et al. *Arch Dis Child* 1991;66:193-196.

**Editor's comments:** The authors indicate that they were unable to determine the pathophysiology of the hypoparathyroidism in these individuals. They point out that since no patient showed clinical evidence of T-cell deficiency, it is unlikely that these patients represent a variant of DiGeorge syndrome. In addition, this syndrome is the only syndrome with hypoparathyroidism in association with intrauterine growth retardation. Although all of the patients to date have been identified in Saudi Arabia, it is important for pediatric geneticists and endocrinologists to be on the lookout for further cases of this interesting syndrome.

William L. Clarke, MD



**Figure 1:** (A) Case 4: girl with deep set eyes, micrognathia, thin lips, and simple malformed posteriorly rotated ears. (B) Case 2: girl with prominent forehead, depressed nasal bridge, deep set eyes, micrognathia, thin lips, and preauricular tags. (C) Case 1: boy with deep set eyes, broad nasal bridge, prominent forehead, and micrognathia. (D) Case 5: boy with deep set eyes, epicanthic folds, broad nasal bridge.

### Address for Correspondence

Please send all correspondence to Robert M. Blizzard, MD, Department of Pediatrics, Box 386 University of Virginia School of Medicine, Charlottesville, VA 22908



## Decreased Growth Velocity Before Insulin-Dependent Diabetes Mellitus

Growth was studied prospectively in 12 nondiabetic identical twins <14 years, and in their cotwins with insulin-dependent diabetes mellitus (IDDM) to determine whether changes in growth occur before the onset of IDDM. Seven of the 12 nondiabetic twins subsequently developed IDDM. A significantly reduced growth velocity (GV) was observed in these 7 as compared with their diabetic cotwins and as compared with those who did not develop IDDM. The nadir of growth in the twins who developed diabetes occurred a mean of 1.2 years before diagnosis (range, 0.3 to 2.3 years). Islet cell antibodies were observed in all 7 of the prediabetic twins in contrast to 0 of 5 of those who did not develop IDDM. In 4 prediabetic twins the decreased growth preceded impaired glucose tolerance. The

prediabetic twins had lower testosterone (males) and estradiol (females) levels at the time of slow growth than did the diabetic, normally growing twins.

David R, et al. *Diabetes* 1991;40:211.

**Editor's comment:** This is the first prospective study of growth prior to the onset of diabetes in monozygotic twins. As such, it is an exceedingly important study. Previous studies of growth before the onset of diabetes were retrospective and reported conflicting findings that children with diabetes were shorter, taller, or the same height as normals. The present study demonstrates that nondiabetic twins who will develop diabetes have a significant tendency for growth retardation (GV less than the 3rd

percentile), as compared with their diabetic twins. In addition, there is some evidence to suggest that testosterone and estradiol may be lower in the prediabetic individuals as compared with their diabetic twins. The etiology of the decreased GV in relation to these findings, however, remains unclear. Regardless, there must be subtle metabolic changes that require more detailed studies of GH and gonadotropin secretion. From these studies one can presume that many children with identified islet cell antibodies will become diabetic and GV will decrease to abnormally low values (less than the 3rd percentile growth velocity for age) before any evidence of diabetes is present.

William L. Clarke, MD

## Estrogen Treatment of Tall Girls: Dose Dependency of Effects on Subsequent Growth and IGF-1 Levels in Blood

This retrospective study compares the effects of ethinyl estradiol 250, 500, or 1,000 µg/d given 3 weeks of each 4-week cycle in very tall girls. Each study consisted of 15 to 21 girls who were in the same age range ( $13.5 \pm 1.1$  years), with similar bone ages ( $12.6 \pm 0.9$  years), similar height predictions ( $186.2 \pm 3.1$  cm), and duration of treatment ( $1.9 \pm 0.6$  years). Most received a progesterone analogue during the third week of each cycle.

In the 3 groups, the difference between final and predicted height was similar:  $5.5 \pm 2.7$ ,  $5.9 \pm 3.3$ , and  $5.6 \pm 2.7$  years. Follow-up of plasma insulin-like growth factor 1 (IGF-1) levels revealed a significant decrease with 500 or 1,000 µg/d of ethinyl estradiol but not with 250 µg/d.

The authors conclude that a dose of 250 µg of ethinyl estradiol per day for 3 of every 4 weeks is as potent in reducing final height in tall girls as higher doses.

Savan H, et al. *Acta Paediatr Scand* 1991;80:328-332.

**Editor's comment:** The use of large doses of estrogen to reduce stature in tall girls was initiated by Wettenhall in 1955 (*J Pediatr* 1975;86:602). He used diethylstilbestrol with limited success in reducing stature (an average of -3.5 cm), as the girls he treated had epiphyses that were nearly fused to the metaphyses when treatment was initiated (mean bone age, 13.2 years). The value of his

study was that 87 girls were followed for >15 years. The only toxicity reported was the development of paraovarian cysts in 2 girls and superficial thrombophlebitis in another.

Bierich (*Pediatrics* 1978;62:1196 and *Gynakologe* 1983;16:72) reported his experience with 41 girls whose predicted heights were >180 cm. Conjugated estrogen (7.5 mg/d) was used (plus 7 days of progesterone each 28 days). Fifty percent of the girls were menstruating when treatment started. Because skeletal maturation advanced 3.7 times faster than body height, growth retardation as compared with predicted height was -8.3  $\pm$  2.1 cm in the premenarcheal girls and -6.8  $\pm$  1.6 cm in the menarcheal girls. Doses of 0.3 to 0.5 mg/d of ethinyl estradiol produced similar results. No side

effects were observed in relation to high-dose treatment including no increases in triglycerides and cholesterol. However, Weninger et al subsequently reported (Acta Paediatr Scand 1987;76:500) hyperlipidemia in association with ethinyl estradiol therapy at this dose level.

An important question is: Could a smaller dose of ethinyl estradiol be as effective as the usually recommended larger dose? Bartsch et al (Eur J Ped 1988; 147:59) evaluated the use of

0.1 mg/d for 2 years in 25 tall girls. They reported that the reduction in predicted adult height achieved by estrogen treatment averaged 7.4 cm in girls whose bone ages were >12.5 years. They concluded that the higher dosages offer little advantage. Gruters et al (Eur J Ped 1989;149:11) studied 2 comparable groups of tall girls: the first group receiving 0.3 - 0.5 mg/d ethinyl estradiol, and the second 0.1 mg/d ethinyl estradiol. The different doses had similar effects on final height reduction.

The current abstract compares several intermediate doses (0.25, 0.50, and 1.0 mg/d) and concludes that the effects are comparable. The consensus seems to be that doses prescribed to treat tall girls over the past 20 years may be unnecessarily high. A response from the readers through the "Letters to the Editor" column is welcome.

Jean-Claude Job, MD

## Growth Hormone Gene Deficiency: Hot Spots for Growth Hormone Gene Deletions in Homologous Regions Outside of Alu Repeats

Most types of growth hormone deficiency (GHD) do not involve the growth hormone gene, but there is a rare familial type of GHD, type 1A, that is caused by deletion of the growth hormone N gene on each chromosome 17 in affected individuals. The authors examined the specific mutation in 10 patients with type 1A GHD. These patients represented different geographic origins. Different size deletions were found in each family. The deletions appear to be related to abnormal pairing in the areas that flank the growth hormone gene, which leads to abnormal cross-overs. These areas have many Alu repeats. Since these are areas of recombination, it is possible to mismatch if there are different numbers of Alu repeats on the chromosomes inherited from mother and father. The mismatch of Alu repeats appears to make for hot spots of abnormal recombination. The areas of Alu repeats are clearly important areas for producing deletions that result in GHD since all the patients studied have this kind of mismatch deletion.

Vnencak-Jones CL, et al. *Science* 1990;450:1745-1748.

**Editor's comment:** Type 1A GHD is quite rare and presents a problem to the clinician in that when growth hormone is given, antibodies to it usually develop. Nevertheless, the study of these families has led to a better understanding of how mutations occur, at least in the case of the growth hormone gene. It

appears that they are likely to occur because of mismatching of the chromosome areas outside the gene. The study is particularly important for understanding what leads to mutations of this type.

Judith G. Hall, MD

## Adult Height in Boys and Girls With Untreated Short Stature and Constitutional Delay of Growth and Puberty: Accuracy of 5 Different Methods

The height predictions of 5 methods were compared with ultimate adult height in 37 boys and 32 girls with short stature associated with constitutional delay of growth and puberty (CDGP). The boys were seen initially at a chronologic age (CA) of  $14.8 \pm 1.7$  years and the girls at  $12.9 \pm 2.6$  years. The groups were seen ultimately at 23.1 years and 21.1 years.

For boys, the adult height was overestimated by calculation of the target height, as compared with the ultimate height, by  $1.7 \pm 5.7$  cm. The overestimate for girls by the target height method

was  $0.65 \pm 4.31$  cm. The Roche-Wainer-Thissen (RWT) method was the most accurate predictor for boys, underestimating the adult height by  $0.53 \pm 4.37$  cm. In girls, the RWT method was less accurate as it overestimated the adult height by  $2.6 \pm 3.2$  cm. The Bayley-Pinneau (BP) method overestimated significantly the ultimate height for boys ( $3.1 \pm 5.5$  cm) and underestimated the height for girls ( $0.8 \pm 3.6$  cm). The TW2 method underestimated significantly for both boys ( $1.76 \pm 3.27$  cm) and girls ( $4.17 \pm 5.35$  cm). The TW1 method was even less

predictable. In girls, all prediction methods gave similar results, with no method being significantly superior to the others. In boys, the RWT method offered the best estimates of adult height.

In the studies reported here in patients with CDGP, the adult height by the BP method was overestimated by 3.1 cm, which compares with the data in other series by this method (overestimations of 2 to 4 cm). The authors conclude that patients with CDGP usually reach an adult height in the lower normal range. The adult height of patients with CDGP is below the target height and does not reach

the height standard deviation score (SDS) for bone age observed at the initial visit.

Bramswig JH, et al. *J Pediatr* 1990;117:886-891.

**Editor's comment:** *The observed discrepancies of overestimations and underestimations of each method may relate to the particular ethnic group evaluated; thus, obtaining data for a particular center and the ethnic group(s) served by that center may be important in determining accurate predictions. Nevertheless, this is an*

*important paper that demonstrates the variability of 5 different methods of height prediction within 1 clinic population for boys and girls with CDGP. These data do not necessarily apply to children who do not have CDGP. Particularly impressive in the data presented are the large measurements related to SDS. It is important for both clinicians and investigators who use height predictions to evaluate growth promoting therapy to know the tendency of each method to underestimate or overestimate adult height.*

Judith G. Hall, MD

## Growth Acceleration and Final Height After Treatment for Delayed Diagnosis of Celiac Disease

Short stature and growth failure may be the only clinical presentation of the so-called occult form of celiac disease (CD). This paper reports on 24 patients over 4 years of age in whom CD was diagnosed. Their initial presentation was short stature or retarded growth, with heights below the 5th percentile but without any other overt symptoms. The effect of treatment with a gluten-free diet (GFD) on catch-up growth and final height was determined.

Small-bowel biopsy demonstrated mucosal atrophy in all 24 patients. Antiglutin antibody (AGA) titers were also found to be abnormal in the 13 of 24 patients whose levels were measured. Weight and height velocities, pubertal staging, bone age (BA), target height (TH, based on midparental height), and predicted height (PH, according to Tanner) were recorded at diagnosis and periodically after treatment was begun with GFD.

At diagnosis, 82% of patients were below the 3rd percentile for height and 58% were below

the 3rd percentile for weight. Nearly all of the patients (95%) had a delayed BA compared with chronologic age (range of delay, 1 to 6 years). All patients had catch-up growth following the institution of GFD, with increased height and weight velocities rapidly achieved during the first year of GFD. After 1 year of treatment, 87% of patients had a stable height velocity above the 50th percentile and their height standard deviation score (HSDS) improved significantly. By the third year, their HSDS showed less stature reduction than that observed at the time of diagnosis (-1.77 vs -2.52). The patients who reached an appropriate TH for midparental height were those in whom the diagnosis was made and treatment was started before puberty. In contrast, the patients who did not achieve a satisfactory final adult height were those who began dietary treatment after the onset of puberty.

Bosio L, et al. *J Pediatr Gastroenterol Nutr* 1990;11:324-329.

**Editor's comment:** *Although the paper by Bosio et al does not address all pertinent issues of CD diagnosis and prognosis, it adds to the large volume of reports indicating that relatively asymptomatic short-statured children without weight deficits for height but with various degrees of retarded BA may have CD as the cause of their poor growth. This paper also confirms that the diagnosis of CD can be established only by a small-bowel biopsy, which shows the typical histologic findings, whereas other measurements of intestinal function (ie, xylose tolerance) may fail to detect any abnormality.*

*The paper by Bosio et al also suggests that when an appropriate diagnosis is made and when timely dietary treatment is given these patients exhibit catch-up growth and attain an appropriate height based on midparental height. In contrast, when there is a delay in the diagnosis and treatment with GFD is initiated after the onset of puberty, there may be an unsatisfactory final adult height.*

Therefore, the clinician must be alert and must consider CD as a cause of short stature. Of course, there are other, perhaps more important reasons besides stature mandating that the accurate diagnosis of CD be made as early as possible. Since the only way to rule out CD is by small-bowel biopsy, the clinician must keep in mind the clinical indications for this procedure when a short child is evaluated. These vary in accordance with the geographic location and with the clinical history of the patient. In areas of the world where CD is frequent, it should be high on the list in the differential diagnosis of short stature. The clinical history usually reveals clues for consideration of CD. This entity

is not "occult" in the majority of these subjects. In the paper by Bosio et al summarized above as well as in other publications on the subject, it is clear that these patients have a frequent history of diarrhea, poor weight gain, and other gastrointestinal symptoms in infancy. These symptoms are often considered not important enough to be thoroughly evaluated by the physician, although patients are often treated by dietary manipulations. Other important clues to alert the clinician include a deteriorating height and weight pattern of growth and the presence of nutritional deficits, ie, iron deficiency associated with short stature. The paper by Bosio et al provided no data on the growth patterns that

preceded the diagnosis of CD in their patients, nor did it contain information regarding the presence or absence of nutritional abnormalities like iron deficiency. Patients with CD usually have a growth pattern typical of nutritional dwarfing with decelerating weight gain and height velocity, although they may not have weight loss or body weight deficits for height. Also, they often exhibit iron deficiency even though there may be no anemia. It is clear that following an appropriate diagnosis and treatment with GFD, the patient will exhibit catch-up growth and weight gain, which may occur rapidly during the initial stages of treatment.

Fima Lifshitz, MD

## Review: Mapping for the Marfan Syndrome Gene

Marfan syndrome is a relatively common inherited connective tissue disorder characterized by tall stature, dislocated lens, and cardiovascular abnormalities including aortic dilatation and dissection. Despite intensive research carried out in various laboratories over the years, nothing has been known about the genetic defect leading to the syndrome. Linkage analyses have excluded many of the suspected genes. Recently Kainulainen et al, using linkage analyses with polymorphic markers of the human genome, mapped the genetic defect to the long arm of chromosome 15 in 5 families with Marfan syndrome. This methodology now serves as a diagnostic test in families in which cosegregation of these markers with the disease has been confirmed.

The basic defect now appears to be in the microfibrillar system, which is widely distributed in the extracellular space. Hollister et al demonstrated that there is a

striking lack of fibrillin in both skin sections and dermal fibroblasts from Marfan patients as compared with normal subjects. However, there are several genes in the identified affected area of chromosome 17, including those for chondroitin sulfate, proteoglycan I core protein, cardiac muscle alpha-actin, and type I collagen receptor, that could be

implicated. The specific gene remains to be isolated.

Kainulainen K, et al. *N Engl J Med* 1990;323:935-939.

Hollister DW, et al. *N Engl J Med* 1990;323:152-159.

Editorial in *Lancet* 1990;336:973.

Judith G. Hall, MD

## Iodine and Selenium Deficiency Associated With Cretinism in Northern Zaire

It is known that endemic cretinism is associated with severe iodine deficiency, but the reason for the variable geographic distribution of its myxedematous form is not clear. This study examined the selenium status of persons living in the endemic goiter belt of northern Zaire, where myxed-

matous cretinism, characterized by goiter, overt hypothyroidism, and stunted growth, is predominant.

The study was conducted in 2 rural villages in the core of the endemic goiter area and included 52 normal schoolchildren (aged 9 to 18 years) and 28 cretins (aged 3 to 25 years). Reference values



were obtained from adults hospitalized for medical checkups ( $n=30$ ) in another, less severely iodine-deficient area and from volunteer healthy medical workers ( $n=9$ ) in an iodine-nondeficient location. Stature and the presence of visible goiter were recorded. Blood was drawn for baseline serum thyroid indexes, serum and red blood cell (RBC) selenium, and erythrocyte glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, pyruvate kinase, and hemoglobin A, A<sub>2</sub>, and S. Urine for iodide was also analyzed. The schoolchildren and cretins in 1 village were supplemented orally for 2 months with 50 µg selenium/d while the participants from the second village received a placebo. All subjects in both villages were subdivided for the next 4 months into a supplemented group (100 µg selenium/d) and a placebo group.

The schoolchildren often presented with visible goiter (41 out of 53) and had biochemical evidence of hypothyroidism (low thyroxine [T<sub>4</sub>] and triiodothyronine [T<sub>3</sub>] and high thyrotropin [TSH]) but the cretins showed biologic signs of severe hypothyroidism (very stunted growth, low T<sub>4</sub>, and markedly elevated TSH). Seven of 30 of the hospitalized adults had goiters and all were euthyroid, whereas none of the volunteers had any evidence of thyroid dysfunction.

The serum selenium of school children and cretins ( $343 \pm 176$  nmol/L vs  $443 \pm 188$  nmol/L,  $P>0.1$ ) was markedly lower than that of the adult patients ( $753 \pm 355$  nmol/L) or medical workers ( $2,555 \pm 347$  nmol/L). The erythrocyte glutathione peroxidase concentrations followed the same pattern as the selenium. However, the other erythrocyte enzyme activity (pyruvate kinase, glucose-6-phosphate dehydrogenase, pyruvate kinase, and glutathione

reductase) and the prevalence of abnormal hemoglobin (consistent with  $\beta$ -thalassemia trait) were similar in all groups. The urine iodine was very low in school children and cretins (0.20 and 0.16 µmol/L) and was moderately decreased in adult patients (0.37), but was high in the medical workers (4.57).

Multifactorial analysis was significant for the effect of iodine on thyroid hormones. The effect of serum selenium was not significant. A supplementary effect of erythrocyte glutathione reductase was also significant for TSH, T<sub>4</sub>, and free T<sub>4</sub> levels in multiple regression analysis. After 2 months of selenium supplementation, the serum selenium concentrations became normal, whereas the erythrocyte glutathione peroxidase continued to increase and reached normal concentrations only after 6 months of treatment. The increase in serum selenium and erythrocyte glutathione peroxidase after 2 months of supplementation was more pronounced in the cretins than in the schoolchildren but was not significantly different after 4 to 6 months.

The authors conclude that there is a severe selenium deficiency in the core of the northern Zaire goiter belt, which reinforces the hypothesis of and association with endemic myxedematous cretinism.

Vanderpas JP, et al. *Am J Clin Nutr* 1990;52:1087-1093.

**Editor's comment:** These data clearly document the presence of selenium deficiency associated with iodine-deficient goiter and cretinism in Zaire. In China, selenium deficiency has been reported to produce a cardiomyopathy called Keshan disease and osteoarthropathy called Kashin-Beck disease. However, the selenium deficiency observed in these 2 conditions may be more severe than the condition documented in association with iodine

deficiency, goiter, and cretinism since there was no cardiomyopathy or osteoarthropathy detected in the Zaire study. Macrocytosis, lightening of hair and skin color, and abnormalities of liver enzymes have also been noted in patients with selenium deficiency given total parenteral nutrition for long periods. These findings were not addressed in the study of Vanderpas et al in Zaire.

In other areas of the world it has also been noted that where iodine-deficiency goiter is prevalent, the presence of cretinism is more frequent when there is also an overlap with selenium deficiency. The converse is also true; in selenium-deficient areas where there is a high supply of iodine there is no increased rate of cretinism or thyroid function abnormalities even when there is endemic goiter.

The possibility of an interaction of combined deficiencies of iodine and selenium leading to derangements of thyroid function should be considered. There are data indicating that hydrogen peroxide generated at the apical membranes of thyroid cells is necessary to oxidize thyrosal residues of thyroglobulin in the formation of thyroid hormones. Thus, the synthesis of excess hydrogen peroxide in a stimulated gland and the lack of hydrogen peroxide-detoxifying enzyme would progressively induce more severe thyroid hormone deficiencies. Additionally, selenium may also play a role in thyroid function by modulating the iodinases necessary for conversion of T<sub>4</sub> into T<sub>3</sub> and reverse T<sub>3</sub> (rT<sub>3</sub>) in extrathyroid tissues.

Further studies remain to be done to ascertain the clinical and public health benefits of selenium supplementation in areas of the world where there is selenium deficiency, as well as in other types of goiters more commonly observed in our population.

Fima Lifshitz, MD

## Nitrogen Kinetics and Growth in Short Children Treated With Growth Hormone

Dempsher et al studied the acute effects of growth hormone (GH) on retention of  $^{15}\text{N}$ -labeled amino acids and its relationship to the response to GH treatment in 37 short children.

The patients were recruited from the Washington University Pediatric Endocrinology Clinic. They were prepubertal, between 6 and 14 years of age, and had heights more than 2 standard deviations (SD) below the mean for age. Patients with chronic medical illnesses were excluded, as were girls with chromosome abnormalities. The group as a whole had a mean bone age (BA) delay of slightly more than 2 years and biologic parents with mean heights below the 50th percentile for normal adults. Pretreatment height velocity measurements were recorded for a minimum of 1 year and averaged  $4.7 \pm 1.2$  cm/yr.

The patients underwent the following studies:

1. GH secretion: GH response was measured by radioimmunoassay (RIA) after clonidine and insulin stimulation. Thirty-four of the 37 children had normal GH levels as measured by provocative stimuli; 3 children had values below 7 ng/mL in response to both tests. These 3 patients were classified as GH deficient (GHD). However, because the subsequent response of this group of 3 children to acute and chronic GH supplementation was indistinguishable from those of the remaining 34 subjects, their results were included with the rest of the study group.
2. GH molecule: In addition to the GH measurement by conventional polyclonal RIA, the highest GH concentrations in the 2 plasma samples from each provocative test were remeasured by monoclonal IRMA or by IM-9 or human liver radioreceptor

assays. All of the 34 non-GHD subjects had a normal GH molecule as estimated from the ratio of IRMA radioreceptor to RIA.

3. Growth hormone-binding protein (GHBP): None of the 37 subjects had GHBP alterations.
4. GH and insulin-like growth factor 1 (IGF-1) genes: The GH gene was analyzed in all 37 subjects and in 10 controls of normal stature. Normal patterns were observed in all subjects with each of the 3 restriction enzymes used. Additionally, the IGF-1 gene was studied in these subjects. Analysis of the chromosomal DNA failed to reveal any abnormal mutation.
5. Fibroblast responsiveness to IGF-1: A punch biopsy skin specimen was obtained in 34 of the 37 children. Cultured fibroblasts were assessed for aminobutyric acid uptake response to recombinant human IGF-1. All the cell lines responded normally.
6. Nitrogen kinetic response: Each child was admitted to the Clinical Research Unit for 9 days to study the acute effects of GH administration on nitrogen kinetics. The patients consumed an isocaloric diet containing 1 g of protein per kilogram daily for the week before the admission, and continued with the same intake during the 9 days of hospitalization. On the second and sixth hospital days, a dose of mixed  $^{15}\text{N}$ -labeled amino acids (1 mg  $^{15}\text{N}$ /kg body weight) was given orally with breakfast. The mixture contained leucine, valine, methionine, phenylalanine, lysine, alanine, aspartic acid, glutamic acid, glycine, serine, and tyrosine. From the morning of the fifth day until discharge, recombinant human GH (rhGH) was injected sc every

12 hours at a dose of 16  $\mu\text{g/kg}$  body weight.

Daily total urinary nitrogen excretion declined slightly but significantly ( $P < 0.05$ ) between the first and second day of hospitalization but remained unchanged from the second to the fourth day. GH injections, begun on the fifth hospital day, produced a second decline in total urinary nitrogen, which achieved a new constant level on days 6 through 8. The total cumulative excretion of  $^{15}\text{N}$  before the administration of GH averaged 20.3% of the dose. During acute supplementation with GH,  $^{15}\text{N}$  excretion declined an average of  $31 \pm 10\%$ , but the change varied considerably among subjects. The  $^{15}\text{N}$  excretion of the 3 GHD children diminished by 31.5, 31.8, and 50.5%. Statistical analysis failed to demonstrate a significant relationship among the subjects' clinical characteristics, BA delay, IGF-1 level, and the degree of nitrogen retention after GH administration.

Whole body protein turnover, synthesis, and catabolism were also evaluated. GH challenge increased the net body protein accretion (synthesis minus catabolism) by more than 200%, from  $0.14 \pm 0.30$  to  $0.35 \pm 0.02$  g/kg $^{-1}$  day $^{-1}$  ( $P < 0.001$ ).

7. Changes in other parameters in response to acute rhGH administration: IGF-1 levels rose significantly with GH administration, but this increment did not correlate significantly with any of the protein kinetic indexes studied.

The acute challenge with rhGH caused no change in circulating GHBP, nor were

any significant changes observed in plasma glucose or insulin values. However, daily urinary C peptide excretion increased significantly ( $P < 0.05$ ) within 24 hours of GH administration and remained elevated throughout the period of GH treatment. Serum osteocalcin levels remained unchanged after 4 days of GH injections.

8. Growth response: On discharge from the hospital, each patient was treated with rhGH at the dose of 75  $\mu\text{g/kg}$  body weight sc tiw. The children were assessed every 3 months over the next 6 to 12 months. Of the 37 subjects who had completed the initial studies, 2 discontinued follow-up. In another 3 children, GH therapy was discontinued at 6 months because height velocities had not increased by more than 2  $\text{cm/yr}$  above pretreatment values. The final examination performed in the remaining "responders" at the 12-month visit showed a mean increase in height velocity from  $4.7 \pm 1.2$  to  $8.3 \pm 1.6$   $\text{cm/yr}$  ( $P < 0.001$ ), producing significant changes in the height Z score and the height velocity Z score for chronologic age (CA) and BA. The 3 GHD children increased their absolute height velocities by 2.6, 6.3, and 9.2  $\text{cm/yr}$ .

9. Predictors of response to GH treatment: Neither the pretreatment IGF-1 nor the acute change IGF-1 to GH challenge predicted the long-term growth response. Only a low pretreatment height velocity correlated significantly with the change in growth rate after 1 year of treatment ( $r = -0.6$ ,  $P < 0.001$ ).

Neither the measured growth rates nor the increments above pretreatment values at 3, 6, or 12 months of therapy correlated

with any of the indices of protein dynamics. However, when the change in height velocity measured after 1 year of treatment was expressed as Z score, there was a weak but significant correlation ( $r = 0.37$ ,  $P = 0.03$ ) with the change in  $^{15}\text{N}$  retention to the acute GH challenge. This relationship was considered by the authors as too weak to be used as a predictor of GH response in individual cases.

Dempsher DP, et al. *Pediatr Res* 1990;28:394-400.

**Editor's comment:** This study is a very comprehensive and sophisticated one. It attempted to ascertain a functional test that would predict the long-term efficacy of GH treatment in short children. The authors measured many of the possible alterations that could lead to disturbed growth and/or short stature in children. They found that in the great majority of instances there was no GHD, nor were there abnormalities in the GH molecule, GHBP, response to IGF, or in the nitrogen kinetic response to GH administration. Yet these children were short.

By sophisticated analysis with stable isotope  $^{15}\text{N}$ -labeled amino acids, total body nitrogen retention as well as protein synthesis, breakdown, and net anabolism were shown to increase with the acute administration of GH. However, the degree of positivity of the nitrogen balance enhanced by GH was extremely variable. Also, the levels of nonabsorption found among short children who had no GH deficits were similar to those who exhibited classic GHD. Moreover, none of the above mentioned measurements were of value in predicting the long-term effects of rhGH in inducing enhanced growth responses.

Unfortunately, there were no measurements of spontaneous

GH secretory rates in these children. Perhaps the spontaneous GH secretion may have shed some light on the variability of response to GH administration. It is possible to speculate that those who had adequate spontaneous GH secretion had the weakest anabolic responses, while those with inappropriate spontaneous secretion may have had the strongest responses.

Once again, there is strong confirmation in this paper of the great value of careful clinical observation and close monitoring of long-term growth in children. The only variable that was of value in predicting the response to GH was the children's pretreatment growth rate. Those who grew at the lowest rate had the most significant enhancement of growth with GH therapy, whereas those who had appropriate growth before treatment had the least significant responses. Thus, the clinician can be thoroughly assured that sophisticated laboratory measurements are no substitute for careful measurements of growth both in assessing short children and in determining the need for therapeutic trials of rhGH. Additionally, caution is advised regarding the questionable improvement of predicted adult height, even among those patients who had significant improvement in growth velocity during the first year of GH treatment.

Fima Lifshitz, MD

#### Erratum

In *GROWTH, Genetics, & Hormones* Vol. 7, No. 2 (June 1991), an error on page 2, Table 1 incorrectly shows the nomenclature for DRBIII italicized and it should not be italicized. DRBIII is not a pseudogene but is expressed.

## Serum Bone GLa Protein (BGP): A Potential Marker of Growth Hormone Deficiency and the Response to Growth Hormone Therapy

Serum bone GLa protein (BGP), a calcium-binding protein of the bone matrix, is the most important noncollagenous protein in the skeleton. BGP has been shown to be an important indicator of the rate of bone formation. In this paper, Johansen and coinvestigators studied the usefulness of BGP in predicting the long-term response of growth hormone (GH)-deficient patients to GH treatment.

Sixty-six GH-deficient children aged 6 to 18 years, 49 boys and 17 girls, were studied before and after 3, 6, 9, and 12 months of daily GH treatment. Patients were divided into 2 groups: those who remained prepubertal throughout the study period were included in group 1 ( $n = 51$ ; mean age, 11.2 years; 35 boys and 16 girls), while those who had entered stage 3 of puberty before starting treatment or those who reached that stage during the study were included in group 2 ( $n = 15$ ; mean age, 14.2 years; 14 boys and 1 girl). Serum BGP concentrations were determined by radioimmunoassay (RIA). Height velocities were estimated from all available height measurements. The change in height velocity from 0 to 12 months of treatment was correlated with the change in BGP concentration at each period.

The mean pretreatment height velocities were 4.5 and 5.1 cm/yr in groups 1 and 2, respectively, while the mean height velocities at 12 months of GH treatment were 8.3 and 8.2 cm/yr in groups 1 and 2, respectively. The mean BGP in patients before treatment was significantly lower than the levels found by other investigators in normal controls ( $P < 0.001$  in prepubertal patients and  $P < 0.01$  in pubertal patients). The BGP levels increased significantly in both groups of GH-deficient children, reaching normal levels at 3 months, and plateaued at a higher level thereafter.

The authors conclude that the present study demonstrates that determination of serum BGP is a valid contribution to the prediction of growth response after 12 months of treatment; the change in serum BGP determined after 3 months of therapy was able to predict the height at 12 months of therapy with the same validity as the prediction at 6 months without using BGP. The present study suggests that determination of serum BGP may help to assess the extent to which bone metabolism is affected in GH-deficient children. Furthermore, serum BGP could be particularly useful to monitor treatment. Measurement of changes in serum BGP after short-term GH administration may thus help to identify those children who will benefit from long-term therapy as well as those who will not respond to therapy.

Johansen JS, et al. *J Clin Endocrinol Metab* 1990;71:122-126.

**Editor's comment:** The efficacy of GH treatment in improving the ultimate height of a child is an important question that has been difficult to address. The response to GH has been widely variable even among patients with GH deficiency. For example, in this study with supposedly GH-deficient subjects there was a normal growth rate before treatment and an improved growth velocity of +3 cm/yr with GH treatment. The growth of these patients and the response to GH is more like that of normal short-statured patients. Usually, GH-deficient patients grow less than 4 cm/yr and exhibit catch-up growth when treated with human GH.

However, it has always been difficult to predict who will benefit most from long-term GH treatment. The good predictive value of serum BGP concen-

trations reported here at 3 months of treatment, if duplicated by other studies, might help the clinician decide which patient will benefit most from treatment. Potentially this would facilitate optimization of dosing regimen, growth response, and monetary expenditures. For example, if the dose of GH being employed does not increase the BGP in an ordinary patient, it may be beneficial to increase the dose; or, if the patient is being treated 3 times per week, it may be better to give it daily. The prompt recognition of poor GH response before wasting many months of treatment would be of great benefit.

Other investigators have studied the usefulness of procollagen levels as a biochemical marker for growth.<sup>1,2</sup> Various methods are available to measure either type I (pColl-1-C) or type III procollagen (P III NP). More studies have been done on P III NP, which also reflects generalized somatic growth, presumably because this assay has been simplified and RIA kits are commercially available. P III NP concentrations have also been found to be good predictors of growth response to GH treatment.<sup>3,4</sup> Comparative studies between BGP and these other procollagen measurements have not been done. This approach is very important.

Fima Lifshitz, MD

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## Meeting Calendar

**November 1-3, 1991** PG Symposium on Ped Diab & Endo (sponsored by the ISGD), New Delhi, India. Info: Prof. I.C. Verma, All India Institute of Medical Sciences, Genetics Unit, Dept of Pediatrics, Old Operation Theatre Building, Ansari Nagar, New Delhi 110 029, India.

**January 3-11, 1992** Introduction to Endocrine Investigations, Pacific Grove, CA. Info: Ann Singer, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

**May 4-8, 1992** Annual Meeting of the APS/SPR/APA, Baltimore Convention Center, Baltimore, MD. Abstract submission deadline 1/3/92. Info: APS/SPR/APA Program Office, 141 NW Point Blvd, PO Box 675, Elk Grove Village, IL 60009-0675. Fax: 708-427-1305

**May 6-8, 1991** Annual Meeting of the LWPES, Baltimore, MD. Info: Dr. G.P. August, Secretary,

LWPES, Children's National Medical Center, 111 Michigan Ave, Washington, DC 20010. Fax: 301-460-8846.

**June 18-23, 1992** 52nd Annual Meeting of the ADA, San Antonio, TX. Info: Meetings Dept, ADA, 1660 Duke St, Alexandria, VA 22314. Fax: 703-836-7439.

**June 24-27, 1992** 74th Annual Meeting of The Endocrine Society, San Antonio, TX. Info: Ann Singer, Meetings Manager, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

**August 30 - September 5, 1992** 9th Int'l Congress of Endocrinology, Nice, France. Info: NICE 92, c/o SOCF1, 14 Rue Mandar, 75002 Paris, France.

**September 7-10, 1992** 31st Annual Meeting of the ESPE, Zaragoza, Spain. Info: Dr. A. Ferrandez-Longas, Endocrine

Unit, Miguel Servet Children's Hospital, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

**September 10-12, 1992** Int'l Congress on Growth Hormone and Somatomedins During Lifespan, Milan, Italy. Info: Drs. D. Cocchi and V. Locatelli, Dept of Pharmacology, School of Medicine, Univ of Milan, Via Vanvitelli, 32, 20129 Milan, Italy.

**June 3-7, 1993** 4th Joint Meeting of the ESPE/LWPES, San Francisco, CA. Info: Prof. M. Grumbach, Dept of Pediatrics, Univ of CA School of Medicine, San Francisco, CA 94143. Tel: 415-476-2244, Fax: 415-476-4009.

**June 9-12, 1993** 75th Annual Meeting of The Endocrine Society, Las Vegas, NV. Info: Ann Singer, Meetings Manager, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

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# GROWTH

## Genetics & Hormones

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## Rickets and Growth

**Dagfinn Aarskog, MD**

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The relationship between growth and the skeletal manifestations of rickets is a time-honored clinical observation expressed in the classic dictum: "No growth, no rickets." Thus, in vitamin D-deficiency rickets the osseous manifestations depend on the age of onset and the relative growth rate of the different bones.<sup>1</sup> In the first year of life, the skull, upper limbs, and ribs are the fastest growing bones and thus prone to be affected. Accordingly, in the youngest infants craniotabes, frontal bossing, thickening of the wrist, and visible enlargement or palpable swelling of the costochondral junction (rachitic rosary or beads) are the characteristic skeletal manifestations. In the second year of life, the legs grow faster and the effect of weight bearing results in bowing of the legs, or genu varum. The angulation is especially pronounced at the junction of the lower third and upper two thirds of the leg. Later in childhood, vitamin D-deficiency rickets is rare but might occur during the growth spurt of puberty. The most prominent osseous manifestation of adolescent rickets is the occurrence of "knock knee," or genu valgum.

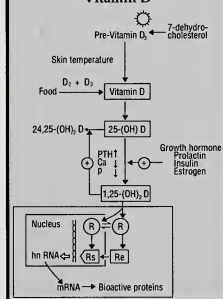
The assessment of the direct effects of vitamin D deficiency on growth is complicated by the degree and duration of bone deformities and the effects of concomitant nutritional deficiencies other than vitamin D. However, the general consensus among the pioneers in pediatrics at the turn of the century, when rickets was endemic in Northern Europe and North America, was that only part of the reduced height in patients with rickets could be ascribed to the deformity of the lower extremities.

### VITAMIN D METABOLISM

Vitamin D was discovered in 1932. For many years, the vitamin was considered to be the active agent in controlling calcium and phosphate metabolism. The discovery of 25-hydroxyvitamin D (25-(OH)D) in 1968 paved the way for new progress, culminating with the identification of 1,25-dihydroxyvitamin D (1,25-(OH)<sub>2</sub>D) in 1971. The appreciation of 1,25-(OH)<sub>2</sub>D as the principal and most potent form of vitamin D, along with the elucidation of the molecular mechanism of action, which is analogous to that of classic steroid hormones, led to the recognition of vitamin D as a precursor of a potent steroid hormone rather than as a vitamin in the context of an essential nutritional substance. Recently, the identification of many new receptor-positive target tissues for 1,25-(OH)<sub>2</sub>D beyond the classic ones of the intestine, the skeletal system, and the kidney implies that the hormone might have functions beyond its classic role in mineral homeostasis.<sup>2</sup>

The general term vitamin D refers to both vitamin D<sub>2</sub> (ergocalciferol), which originates in plants, and to vitamin D<sub>3</sub> (cholecalciferol), which is produced in the body. In humans, both compounds appear to be equipotent, and the requirements for the vitamin can be satisfied by either. Vitamin D undergoes 2 hydroxylation steps before it becomes biologically active (Figure 1). The first takes place in the liver to form 25-(OH)D,

**Figure 1**  
**Synthesis of 1,25-(OH)<sub>2</sub> Vitamin D**



which is the major circulating metabolite of vitamin D. The serum concentration of 25-(OH)D reflects the vitamin D status of an individual, and is primarily determined by sunlight exposure and dietary supply of parent vitamin D. In temperate zones, the mean serum level is approximately 30 ng/mL (75 nmol/L), with a range of 10 to 50 ng/mL (25 to 125 nmol/L). Seasonal variation has to be taken into account, with the highest levels occurring in late summer and the lowest in late winter. A serum level

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## Letter to the Editors

Dear Drs. Blizzard, Stanhope,  
and Hindmarsh:

In your article on oxandrolone therapy (*GGH* 1991; Vol 3, No. 1:1-6) you gave significant discussion to studies supporting the use of oxandrolone to increase final adult height. However, you gave only the statement, "The effect of oxandrolone treatment on final adult height in Turner syndrome is somewhat controversial," in response to a significant body of literature suggesting oxandrolone makes no difference in adult height. In my study (*J Pediatr* 1984;104:365) of 66 adult patients with Turner syndrome, of whom 29 were treated with androgens (28 oxandrolone), no significant difference in adult height was noted. Moore previously reported some of these adult patients (whom you cite in defense of oxandrolone use) as successful results of oxandrolone therapy. In the end, these patients had no difference in ultimate height compared to untreated patients.

Others also found no significant difference in adult height (Lev-Ran; Mauri et al; Muritano and Job). They were similarly unimpressed with the effects of oxandrolone on final adult height.

The way in which the studies are described about patients with Turner syndrome in your article suggests that Stahnke et al found that oxandrolone therapy "increased final adult height in many patients." Stahnke did not present any data about final adult height in his study (in a letter to the Editor in the *Journal of Pediatrics*, 1980). He also stated that Joss and Zuppinger showed a significant increase in final adult height in all 15 oxandrolone-treated patients versus the untreated controls. This was true only for 8 patients who received 2 years of therapy. The difference between the control group and those who received only 1 year of therapy was not significant.

I agree that oxandrolone therapy increases growth velocity in Turner syndrome, but the evidence supporting its beneficial effect on adult height is limited and there is considerable evidence against this hypothesis. I also recognize that many feel that the "psychological

*continued on page 4*

below 8 ng/mL (20 nmol/L) indicates a state of vitamin D deficiency. Although 25-(OH)D is 2 to 5 times more potent than the parent hormone, it is not active at physiologic concentrations. To achieve full potency, 25-(OH)D is further hydroxylated in the kidney to 1,25-(OH)<sub>2</sub>D. The circulating concentration is approximately one thousandth that of 25-(OH)D. In normal children, the serum level of 1,25-(OH)<sub>2</sub>D ranges between 25 and 85 pg/mL (60 to 120 pmol/L). The higher values in infancy and adolescence probably reflect the need for increased intestinal calcium absorption during these periods of rapid growth.<sup>3</sup>

The conversion of 25-(OH)D to the active hormonal form 1,25-(OH)<sub>2</sub>D is strictly regulated. The regulatory factors include parathyroid hormone (PTH), calcium, phosphate, and 1,25-(OH)<sub>2</sub>D itself (Figure 1). PTH is the main stimulatory factor, and accordingly the serum level of 1,25-(OH)<sub>2</sub>D is elevated in both primary and secondary hyperparathyroidism. The regulatory effect of calcium is probably indirect and mediated by stimulation of PTH secretion in hypocalcemic states. Dietary phosphate restriction and hypophosphatemia increase the serum concentration of 1,25-(OH)<sub>2</sub>D, whereas high intake of phosphate decreases the level. Several endocrine factors have a direct or indirect effect on 1,25-(OH)<sub>2</sub>D production (Figure 1). Children with growth hormone deficiency have normal serum concentrations of 1,25-(OH)<sub>2</sub>D. High doses of growth hormone raise their serum levels of 1,25-(OH)<sub>2</sub>D over the first week of treatment, whereas long-term replacement therapy does not cause such an effect.

Another major metabolite of 25-(OH)D formed in the kidney is 24,25-dihydroxyvitamin D (24,25-(OH)<sub>2</sub>D). In normal children and adolescents, the serum concentration of 24,25-(OH)<sub>2</sub>D is approximately 3% to 6% of the 25-(OH)D level. The production of 24,25-(OH)<sub>2</sub>D is regulated by the same factors as the 1,25-(OH)<sub>2</sub>D formation, but in the opposite direction (Figure 1). Although the physiologic role of 24,25-(OH)<sub>2</sub>D remains unclear, it is regarded as an alternate path to the formation of 1,25-(OH)<sub>2</sub>D to form a minimally potent instead of a maximally potent steroid.

## BONE AND MINERAL METABOLISM

The importance of vitamin D for the maintenance of mineral homeostasis and normal bone growth and mineralization is apparent during states of either

deficiency or resistance to vitamin D. Much of the effect of 1,25-(OH)<sub>2</sub>D on bone mineralization is probably indirect, ie, providing minerals for incorporation into bone matrix through increased intestinal absorption of calcium. The essential bone lesion in rickets is an accumulated excess of osteoid tissue resulting from a lag in the mineralization of the cartilaginous epiphyseal plate.

The formation of new bone is a function of the osteoblasts, which possess 1,25-(OH)<sub>2</sub>D receptors, and probably are the primary target cells for 1,25-(OH)<sub>2</sub>D in bone. Receptor-mediated effects of the hormone include modulation of the proliferation of osteoblasts and the production of alkaline phosphatase and osteocalcin.<sup>2</sup>

## CLASSIFICATION OF RICKETS

Vitamin D-deficiency rickets was once extremely prevalent in Northern Europe and North America, but was nearly eradicated following the introduction of prophylactic vitamin D supplementation in the 1930s and early 1940s. Today the children at risk of developing vitamin D-deficiency rickets are mainly immigrants of Asian origin and children on strict vegetarian diets, cult diets, or other fad diets (Table 1). Provided prompt diagnosis and proper treatment, these children will experience only a short and transient slowing of the growth rate having little impact upon final adult height.

**Table 1**  
**Etiologic Classification**  
**of Rickets**

### **Vitamin D Deficiency**

Lack of sun exposure  
Dietary vitamin D deficiency  
Vitamin D malabsorption  
Anticonvulsant therapy

### **Decreased Synthesis of 1,25-(OH)<sub>2</sub>D**

Vitamin D-dependent  
rickets type I

### **End-Organ Resistance**

Vitamin D-dependent  
rickets type II

### **Phosphate Deficiency**

X-linked hypophosphatemia  
Tumor-associated  
hypophosphatemia  
Fanconi's syndrome  
Hypercalciuric hypophosphatemia

Vitamin D-dependent rickets type I is a rare autosomal inborn error of metabolism: 25-(OH)D is not converted to 1,25-(OH)<sub>2</sub>D and the serum concentration of 1,25-(OH)<sub>2</sub>D is low. The resulting hypocalcemia leads to secondary hyperparathyroidism, increased phosphate excretion, and subsequent hypophosphatemia. The clinical manifestations, which are similar to those in vitamin D deficiency, usually appear before 1 year of age and include hypotonia and growth failure.<sup>45</sup>

1,25-(OH)<sub>2</sub>D or its synthetic analogue, 1- $\alpha$ -hydroxyvitamin D, is used for treatment. The latter is available in solution and is more convenient for infants and young children. The biologic activity of 1- $\alpha$ -(OH)D is about one half to two thirds that of 1,25-(OH)<sub>2</sub>D. The recommended doses in treatment of active rickets are 2 to 8  $\mu$ g/d of 1- $\alpha$ -(OH)D, or 1 to 4  $\mu$ g/d of 1,25-(OH)<sub>2</sub>D until radiologically demonstrated healing occurs. This initial treatment takes about 2 to 5 months and is followed by a lifelong supplemental dose of 0.5-2  $\mu$ g/d of 1,25-(OH)<sub>2</sub>D. To avoid overtreatment, the serum concentration of calcium and phosphate and the urinary Ca:Cr ratio should be measured periodically. When Ca:Cr exceeds 0.25, the dose should be reduced.

Since replacement therapy in physiologic amounts of 1,25-(OH)<sub>2</sub>D results in complete correction of the phenotype, including normalization of growth, this disorder or "experiment by nature" probably offers the best human model to assess the effect of 1,25-(OH)<sub>2</sub>D on growth.

Vitamin D-dependent rickets type II is caused by end-organ resistance to 1,25-(OH)<sub>2</sub>D due to defective cellular receptor hormone binding and/or expression. At present, 5 different patterns of defects in this rare disorder have been outlined. The clinical hallmarks include early onset of rickets, hypocalcemia, secondary hyperparathyroidism, and very high levels of 1,25-(OH)<sub>2</sub>D. Alopecia has been noted in about half of the patients. Symptoms, including growth failure, usually appear before 1 year of age.<sup>4</sup>

The patients usually are responsive to high doses of 1,25-(OH)<sub>2</sub>D, or 1- $\alpha$ -(OH)D, with or without calcium supplementation. Such treatment can heal the rickets, but alopecia never improves. In patients who respond to therapy, the prognosis appears to be good for growth and development. X-linked hypophosphatemic rickets presents with shortness of stature, bowlegs, and hypophosphatemia. Roentgenologic manifestations of rickets are evident by 1 to 2 years of age. Because this is an X-linked dominant trait, males are more severely affected than females.

The biochemical findings are characterized by low serum phosphate, normal or low-normal calcium level, and mild elevation of alkaline phosphatase activity. The serum concentration of PTH is usually normal. The level of 1,25-(OH)<sub>2</sub>D is also within normal limits, but often this is inappropriately low in view of the hypophosphatemia.

Combined treatment with adequate doses of oral phosphate and 1,25-(OH)<sub>2</sub>D or 1- $\alpha$ -(OH)D increases growth rates and heals rickets. If treatment starts before 5 years of age, catch-up

growth and correction of lower limb deformities can be achieved.<sup>3</sup> The recommended daily phosphate supplement of 1 to 3 g of elemental phosphorus is administered in 4 to 6 divided doses. To avoid gastrointestinal intolerance of phosphate, the initial dose should be small and increased over several months. The initial dose of 1,25-(OH)<sub>2</sub>D is 15 to 20 ng/kg/d, and is increased over several months to a maintenance dose of 30 to 60 ng/kg/d. During long-term treatment, the serum concentration of calcium and phosphate and the alkaline phosphatase activity should be monitored at regular 1- to 3-month checkups. Radiologic evaluation of the wrists, ankles, and knees, as well as renal ultrasound examinations, should be carried out at 12-month intervals to assess healing of rickets and provide early detection of nephrocalcinosis.

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# Obesity in Childhood and Adolescence

## Part 2: Pathophysiology, Associations, and Complications

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Childhood obesity now may be the most prevalent nutritional disease in the United States. In the past, the sense of futility that has accompanied unsuccessful attempts at therapy has led

many to ignore the disease and its complications. However, recent advances in successful therapy<sup>1,2</sup> suggest that the persistence of the disease is not inevitable. Furthermore, new information regarding its aftereffects provides new insights and poses additional questions regarding its physiology.

## PATHOPHYSIOLOGY

Although the similarity in fatness within families<sup>3</sup> and twins<sup>4</sup> is popularly interpreted as evidence that a genetic cause

exists for obesity, genetics probably confers only an increased susceptibility to obesity. The causes of obesity are those factors that either increase energy intake, reduce energy expenditure, or impair the regulation of energy balance. In a previous issue of *GROWTH, Genetics, & Hormones* (Vol 7, No. 1), we examined host factors that might increase susceptibility. Basal metabolic rate (BMR) and the thermic effect of food (TEF) appear to be genetically mediated components of energy expenditure, but no significant differences of



## Letter to the Editors

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beneficial effects" of an induced growth spurt justifies its use in Turner syndrome. This contention has not been rigorously studied. What I am most concerned about in your article is that you have not played fairly with the studies that are available in the literature and have presented a biased view in what purports to be an even-handed review.

Virginia P. Sybert, MD  
Associate Professor  
Department of Pediatrics  
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Seattle, Washington

### Response From the Editors

Dr. Sybert believes that the recent review concerning oxandrolone in GGH ignores a considerable body of evidence that does not support the use of oxandrolone to increase final adult height in Turner syndrome. We respond as follows.

Several published studies on low-dose oxandrolone therapy in Turner syndrome reported improvement in adult stature. Recent reviews of the use of anabolic steroids to manage Turner syndrome from Joss (1988) and Naeraa et al (1990) imply that oxandrolone may increase ultimate height but acknowledged, as did we in GGH, that the issue is not settled. Just as importantly, authors in the 7 published articles in the world literature addressing

the effect of oxandrolone on final adult height in Turner syndrome reported no adverse effect on final height, and the majority reported a significant improvement, on the average of approximately 4 cm over that expected (ie, Naeraa et al, 1990; Heidemann et al 1987; Joss et al, 1984; Sybert, 1984; Urban et al, 1979; Moore et al, 1977; Stahnke et al, 1985). Stahnke et al did in fact report in abstract form that oxandrolone increased final adult height in Turner syndrome (Pediatr Res 1985;19:620) although as stated by Dr. Sybert, not in a letter to the editor in the Journal of Pediatrics in 1980.

Regarding the data of Joss and Zuppinger (1984), the conclusions stated in the GGH review are in accord with the authors' statement, namely, that final adult height was significantly improved in girls treated with oxandrolone for 12 months ( $n = 7$ ;  $P < 0.05$  for the index of predicted height [IPH] method, but not in comparison to the control group) and 24 months ( $n = 8$ ;  $P < 0.01$  for IPH and Bayley Pinneau [BP] methods). While true that the final adult height difference at 12 months was not significant between groups (using the BP method), Joss and Zuppinger found that the IPH method is more predictive in girls with Turner syndrome. Thus, our presentation of the data is in full accord with the authors' interpretation of the data.

We wish to emphasize that all anabolic steroids are not necessarily equal in their actions. Thus, it may be inappro-

priate to generalize about potential effects of this class of drugs, which is what Dr. Sybert has done in her letter. Dr. Sybert references, for example, the work of Lev-Ran (1977) and Muritano and Job (1985) as evidence against a positive effect of oxandrolone on final adult height. Oxandrolone was not used in these studies. Instead, other anabolic steroids were evaluated. These studies were not included in our review in GGH since, in our opinion, all anabolic steroids are not necessarily equal in their action.

Last, and perhaps most importantly, the doses of oxandrolone utilized in patients with Turner syndrome in the Sybert report were 0.13 to 0.29 mg/kg/d and not  $\leq 0.1$  mg/kg/d, which is the maximum dose we believe should be used in order to prevent rapid skeletal maturation.

On the basis of these points, we take exception to Dr. Sybert's statements. While the data on the effect of oxandrolone on final adult height in Turner syndrome is inconclusive, the findings with low doses (ie,  $\leq 0.1$  mg/kg/d) in girls with bone ages  $\geq 8$  to 9 years at initiation of therapy suggest final adult height can be increased or, at a minimum, not adversely affected.

The authors sincerely thank Dr. Sybert for expressing her views, and invite others to do similarly.

Robert M. Blizzard, MD  
Peter C. Hindmarsh, MD  
Richard Stanhope, MD

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either BMR or TEF have been demonstrated among obese and nonobese children or adolescents. The energy spent on physical activity represents the most variable and discretionary component of daily expenditure. Therefore, increases in food intake, reductions in the quantity of energy spent on activity, or impaired regulation of energy balance are the most likely sources of energy imbalance in obesity.

Defective regulation of energy balance appears to cause obesity in patients with hypothyroidism, Prader-Willi syndrome (PWS), or hypothalamic tumors, or after removal of craniopharyngiomas.

Patients who have PWS are short, mildly retarded, sexually underdeveloped, and massively obese. The chromosomes of approximately half of PWS patients carry a partial deletion of the paternally derived chromosome 15 (15q11-13). However, patients with the syndrome who

lack the deletion are identical in every respect to those who demonstrate it.<sup>4</sup> This susceptibility to obesity may be increased by reductions in fat-free mass and a low metabolic rate,<sup>5</sup> but obesity is caused by a ravenous appetite. A second small group of patients who demonstrate the same deletion in the maternally derived chromosome 15 develop Angelman's syndrome,<sup>6</sup> a genetic disorder characterized by mental retardation, ataxia, and inappropriate laughter. Obesity in Angelman's syndrome has not been reported. Although both disorders are rare, they offer a rich and fascinating opportunity to understand how genotype affects not only phenotype but behavior.

The majority of obese children lack any evidence that the regulation of food intake is impaired by intracerebral abnormalities. The observation that some obese children gain weight rapidly along curves of unvarying slope suggests that we should not dismiss the possibility that disordered

regulation of food intake may account for a small percentage of childhood obesity. Nonetheless, the strong associations of environmental variables with obesity indicate the interaction of strong behavioral determinants.

### ASSOCIATIONS

Environmental variables act on the susceptible host to produce obesity. Clues to these variables, although not their action, are found in the environmental variables associated with obesity. Childhood obesity is associated with region, season, and population density. Obesity is more prevalent in the winter and spring, followed by the summer and fall, and is more prevalent in the Northeast, followed in descending order by the Midwest, South, and West.<sup>8</sup> In each region, obesity is more frequent in large metropolitan areas than in any other sampling area. Obesity is also strongly associated with

family variables, such as parental obesity, parental age, family size, socioeconomic class, and parental education.<sup>3</sup> Whether the behaviors linked to these epidemiologic characteristics act to increase food intake, reduce the energy spent on activity, or impair the regulation of energy balance remains unclear.

Television viewing and parental exercise patterns may act to decrease activity. Television viewing is directly related to the prevalence of obesity, and appears to reduce activity and increase food intake.<sup>10</sup> Fatness in children is also inversely related to parental exercise patterns, although fitness is related only to the mother's pattern of exercise.<sup>11</sup>

## COMPLICATIONS

Obesity is associated with a variety of physiologic consequences, many of which impact adversely on health. These consequences can be broadly divided into those that result from the aurogenic effects of obesity, the mechanical effects of increased fat mass, and the metabolic effects of increased body fat.

## AUROGENIC EFFECTS

The effects of obesity on growth have been well described. Obese children and adolescents have increased height velocities,<sup>12</sup> although usually ultimate height is not increased. As a result, they are taller than their nonobese peers prior to epiphyseal closure. Bone ages are generally advanced,<sup>13</sup> although probably not in excess of height age. Fat-free mass is also increased,<sup>14</sup> and accounts for the greater BMR observed in obese children and adolescents.<sup>15</sup> However, even when fat-free mass is controlled, metabolic rate appears greater in obese than in nonobese adolescents.<sup>16</sup> The apparently constant rate at which fat-free mass increases with weight gain and decreases with weight loss<sup>16</sup> suggests that adipose tissue changes in concert with fat-free mass. Likewise, the parallel changes in bone age and height velocity suggest that the normal process of growth can be systematically advanced by overnutrition. Nonetheless, the mechanisms by which increases in fatness produce these other effects of growth remain unclear.

Menarche occurs earlier in obese adolescent females. Although earlier menarche has been attributed to the acquisition of body fat necessary to support a pregnancy,<sup>17</sup> this explanation is probably overly simplistic. Evidence to support this hypothesis was derived from estimates of fatness based on weight and height,<sup>18</sup> which are considerably less reliable than direct measures of fatness.<sup>19</sup>

Furthermore, changes in relative weight may be less influential than skeletal maturation as a determinant of menarcheal age.<sup>20</sup>

## MECHANICAL EFFECTS OF OBESITY

The mechanical effects of obesity can be grouped into effects on bone growth, respiratory status, and psychosocial function. Increased weight bearing generated by obesity causes bowed femurs, Blount's disease (or tibia vara), and slipped caput femoral epiphysis. Increased weight acting on cartilaginous bone in young children produces bowed tibia, and increased bone deposition on the medial side of the proximal tibia. Interestingly, the degree of bowing correlates well with the degree of obesity. Obesity probably acts on the femurs in a similar fashion. Slipped caput femoral epiphysis results from the effects of increased weight across the femoral neck, and the caput of the femur slips on its epiphysis. Although Blount's disease and slipped caput femoral epiphysis may occur in nonobese individuals, the majority of patients affected with these disorders are obese.

The most frequent respiratory disorder associated with obesity is sleep apnea,<sup>21</sup> which is probably caused by increased peripharyngeal fat that narrows the airway. In the pickwickian syndrome, which occurs less frequently, increased intra-abdominal fat decreases the diaphragmatic excursion to produce CO<sub>2</sub> retention. Increased PCO<sub>2</sub> produces CO<sub>2</sub> narcosis and, like sleep apnea, daytime somnolence. In sleep apnea, daytime somnolence results from sleep deprivation incurred by recurrent arousals in response to sleep apnea. In the pickwickian syndrome, daytime somnolence results from CO<sub>2</sub> narcosis. In both disorders, hypoxia may produce cardiac arrhythmia and death.

Peer discrimination and low self-esteem also result from increased body fatness. Our culture is highly sensitized to fatness, and discrimination against the obese begins at an early age.

## METABOLIC EFFECTS

In adults, the distribution of fat is directly related to the risk of hypertension, diabetes mellitus, hyperlipidemia, and atherosclerotic cardiovascular disease.<sup>22</sup> Furthermore, the regional deposition of body fat in response to overfeeding appears genetically determined.<sup>23</sup>

In the past several years, a cohesive explanation has evolved to explain the relationship between the regional distribution of fat and its pathologic consequences.<sup>22,24</sup> The distribution of body fat is

most commonly assessed by the waist:hip ratio. Excess intra-abdominal fat (android or "apple-shaped" obesity) carries a substantially greater risk of these complications than femoral fatness (gynoid or "pear-shaped" obesity). In android obesity, intra-abdominal fat may be more sensitive to factors that promote lipolysis.<sup>25</sup> High free fatty-acid levels may reduce insulin uptake by the liver. Increased insulin levels may affect blood pressure by increasing sodium reabsorption by the kidney,<sup>26</sup> and by increasing sympathetic nervous system activity in the heart and peripheral vasculature.<sup>24,27</sup>

Hypertension, abnormal glucose tolerance, and hyperlipidemia also occur in obese children and adolescents, but the frequency of these complications is lower than in adults. Furthermore, the relationship of fat distribution to morbidity in the pediatric age group remains unclear. Central obesity has been associated with the insulin response to a glucose load in normal adolescents,<sup>28</sup> and with blood pressure in normal children<sup>29</sup> and normal and obese adolescents.<sup>30</sup> Because none of these studies controlled for total body fat, it is not clear whether fat distribution affects morbidity more than total body fat.

In males, puberty is accompanied by an increase in skinfolds on the trunk, whereas in females, the increase in body fat is more uniform.<sup>31</sup> However, it is not yet clear whether the pattern of fat deposition increases the likelihood of the metabolic aftereffects of adolescent-onset obesity, or whether the effect of fat localization on morbidity is independent of the degree of excess fat.

The metabolic effects of body fat also play an important role in the genesis of polycystic ovary disease (PCOD).<sup>32,33</sup> PCOD may begin in adolescence, and may affect 4% of women. One third of affected patients have android obesity.<sup>34</sup> Affected women tend to have amenorrhea, hirsutism, and infertility. Fat contains an aromatase that converts androgens, particularly androstenedione, to estrone. In addition, the hyperinsulinemia of obesity may increase androstenedione synthesis by the ovary. The net effect of increased estrone may be to alter the rhythmicity or levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to produce abnormal stimulation of the ovary and PCOD. Weight loss may reverse the cycle entirely.<sup>32,33</sup> In some families, PCOD appears to be inherited as an autosomal dominant trait. However, primary abnormalities of insulin action that lead to obesity are suggested by the finding of acanthosis nigricans, insulin resistance, and polycystic ovaries in a woman with an abnormality of the insulin receptor.<sup>35</sup> The finding that 5% of all

women with PCOD had acanthosis nigricans, obesity, and hyperinsulinemia<sup>26</sup> suggests that this defect may be among the most common genetic syndromes associated with obesity.

## SUMMARY

The diversity of central and peripheral host factors that can influence the susceptibility and causes of obesity is aptly illustrated by PWS and PCOD. The extent to which these factors operate in the general population and the behavioral linkages that connect the epidemiologic variables associated with obesity to these or other host factors remain uncertain. However, these observations suggest that even the prevalent and apparently mundane disease of obesity is still surrounded by a multiplicity of questions, the answers to which promise new insights into the metabolic interactions of genetics and growth.

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# Annual Meeting of the Endocrine Society: Highlights

Dr. Seymour Reichlin opened the symposium with an elegant review of the endocrine physiologic factors that interact in response to stress. The entire brain-hypothalamic-pituitary end-organ axis is activated. Much of the presentation centered on the neuroendocrine aspects, with emphasis on the interactions between the neuroendocrine axis for corticotropin release and the immune regulatory system. Interleukins 1 and 6 (IL-1 and IL-6) are particularly involved in the activation of the neuroendocrine system in response to stress; however, it is not a global activation. Data were presented to show that IL-1B activation through specific IL-1 receptors differed substantially from the bacterial lipopolysaccharide-stimulated IL-6 release of rat anterior pituitary cells.

Exciting new insights into the interactions among the great integrative systems of physiology (endocrine,

neuroendocrine, and immunologic) will be forthcoming in the near future as some of the endocrine, paracrine, and possibly autocrine activities are uncovered and the corresponding physiologic mechanisms described.

Dr. Larry Parker reviewed the past decade's data on the elusive pituitary factor that stimulates adrenal androgen production. This factor, alternatively called AASH (adrenal androgen-stimulating hormone) and CASH (cortical androgen-stimulating hormone), reportedly is synthesized in the anterior pituitary gland, differs from corticotropin, and is regulated by mechanisms that differ from those described for corticotropin. Partial amino-acid sequence data were presented that indicate/identify with a part of the joining peptide of pro-opiomelanocortin (POMC), but at present there are no unequivocal chemical or biologic data to consider the AASH (activity) a single, distinct entity with a

proven structure and physiologically relevant activities.

Dr. Lynn Loriaux delivered another of his comprehensive, well-balanced lectures on the vast experience of the National Institutes of Health (NIH) group using corticotropin-releasing hormone (CRH). The subjects chosen — patients with Cushing's syndrome and those with depression — reflect the great numbers of patients referred to the NIH and the close working relationship between the psychiatrists and the endocrinologists. Responses to exogenous CRH are abnormal in both groups of patients. Exogenous CRH is probably more helpful in categorizing patients with Cushing's syndrome into specific pathophysiologic entities, eg, ectopic Cushing's syndrome and Cushing's disease. Emphasis was placed upon the

clinical presentation and the sum of the biochemical and imaging tests rather than upon the CRH (or any other single) test.

Suffice it to say that there remains significant biologic variability even within seemingly single biologic entities and that the CRH test is but another "window" into the disordered neuroendocrine axis for adrenal function in both of these syndromes.

Dr. Louis Underwood opened the symposium with a review of the expanding role for growth hormone (GH) in non-GH-deficient states. Great controversy still exists in the definition of some of these states and upon the efficacy of both short- and long-term hormonal treatment. GH is efficacious in accelerating growth in girls with Turner syndrome and may increase adult height in these girls. A particularly exciting new

use is to increase the efficient harvesting of ova for in vitro fertilization.

Dr. Richard Fine presented an update of his and other studies using GH in growth-retarded children with chronic renal insufficiency. There are a number of relatively short-term studies that indicate a growth-promoting effect of this hormone in these children. None has followed children to adult height to see if the predicted gains actually continue to final height. The data were exciting, and the role of GH treatment in this and other conditions is but one of the avenues being explored.

Alan D. Rogol, MD, PhD

## Abstracts From the Literature

### Pubertal Growth in Chronic Renal Failure

This paper analyzes the height growth of 15 boys and 14 girls with end-stage renal failure first studied before puberty and followed at 3- to 6-month intervals until growth ceased or nearly ceased. The height data were smoothed by the kernel estimation method, which is a form of moving average. The records were from Heidelberg, and the curves were compared with those from the Zurich Longitudinal Growth Study. This made possible a comparison with late normal maturers as well as with the average maturers in a normal growth study.

The start of the pubertal growth spurt was delayed by 2.5 years in both the girls and boys, and its duration and intensity were also very significantly reduced, with the mean height gain at around 50% of that observed in the late-maturing control group. However, mean height at the onset of the spurt was approximately the same as that in the late-maturing control group. The data indicate that most patients with end-stage renal failure occurring before or during puberty irreversibly lose growth potential. Renal transplantation did not consistently improve pubertal growth.

Schaefer F, Seidel C, Binding A, et al. *Pediatr Res* 1990;28:5.

**Editor's comment:** This paper is particularly striking because of the use of the kernel estimation method, which, in my opinion, is currently the most advanced technique for analyzing growth curves. Since it is nonparametric, it is

*particularly applicable in cases of growth disorder, and this paper constitutes a real model for other research workers studying growth in chronic disease. It is interesting that in the patients with renal failure, puberty did not start until their height had reached virtually that of the controls when they started puberty; however, by this time height velocity was far below normal and the subsequent pubertal spurt was very much reduced. Such a fine analysis does require many measurements of height to be made during the growth period but results in a much better understanding of the dynamics associated with the disorder than has previously been possible.*

James M. Tanner, MD

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#### In Future Issues

**Insulin-Like Growth Factors and In Utero Growth**  
by Joseph D'Ercole, MD

**Testis-Determining Factor: An Update**  
by Barbara C. McGillivray, MD

**GH Deficient-Like Syndromes and Their Etiologies**  
by William H. Daughaday, MD

**Intrauterine Growth Restriction Revisited**  
by Joseph D. Warshaw, MD



## X Inactivation Is Not Really Complete Inactivation of the Whole Chromosome

X-chromosome inactivation in mammals is a regulatory phenomenon in which gene expression from 1 of the 2 X chromosomes in female cells is inactivated, resulting in dosage compensation for X-linked genes between females (with 2 X chromosomes) and males (with only 1). However, a series of papers recently indicates that the inactivated X is not completely silent. There are now at least 4 regions on the short arm and 2 regions on the long arm of the inactivated X that contain actively expressed genes.<sup>1</sup> One of these genes, cloned by Fisher and colleagues, codes for a ribosomal protein, which has a homologue on the Y chromosome. The authors suggest that some or all of the features of Turner syndrome (45,X) could be the result of haploinsufficiency for this type of protein.<sup>2</sup> Another interesting area on the long arm of the X chromosome is a region that has been identified by Brown and colleagues as the putative X-inactivation center (XIC), mapped to Xq13.<sup>3</sup> This is believed to be a locus that is blocked by

a *trans*-acting factor in the active X, ie, unless it is blocked the chromosome is inactivated.<sup>4</sup> Brown et al have also identified a gene that produces an RNA transcript only from the *inactive* copy of the X chromosome. In addition, this gene, called XIST (for X inactive-specific transcript), appears to lie in the same region as the putative XIC. Thus, it is a candidate for a gene either involved in or uniquely influenced by the process of X inactivation.<sup>5</sup>

**Editor's comment:** *The mechanism by which the genes on the inactive copy of the X chromosome are silenced has long been an enigma. The mystery has been heightened by the discovery that many genes escape inactivation, ie, it is not an "all-or-none" phenomenon. The localization of the XIC and of a gene that is expressed only by the inactive copy of the X chromosome will hopefully provide tools with which to dissect the process of X inactivation on a molecular level.*

Judith G. Hall, MD

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## IGF-2 and IGF-2 Receptor: Evidence for Genomic Imprinting and Complementarity in Parental Genetic Contribution to Growth

A compelling body of evidence from widely diverse areas of research suggests that the expression of some genes depends upon their parental origin, ie, whether they have been inherited from the mother or from the father. Recent developments in studies of a growth factor gene and its receptor indicate that genomic imprinting is involved in the regulation of expression of these genes.

Horton reported in the March 1991 issue of *GGH*<sup>1</sup> on the experiments by DeChiara et al that provided direct evidence for the role of insulin-like growth factor 2 (IGF-2) in antenatal growth.<sup>2</sup> These experiments demonstrated that transgenic mice carrying 1 normal and 1 disrupted copy of the IGF-2 were much smaller than normal controls with 2 functional copies of the gene. Subsequently, these same authors found that when the disrupted IGF-2 gene is transmitted to offspring through the male germ line, progeny are growth deficient, as shown previously. However, when the disrupted gene is transmitted maternally, the heterozygous offspring (ie, 1 normal copy and 1 disrupted copy) are phenotypically

normal. They also found that only the paternal allele is expressed in embryos, while the maternal allele is silent. In addition, homozygous offspring carrying 2 copies of the disrupted gene were indistinguishable from the heterozygotes. They thus concluded that the IGF-2 gene is subject to parental imprinting.<sup>3</sup>

At approximately the same time, Barlow et al began studying the T-associated maternal effect (*Tme*) mutation in mice. This defect is nuclear-encoded, and embryos that inherit a deletion of the *Tme* locus from their mother die at day 15 of gestation. Barlow and colleagues found that the genes for the IGF-2 receptor (IGF-2r) lie within the region of this deletion, thus making it a candidate for the *Tme* gene. They also demonstrated that embryos express IGF-2r only from the maternal chromosome, and it is therefore paternally imprinted.<sup>4</sup>

**Editor's comment:** *These studies of a growth factor gene and its receptor provide further support for the theory of genomic imprinting, and indicate that the maternal and paternal genetic contri-*

*butions may play complementary roles in growth regulation, acting to balance each other so that growth proceeds in a controlled fashion but does not proceed beyond certain carefully regulated boundaries. The identification of specific genes that are imprinted also provides excellent tools for determining the molecular mechanism(s) of imprinting.*

Judith G. Hall, MD

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## The Protective Effect of Growth Hormone on Steroid Damage to Bone and Cartilage in Mice

Glucocorticoid therapy in children and young animals causes growth deceleration and degenerative changes of bone and cartilage. To evaluate the possible therapeutic effect of growth hormone (GH) in the presence of excessive glucocorticoids, 3-week-old female ICR mice were treated IM for 4 weeks as follows ( $n = 5$  each group): (1) Control, saline. (2) Dexamethasone (DEX), 1 mg/kg/d. (3) recombinant GH (rGH) 1 mg/kg/d. (4) bovine GH (bGH), 1 mg/kg/d. (5) DEX + rGH. (6) DEX + bGH. Tibiae and vertebrae were analyzed for morphometric and biochemical parameters. Growth, as measured by weight and tibial length, was compromised in the DEX group, but reversed to control growth rate by rGH and bGH. Epiphyseal growth plate width was  $295 \pm 17 \mu$  in the DEX group,  $360 \pm 26 \mu$  in the DEX + rGH ( $P < 0.01$ ), and  $352 \pm 34 \mu$  in the DEX + bGH group ( $P < 0.02$ ). Cortical bone width was  $265 \pm 22 \mu$ ,  $292 \pm 27 \mu$  and  $285 \pm 31 \mu$ , respectively ( $P > 0.05$ ). Trabecular bone volume, as percent of total bone volume, was  $9.8 \pm 2\%$  in the DEX group,  $22.1 \pm 4.7\%$  in the DEX + rGH group ( $P < 0.01$ ), and  $18 \pm 2\%$  in the

DEX + bGH group ( $P < 0.001$ ). Minerals from the lumbar vertebrae after ashing were  $27 \pm 2.3$  mg,  $34 \pm 2.3$  mg ( $P < 0.01$ ), and  $35 \pm 3$  mg ( $P < 0.01$ ), respectively. Bone soluble protein was  $14.9 \pm 2.4 \mu\text{g}/\text{mg}$ ,  $20.7 \pm 0.56 \mu\text{g}/\text{mg}$  ( $P < 0.01$ ), and  $20.6 \pm 0.6 \mu\text{g}/\text{mg}$  of bone ( $P < 0.01$ ), respectively. Bone acid phosphatase was  $0.5 \pm 0.012$  U/g bone weight in the DEX group,  $0.9 \pm 0.027$  U/g in the DEX + rGH group ( $P < 0.001$ ), and  $0.74 \pm 0.054$  U/g bone in the DEX + bGH group ( $P < 0.001$ ). Bone alkaline phosphatase was  $1.56 \pm 0.09$  U/g,  $4.05 \pm 0.05$  U/g ( $P < 0.001$ ), and  $4.04 \pm 0.02$  U/g ( $P < 0.001$ ), respectively. It is concluded that GH treatment to a large extent prevents the damage inflicted on bone and cartilage by dexamethasone.

In summary, the increases in weight and bone length reflect the growth-saving effect of GH. The increases in trabecular bone volume and epiphyseal growth plate width indicate an increase in bone formation, and the protein and phosphatase increases reflect the protective effect of GH on the tissue destruction by dexamethasone.

Altman A, Silbermann M, Hochberg Z. 30th Annual Meeting of the ESPE, 1991.

**Editor's comment:** This abstract was published in the proceedings of the 30th Annual Meeting of the European Pediatric Endocrine Society (EPES). It was the basis for a special presentation and an award from the EPES. Dr. Raphael Rappaport presented the paper. The importance of the paper is that it is another of a few (Horber et al, J Clin Invest 1990;86:265; Horber et al, Diabetes 1991;40:141) that suggest GH can overcome the antianabolic and growth-inhibiting effects of glucocorticoids. What a blessing it will be if GH can reverse the devastating effects of chronic corticosteroid therapy in childhood. Before capitalizing upon the substance of these reports, however, investigators are urged to set up controlled protocols. Now is the time to do exactly that. It is not the time to muddy the therapeutic waters.

Robert M. Blizzard, MD

## The Strange Case of Fragile X Syndrome: Increased Mutation Frequency, Increased Fragment Size, and/or Genomic Imprinting?

The fragile X syndrome may be the most frequent cause of inherited mental retardation; the incidence is about 1 in 1,500 males and 1 in 2,500 females.<sup>1</sup> It is a most puzzling syndrome for a number of reasons. Affected males have been characterized as having a relatively normal phenotype except for megalotestes and the presence of a large head, high prominent forehead, prominent jaw, and large protruding ears. Affected carriers have been diagnosed only by the finding of a characteristic abnormality in which the tip of the long arm of the X chromosome seems to be connected to the rest of the chromosome by a slender thread when the cells are cultured under specific conditions. Such chromosomes are easily broken — hence the name fragile X. This gross chromosomal change is rarely evident, however, in asymptomatic carriers of the defect.<sup>2</sup>

The inheritance pattern of the fragile X syndrome seems bizarre in terms of traditional expectations for X-linked diseases. Surprisingly, between 20% and 50% of males who carry the fragile X mutation are asymptomatic. These asymptomatic male carriers can pass the gene along to their daughters, who are also asymptomatic, as would be expected for an X-linked disease. But in the third

generation, the children of those daughters — both males and females — are likely to express the facial features of the syndrome,<sup>2</sup> and the affected males will have megalotestes.

Recently, 2 groups have independently identified the site of the fragile X mutation.<sup>1,2</sup> When Yu and colleagues used their probe, specific for the fragile X region, on the chromosomal DNA of normal and fragile X genotypic individuals, alterations in the mobility of the sequences detected by Southern blotting were found only in fragile X genotype DNA. These sequences were of an increased size in all fragile X individuals and varied within families, indicating that the region was unstable.<sup>3</sup> Oberle and colleagues have linked the phenotypic expression of the syndrome to abnormal cytosine methylation of a single CpG island, at or very near the fragile site. Probes adjacent to this island detected very localized DNA rearrangements that constituted the fragile X mutations, within a 550-base pair, GC-rich fragment. They found that normal male carriers had a 150- to 400-base pair insertion that was inherited by their daughters either unchanged or with small differences in size. Fragile X-positive individuals in the next generation had much larger fragments that differed among siblings

and showed a generally heterogeneous pattern, indicating somatic mutation. The mutated allele appeared unmethylated in normal male carriers, methylated only on the inactive X chromosome in their daughters, and totally methylated in most fragile X males. They thus concluded that expression of the fragile X syndrome appears to result from a 2-step mutation as well as a highly localized methylation.<sup>1</sup> Kremer et al have further characterized this region, and have found that the instability was localized to a trinucleotide repeat, p(CCG)n. The sequences flanking this repeat were identical in normal and affected individuals. The break points in 2 somatic cell hybrids constructed to break at the fragile site also mapped to this repeat sequence. The repeat exhibits instability both when cloned in a nonhomologous host and after amplification by the polymerase chain reaction.<sup>4</sup> These results suggest variation in the trinucleotide repeat copy number as the molecular basis for the instability and possibly the fragile site. This would account for the observed properties of this region in vivo and in vitro. These studies do not explain why such an unstable sequence would be maintained in the genome, let alone further amplified in fragile X pedigrees. Nor

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do they address the issue of methylation of the region in fragile X syndrome individuals. The composition of the unstable sequence, which contains many targets for methylation, provides a link between the instability seen in the fragile X genotype and the methylation of this region associated with the fragile X syndrome phenotype.<sup>4</sup>

**Editor's comment:** The discovery of the fragile X site will allow testing for both affected and asymptomatic carriers of the mutation. This will aid in prenatal diagnosis and in genetic counseling for carriers who are contemplating having children. Because it often had been impossible in the past to detect asymptomatic carriers of the fragile X mutation, the ascertainment of complete pedigrees has been difficult. It represents a

new type of mutation in which amplification of an abnormal site occurs. Time will tell how many other disorders will share this mechanism of disease production.

Judith G. Hall, MD

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## Insulin-Like Growth Factors and Their Binding Proteins in Human Fetal Serum: Relationships With Fetal Growth

Cord blood, obtained by direct transperitoneal puncture of the umbilical cord for purposes of prenatal diagnosis, allowed this study of 119 human fetuses between 20 and 37 weeks of gestation, among which 103 were of normal size for gestational age and 15 had intrauterine growth retardation. Neonatal cord blood of 37 normal-term newborns also was studied. The authors measured in these sera: insulin-like growth factor 1 (IGF-1) by radioimmunoassay, IGF-2 by competitive protein binding assay, and their specific carrier proteins (IGF-binding proteins [IGFBPs]) by binding to <sup>125</sup>I-labeled IGF-1 after separation by elution from a column of Ultragel. The respective association of IGF-1 and IGF-2 with their BPs was determined by electrophoretic separation obtained with western-ligand blotting. Placental lactogen (PL) was measured by radioimmunoassay.

The serum levels of both IGFs were steady from 27 to approximately 33 weeks of gestation, close to 50 ng/mL for IGF-1 and 350 ng/mL for IGF-2. Thereafter both increased to reach, at term, values 2 to 3 times higher. The profiles of these age-related changes were roughly parallel along an exponential regression curve. PL in the serum of fetuses followed a similar curve. Significant correlations were found between the levels of PL and those of both IGFs, suggesting that PL may be involved in the regulation of circulating IGF-1 and IGF-2 in the human fetus.

Total concentrations of the IGFBPs in the fetuses were low. Binding activity was

approximately half of that found in normal adults. Qualitatively, the BP profiles in fetuses resembled those of patients with growth hormone deficiency, with small amounts of 41.5- and 38.5-kd forms, contrasting with relatively increased 34-kd and 30-kd forms. The distribution of the IGFBP in the newborns' complexes showed a low proportion of the 150-kd complex, which is the predominant form in normal children and adults.

The relationships with fetal weight were studied using both echographic data and the birth weight. The levels of IGF-2 during the latter months of gestation did not relate to the fetal weight. However, IGF-1 levels in the fetuses with subnormal weight were significantly lower than in those of the same age whose weight was appropriate, mainly after 25 weeks. This suggested to the authors that during the 3 or 4 last months in utero, IGF-1 but not IGF-2 is involved in the control of fetal size.

Lassare C, et al. *Pediatr Res* 1991;29:219-225.

**Editor's comment:** This work yields new data of significance for understanding how human fetuses grow during the second half of gestation. Several points are to be stressed: IGF-2 levels in fetal serum are 4 to 7 times higher than the levels of IGF-1. Both rise in parallel fashion in the late intrauterine period, contrary to what occurs in the rat fetus, whose hepatic synthesis switches from IGF-2 to IGF-1 at the end of gestation. PL

is likely to be a regulatory factor; the amount of specific IGF carrier proteins, their profile, and the complexes that they form with IGFs suggest a physiologic situation of high bioavailability of IGF at a time when it is probably most needed. In addition to these physiologically relevant results, the data obtained by comparing small-for-gestational age and normal-for-gestational age fetuses confirm some previous studies strongly suggesting that insufficiency of circulating IGF-1 is found in fetal hypotrophy. However, this does not preclude the role of local factors having paracrine or autocrine effects. Fetal growth, its regulatory mechanisms, and especially the role of the placenta, the various factors that may cause intrauterine growth retardation, and the changes occurring during the last 3 months of pregnancy are considered in this report. This work demonstrates that clinical investigations in this field may provide very relevant contributions.

Jean-Claude Job, MD

**Editor's comment No. 2:** This article emphasizes that IGF-1 may play a more important role in fetal growth than IGF-2. The studies of DeChiari et al which were abstracted in GGH (1991;7(1):13) suggest IGF-2 is more important than IGF-1, at least in mice. What is the relationship between fetal growth, IGF-1, and IGF-2, and other growth factors in humans? In rodents? Dr. D'Ercole will address these issues in the March issue of GGH (8:1).

Robert M. Blizzard, MD

## Function of GH-IGF-1 Axis in the Profoundly Growth Retarded Diabetic Child: Evidence for Defective Target Organ Responsiveness in the Mauriac Syndrome

Mauriac syndrome consists of a triad of poorly controlled insulin-dependent diabetes mellitus (IDDM), profound growth retardation, and hepatomegaly, and is seen relatively rarely in 1991. However, it still occurs and the etiology of the growth failure defies explanation. Mauras et al attempted to determine whether the growth retardation was secondary to decreased or abnormal growth hormone (GH) secretion and/or action. The study described compared data in 2 patients with Mauriac syndrome with data from 5 age-matched diabetic boys who were growing well.

Overnight GH profiles in the Mauriac patients and in the normally growing diabetics were similar in respect to mean 12-hour GH concentration, pulse amplitude, and pulse frequency and did

not change during an acute normalization of the serum glucose overnight. The GH-binding proteins (relative binding) were similar in all patients and comparable to those in normal nondiabetics. Insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein (IGFBP) concentrations were comparable in both groups of patients. One patient with Mauriac syndrome treated with GH failed to respond with increased growth velocity (GV) over a 1-year period.

The authors concluded that the data suggest a GH-resistant state either secondary to impaired bioactivity of IGF-1 or a defect at or distal to the IGF-1 receptor.

Mauras N, et al. *Metabolism* 1991;40:1-6.

**Editor's comment:** These studies are very important even though no explicit explanation is found for the severe growth failure manifested in patients with the Mauriac syndrome. I have been perplexed through the years when observing these patients. I still am, but thanks to Mauras and colleagues we now know that the complex cascade of events that controls growth, starting with a positive hypothalamic signal, followed by pulsatile GH release, and GH binding to its protein through IGF-1 and IGFBP generation, is intact in these youngsters. If the defect is truly at or distal to the IGF-1 receptor, advances in technology should help us elucidate that defect in years to come.

Robert M. Blizzard, MD

## Repeated Subcutaneous Administration of Recombinant Human Insulin-Like Growth Factor 1 (IGF-1) to Human Subjects for 7 Days

This paper presents the results of a preliminary trial of insulin-like growth factor 1 (IGF-1) obtained by recombinant DNA technology in adult volunteers. Although biosynthesis of human IGF-1 was reported in 1986 and several experimental studies have been done, human trials have been delayed because of the risk of insulin-like metabolic effects.

This study was conducted in 9 healthy young adult volunteers and 2 growth hormone-deficient (GHD) young adults who had not received GH for 3 years. IGF-1 (0.1 mg/kg) was injected sc after breakfast into 2 patients and into 6 of the healthy subjects. The other 3 controls received placebo. Dietary intakes were of an average type and were controlled. The IGF-1 of 97% purity was dissolved in saline just before use. Blood glucose, plasma insulin, and plasma IGF-1 were measured before treatment and then after the first and seventh injections of recombinant IGF-1 (rIGF-1). The method used for assaying total IGF in plasma was radioimmunoassay after acid ethanol extraction. Free IGF-1 was measured after separation on a Sep Pak C 18 cartridge.

Free IGF-1 increased after injection but rapidly decreased thereafter. Total IGF-1 increased later to reach a peak 2 hours after injection and remained elevated above the baseline for 6 to 24 hours, probably due to its binding on carrier proteins. The GHD patients had lower total IGF-1 levels

than the normal subjects following injection of IGF-1, possibly since they lacked the GHD main binding protein (IGFBP-3) for IGF-1. The effects of the seventh injection were similar to those of the first, although the basal levels of free and total IGF-1 were slightly higher at the end of the 1-week trial than at the onset of the trial.

Significantly, rIGF-1 (0.1 mg/kg) injected sc after breakfast did not induce hypoglycemia in the GHD patients or healthy subjects. There was only a small decrease in the fasting glucose level. The authors stress this point since in a previous trial using a slightly higher dose of rIGF-1 (0.12 mg/kg) given after an overnight fast they had observed a drop in blood glucose to <50 mg/dL in 3 of 5 normal subjects.

Serum insulin levels decreased slightly at the end of the trial. The authors discussed the role of 2 possible factors: a direct effect of IGF on insulin release by the pancreatic islets and an indirect effect mediated by the slightly lower level of fasting blood glucose. They concluded that the repeated administration of rIGF-1 (0.1 mg/kg) after breakfast appeared to be safe and to have some biologic effect.

Takano K, et al. *Growth Regulation* 1991; 1:23-28.

**Among the questions to be asked are (1) will IGF-1 therapy increase growth in syndromes of GH insensitivity (eg, Laron's dwarfism); (2) will IGF-1 replace GH as the favored therapeutic agent in the treatment of GHD or other types of growth failure; and (3) is IGF-1 safe, or will the insulin-like effects preclude its use?**

After 7 days of daily injections at the dose used (0.1 mg/kg), the blood glucose values were not depressed significantly. However, as reported by Walker et al (*N Engl J Med* 1991; 324:1428), a continuous intravenous injection of 0.05 mg/kg daily did produce mild chemical but clinically asymptomatic hypoglycemia in 1 patient with Laron's dwarfism (a GH-resistant syndrome). The patient retained nitrogen, developed hypercalciuria, and had decreased phosphate and sodium excretion.

The tentative answers to the questions are (1) we don't know as yet, but preliminary data indicate that GH will probably increase growth in the GH-resistant syndrome of Laron's dwarfism; (2) currently, there seems to be no theoretical advantage to using IGF-1 to treat GHD; and (3) clinical hypoglycemia secondary to the use of IGF-1 at doses that are metabolically active is probably not going to be a problem.

Jean-Claude Job, MD



## Is SRY the Testis-Determining Factor?

The initiation of male development in mammals requires 1 or more genes on the Y chromosome. A recently isolated gene, termed *SRY* in humans and *Sry* in the mouse, has many of the genetic and biologic properties expected of a Y-located testis-determining factor (TDF) gene. The *SRY* gene lies in the 35-kb interval near the Y pseudoautosomal boundary. A number of genes have been isolated in this region and it is the *SRY* gene that is consistently present in XY individuals who are male.<sup>1</sup> Abnormalities in the *SRY* gene have been found in several XY females.<sup>1,3</sup> In addition, the *Sry* gene is expressed during testes development in the mouse.<sup>4</sup> Expression of the *SRY* gene is confined to gonadal tissue. The gene is highly conserved across species.

Recently, Koopman and colleagues<sup>5</sup> have demonstrated that the *Sry* gene contained in a 14-kb genomic DNA fragment is sufficient to induce testis differentiation and subsequent male development when introduced into

chromosomally female mouse embryos. Sequencing failed to detect any other gene sequences in the 14-kb fragment. Since this fragment alone was able to cause sex reversal, the authors postulate that it contains the entire *Sry* gene, including all of the regulatory elements required for appropriate embryonic expression. Interestingly, these phenotypically male XX mice proved to be sterile despite normal mating behavior, as have all other XX males tested.

**Editor's comment:** *The search for TDF has been a long and hard one. While there may be multiple factors involved in complete sexual differentiation (as evidenced by the failure of maturation of the germ cells in the XX males),<sup>5</sup> these recent developments provide compelling evidence that the *Sry* gene is necessary and sufficient for external male development, at least in the mouse. This topic will be covered more fully by Dr. B. McGillivray in GGH Volume 8, Number 2.*

Judith G. Hall, MD

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4. Koopman P, et al. Expression of candidate sex-determining gene during mouse testis differentiation. *Nature* 1990; 348:450-452.
5. Koopman P, et al. Male development of chromosomally female mice transgenic for *SRY*. *Nature* 1991; 351:117-121.

## The Gene of Insulin-Like Growth Factor 1 and the Gene Cluster of Human Growth Hormone in Children With Constitutional Short Stature

Abnormality of the growth hormone (GH) gene cluster or of the gene of insulin-like growth factor 1 (IGF-1) has long been considered as a thematically possible cause for constitutional or familial short stature. A study of 17 children from 10 constitutionally small families fails to support this hypothesis. The children studied (10 males and 7 females) were ages 4.1 to 11.9 years, with a height -2.5 to -3.6 standard deviations (SD) and a mean height velocity  $-0.35 \pm 0.25$  SD. They had no detectable cause of short stature other than having 1 parent with a height insufficiency of -2.5 to -3.4 SD. They were compared with 3 groups of controls: 25 adults of normal height, 50 unrelated children of normal height, and 60 unrelated children with isolated growth hormone deficiency (IGHD).

The technique used for genetic study was Southern blotting and linkage analysis of restriction fragment length polymorphism (RFLP) on peripheral blood leukocytes.

The patterns of hybridizing DNA fragment generated by 7 different restriction enzymes did not show any difference between the group of constitutionally short children and the 3 control groups. Linkage analysis excluded the possibility that the IGF-1 gene would exert a dominant effect in these short children.

The frequency of the different GH gene cluster haplotypes within the 10 families having 1 parent and 1 child constitutionally short did not show a significant difference between the short and the non-short members, nor between constitutionally short individuals and the various controls. These data, and an analysis of previously published studies in the same field, led the authors to conclude that structural abnormalities of the GH gene cluster detectable by restriction endonuclease analysis are only very rarely a cause for growth failure.

Mullis PE, et al. *Pediatr Res* 1991; 29:412-415.

**Editor's comment:** *Hereditary short stature has been the subject of several recent studies, all more or less related to the question of the possible effects of additional GH in these endocrinologically normal children demonstrating sometimes extreme height insufficiency.*

*Most studies based on hormonal measurements, evaluation of cell receptivity, and other factors participating in the "growth regulation cascade" have not yet given any answers to the questions raised about constitutionally small people. Even if some authors have found a somewhat*

*lower level of GH secretion or circulating IGF-1 in the "short" groups, up to now it has not been considered as a main characteristic of this human group. Since it obviously relates to some kind of hereditary transmission, genetic shortness must have its cause in the genome or its expression. The negative results of the research done by Mullis and colleagues are important, since they strongly suggest that this genetic cause is not a deletion affecting the GH gene cluster or the structural gene of IGF-1. There are still many "candidates" inside the human genome. Even if there is a gap between the clinical facts and the known animal models of body size heredity today, constitutional or familial short stature remains an important field for clinical research.*

Jean-Claude Job, MD

**Editor's comment No. 2:** *The term constitutional short stature should be discarded. It is confused with constitutional delay of growth (and puberty later). Genetic or familial short stature (FSS) are acceptable terms and FSS is the entity discussed in this manuscript.*

Robert M. Blizzard, MD

## Evidence of Hypothalamic-Pituitary Thyroid Abnormalities in Children With End-Stage Renal Disease (With Growth Retardation)

The authors report studies of thyroid hormone levels and thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) injection in 9 children (7.5 to 17 years of age) with end-stage renal disease (ESRD). All were receiving either hemodialysis or peritoneal dialysis. The bone age (BA) was at least 2 1/2 years less than the chronologic age. Height, expressed as standard deviations (SD) from the mean for age and gender, was  $2.9 \pm 1.1$  SD below the mean. The mean growth velocity was  $2.8 \pm 1.6$  SD below the mean for BA and gender.

As listed in the table below, thyroxine ( $T_4$ ) levels were low in almost half of the patients and free thyroxine levels were low in all. However, triiodothyronine ( $T_3$ ) levels were in the normal range. All but 1 patient had basal TSH levels within the normal range. Three patients had a deficient TSH response to TRH. The TSH response was prolonged in all 9. The mean ( $\pm$  SD) nocturnal TSH surge was  $50 \pm 68\%$ , as compared with a mean of 124% in the normal controls. The TSH

surge was below the normal range in 5 of 8. Serum free  $T_4$  values correlated with the nocturnal TSH surge. The authors conclude that their findings support the hypothesis that some patients with ESRD have central hypothyroidism.

Pasqualini T, et al. *J Pediatr* 1991; 118: 873-878.

**Editor's comment:** These authors have extended other studies concerning circulating thyroid hormones in children with ESRD. The study nicely demonstrates that several of the parameters we use to measure low thyroid function indicate central hypothyroidism exists in some patients with ESRD. In addition, the authors commented that the alterations in thyroid hormone levels observed in these patients differ from those found in patients with altered thyroid function due to other forms of acute or chronic illness. In the latter, there frequently is a decrease in total serum  $T_3$  concentration and a rise in serum reverse  $T_3$  concentration. In ESRD there appears

to be a predominant decrease in  $T_4$ , as opposed to  $T_3$  levels. A plausible explanation for the low level of circulating thyroid hormone seems to be that patients with ESRD have a defect in the pituitary secretion of TSH. Because the patients predominantly had prolonged elevations of TSH following TRH administration, the primary site of the metabolic defect is presumed to be the hypothalamus.

My interpretation of these data is that thyroid function is probably abnormal (low in many instances) secondary to a central deficiency of TSH release or production. The growth delay these patients experience is not necessarily related to this, although it might be. In such patients, a trial of thyroid hormone treatment and observation of its effect, if any, on growth would be worthwhile.

Robert M. Blizzard, MD

|          | $T_4$ (nmol/L)  |              | $FT_4$ (pmol/L) |              | $T_3$ (nmol/L) |              |
|----------|-----------------|--------------|-----------------|--------------|----------------|--------------|
|          | No. Below       |              | No. Below       |              | No. Below      |              |
|          | Average         | Normal Range | Average         | Normal Range | Average        | Normal Range |
| Normal   | $118.7 \pm 22$  | 0            | $18 \pm 49$     | 0            | $2.3 \pm 0.5$  | 0            |
| ESRD (9) | $76.7 \pm 16.9$ | 4            | $10.4 \pm 2.6$  | 9            | $1.6 \pm 1.3$  | 0            |

## The Somatostatin Analogue Octreotide: Possible Use for the Treatment of Excessive Body Growth: A Compilation of 3 Reports

An early report of preliminary results was obtained with the long-acting somatostatin (SRIH) analogue, octreotide, in 7 adolescents with excessive height and height velocity<sup>1</sup> and was summarized in *GGH* (Vol 6, No. 2). Another report was published a few weeks later.<sup>2</sup> It included 6 constitutionally tall girls, ages 12.7 to 13.6 years, with a mean predicted height of  $184.5 \pm 4.8$  cm, and 4 constitutionally tall boys, ages 14 to 15.5 years, with a mean predicted height of  $198.7 \pm 6.2$  cm. All were treated with twice daily subcutaneous (sc) injections of 150  $\mu$ g of octreotide for 6 to 12 months. The 24-hour mean integrated concentration of growth hormone (GH) decreased from

5.3 to 3.6 ng/mL/min after 6 months and to 3.9 ng/mL/min after 12 months, with large individual variations. The GH peak following injection of the peptide thyrotropin-releasing hormone (TRH) reached 8.9 ng/mL before treatment and dropped to 3.1 and 2.7 ng/mL after 6 and 12 months, respectively, with octreotide. The GH response to GH-releasing hormone (GHRH) did not change significantly. The clinical effects included a decrease in the mean growth velocity from 7.1 cm/yr to 2.7 cm/yr after 6 months and to 2.4 cm/yr after 1 year. The mean bone maturation progressed 1 year after 6 months of treatment and more than 2 years after 12 months. A mean reduction of

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predicted adult height (according to Bayley-Pinneau) of  $4.9 \pm 2.9$  cm resulted. These results are in agreement with those of the previously related trial.<sup>4</sup> Although the tolerance of octreotide was said to be good, diarrhea during the first 10 days of treatment occurred in the 10 adolescents, and persisted in 1. Three reported nausea for 5 minutes following the injection. Transitory biliary microlithiasis was found in 1 female patient.

A recent comprehensive review<sup>2</sup> gives data on the pharmacology of octreotide. This somatostatin analogue has a half-life of 113 minutes after sc injection. In adult acromegalic patients, it is able to suppress GH secretion for 6 hours. No significant rebound secretion of GH occurs, and SRIH has few inhibiting effects on insulin secretion. The known effects of the analogue in acromegaly and in various cancers are summarized, and the results obtained in constitutionally tall adolescents in the first published study are reported.<sup>1</sup> It is believed that octreotide not only inhibits or suppresses the pituitary secretion of GH for several hours but also may act directly on bone formation. The presence of high-affinity binding sites for [<sup>125</sup>I]-labeled octreotide in long bones of neonatal rats and the inhibiting effect of octreotide on the forskolin-induced adenylate cyclase activity in bone cell preparations from newborn rats have been demonstrated. The number of receptors decreases with age in the rat. This leads one to question if the clinical results obtained with octreotide in excessively tall adolescents could result

in part from a direct effect of the peptide on long bone formation.

#### References

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2. Tauber MT, et al. *Acta Paediatr Scand* 1990;79:176-181.
3. Lamberts SWJ. *Growth Regulation* 1991;1:3-10.

**Editor's comment:** To treat or not to treat excessive growth at adolescence is a controversial question. The ethics for such treatment, the means proposed, the efficacy, and the safety have long been discussed — without developing a consensus. The 2 clinical trials with octreotide reported in 1990 initiated a new approach. However, both trials were limited to a small number of excessively tall individuals who were treated for a relatively short period and without sufficient posttreatment follow-up. In both trials, a decrease in GH secretion during treatment was reported, accompanied by a striking reduction in growth velocity. But the deductions on predicting adult height are questionable, as are all predictions of this kind when concerning patients who are still growing. Moreover, the second report<sup>2</sup> reveals more inconsistencies than did the first one.

It certainly must be pointed out that the use of octreotide for limiting growth is still at the stage of preliminary short-term trials. Larger studies, extended for years, are obviously needed. The experimental

data show that SRIH analogue may have multiple effects. Those pertaining to the digestive tract and its secretions are probably of clinical importance, since SRIH has been used in several therapeutic approaches for several years. The limited data about possible side effects modifying the secretion of insulin and about other peptides that are regulated by somatostatin have not established adequately the safety of using a somatostatin analogue in the control of growth. However, since excessively tall stature is sometimes a social handicap, and the physiology and pathophysiology are important, the preliminary data reported are of significant interest.

Jean-Claude Job, MD

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## Growth Characteristics and Response to Growth Hormone Therapy in Patients With Hypochondroplasia: Genetic Linkage of the Insulin-Like Growth Factor 1 Gene at Chromosome 12q23 to the Disease in a Subgroup of These Patients

Mullis et al performed restriction enzyme analysis of the insulin-like growth factor 1 (IGF-1) gene of 20 hypochondroplastic white British children, 60 unrelated children with isolated growth hormone deficiency (IGHD), and 50 unrelated normal adults. Adult family members of the hypochondroplastic children were studied as well. All children with hypochondroplasia had normal growth hormone (GH) responses to insulin-induced hypoglycemia and plasma IGF-1 levels that were in the upper range of normal. Pretreatment height velocities were calculated for the hypochondroplastic and IGHD children for 1 year prior to treatment with recombinant human GH (rhGH) in doses between 18

and 32 U/m<sup>2</sup> per week. Since previous studies have shown that pretreatment height velocity is a predominant determinant of growth responses to rhGH in GH-deficient children, the hypochondroplastic children were compared with a control group who had similar pretreatment height velocities. The groups also were matched for age, sex, and pubertal status. Sitting height and subischial leg length were determined.

Human lymphocyte DNA was isolated and digested with restriction enzymes *HindIII* and *Pvu II*, under conditions recommended by their suppliers. Southern blots of *HindIII*-digested DNA hybridized with a human IGF-1 cDNA probe showed nonpolymorphic fragments

of 8.2 and 3.2 kb and a restriction fragment length polymorphism (RFLP) with alleles of 4.8 and 5.2 kb. *Pvu II* digests showed nonpolymorphic fragments of 8.4 and 2.5 kb and an RFLP with alleles of 4.7 and 5.1 kb. The frequencies of the heterozygous pattern (*HindIII*: 8.2, 5.2, 4.8, 3.2-kb fragments; *Pvu II*: 8.4, 5.1, 4.7, 2.5-kb fragments) in IGHD children and controls were 22% and 20% respectively, in contrast to 45% in the children with hypochondroplasia ( $P < 0.05$ ). No individuals were homozygous for the 5.2-kb *HindIII*/5.1-kb *Pvu II* allele. The children with hypochondroplasia could be subdivided into 2 groups according to the IGF-1 RFLP alleles they possessed.

The heterozygous group (*Hind*III: 5.2, 4.8 kb; *Pvu* II: 5.1, 4.7 kb) included 4 girls and 5 boys. The second group was homozygous (*Hind*III: 4.8, 4.8 kb; *Pvu* II: 4.7, 4.7-kb), and included 6 girls and 5 boys. These 2 groups of children did not differ in pretreatment height velocity standard deviation scores (SDS) for chronologic age, sitting height, or subischial leg length. However, following 1 year of rhGH treatment, the heterozygous group had a proportionate increase in back and leg length while the homozygous children had a disproportionate increase in back length with respect to leg length ( $P=.009$ ). Five of the 7 families whose 9 children were heterozygous for the IGF-1 allele were studied. Those who possessed the 5.2-kb

*Hind*III and 5.1-kb *Pvu* II alleles were slightly disproportionate and significantly shorter than parents and adult relatives without these alleles ( $P<0.005$ ). Parents of affected homozygous children did not present with body disproportion and were of normal stature. The authors point out that the data suggest that there are 2 subgroups of children with common features of hypochondroplasia but with differences in their response to rhGH. They further state that the data indicate that a gene involved in a form of short stature with hypochondroplasia characterized by proportionate response to rhGH is linked to the IGF-1 gene locus at chromosome 12q23. They conclude that this polymorphism itself is not responsible for the hypochondroplasia since both sets

of alleles are present in the normal population and since some of the parents of the affected homozygous children were heterozygous and of normal stature.

Mullis P, et al. *Clin Endocrinol* 1991; 34: 265-274.

**Editor's comments:** This is an interesting study that presents fascinating data concerning the response of children with hypochondroplasia to rhGH. It is an excellent example of geneticists and endocrinologists working in tandem to understand the etiology of the heterogeneous disease entities that may affect human growth.

William L. Clarke, MD

## Abnormal Growth Patterns and Adult Short Stature in 115 Long-Term Survivors of Childhood Leukemia

Schriock et al evaluated final height in 115 long-term survivors of acute lymphoblastic leukemia (ALL) treated at St. Jude's Children's Research Hospital during the years from 1967 to 1975. Subjects with trisomy 21, central nervous system (CNS) leukemia at diagnosis, or those older than 12 years of age at diagnosis were excluded from the analysis as were children who had not completed growth. None of the 115 patients had been treated with growth hormone (GH) and all experienced spontaneous puberty. A variety of chemotherapeutic protocols were utilized, although all patients received induction chemotherapy with prednisolone, vincristine, daunorubicin, and/or asparaginase. CNS prophylaxis consisted of 2,400 cGy cranial irradiation plus 5 concomitant doses of intrathecal methotrexate or 2,400 cGy craniospinal irradiation alone. Patients' heights were measured at diagnosis and at least annually utilizing a stadiometer. Heights were expressed as standard deviation scores (SDS). The final cohort consisted of 39 males and 76 females who had been followed for a mean of  $13.8 \pm 2.1$  years since diagnosis.

Significant retardation was observed in height SDS from diagnosis to the completion of chemotherapy ( $P<.0001$ ) and from the end of therapy to the last evaluation ( $P<.0001$ ). Heights at diagnosis were  $>1$  SD below population norms for 19% and  $>2$  SD for 2%. At final evaluation, 74% of these patients had SDS  $\leq 1$  SD, and 37% had SDS  $\geq 2$

SD. Chemotherapeutic regimens did not appear to have differential effects on the findings; however, height SDSs were significantly different for those receiving cranial versus craniospinal irradiation. Six patients in the craniospinal group did not receive prophylactic irradiation until chemotherapy had been completed. Despite their growth decrement during chemotherapy ( $P<0.03$ ), they had no significant overall change in final height SDS. Height SDS had decreased at the end of chemotherapy in 90% of children treated with cranial irradiation, and 30% had final height scores of  $\geq 2$  SD below population means. Changes in height SDS were correlated with age at diagnosis for the patients who received cranial irradiation. Growth retardation was most prominent in those with early onset disease. In addition, girls whose disease was diagnosed before age 8 had significantly greater decreases in height SDS after chemotherapy than those who were older at diagnosis.

The authors state that the median change in height SDS from diagnosis to the last evaluation was  $-1.5$ , corresponding to a mean height decrement of 9.1 cm. Their data contrast with that of other studies, which predict minimal effects on adult height in survivors of childhood leukemia. The authors state that this may be due to the failure of other investigators to follow patients until growth was complete. They further note that there have been changes in CNS prophylaxis over the last few years,

including a reduction in total cranial irradiation and the elimination of spinal irradiation.

Schriock E, et al. *J Clin Oncol* 1991; 9: 400-405.

**Editor's comments:** This well-conducted study demonstrates separate effects of chemotherapy and irradiation on final height in long-term survivors of childhood ALL. It is particularly interesting because only children who were younger than 12 years of age at diagnosis were studied, thus eliminating potentially minimal changes in height decrements that might be observed in pubertal children. GH evaluations were not reported for any of the subjects; thus, it is not known whether any had permanent loss of GH secretion. It is noteworthy, however, that all patients entered spontaneous puberty. It is hoped that future prospective studies will include the determination of GH secretion as well as insulin-like growth factor 1 levels, so that these findings might be more fully explained. In addition, it will be interesting to evaluate the effect of omitting spinal irradiation and lowering cranial irradiation doses on final height in survivors of childhood ALL. Dr. Stephen Shalet will cover the entire topic of growth and treatment of cancer, particularly leukemia, in GGH Volume 8, Number 3.

William L. Clarke, MD



## MEETING CALENDAR

**Jan 3-10, 1992** Intro to Molecular and Cellular Research, Pacific Grove, CA. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

**Apr 8-12, 1992** 16th Training Course on Hormonal Assay Techniques, Rockville, MD. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

**May 4-7, 1992** Ann Mtg of the APS/SPR/APA, Baltimore Convention Ctr, Baltimore, MD. Info: APS/SPR/APA Program Office. Fax: 708-427-1305.

**May 6-8, 1992** Ann Mtg of the LWPES, Baltimore, MD. Info: Dr GP August, LWPES, Children's Nat'l Med Ctr. Fax: 301-460-8846.

**May 26-31, 1992** 5th Int'l Conf on the Cell and Molecular Bio of Chlamydomonas, Pacific Grove, CA. Info: G. Witman, Worcester Foun Exp Biol. Fax: 301-530-7079.

**June 11-14, 1992** 7th Ann Mtg of the Assoc of ACT, Rochester, MN. Info: C.R. Schad, ACT. Phone: 507-284-2950.

**June 18-23, 1992** 52nd Ann Mtg of the ADA, San Antonio, TX. Info: Mtgs Dept, ADA. Fax: 703-836-7439.

**June 24-27, 1992** 74th Ann Mtg of the Endo Soc, San Antonio, TX. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

**July 12-15, 1992** 24th Ann March of Dimes Clinical Genetics Conf, Stanford, CA. Info: Prof Svs Dept, March of Dimes Birth Defects Found. Tel: 914-428-7100.

**Aug 30 - Sept 5, 1992** 9th Int'l Congress of Endocrinology, Nice, France. Info: NICE 92, c/o SOCF1, 14 Rue Mandar, 75002 Paris, France.

**Sept 7-10, 1992** 31st Ann Mtg of the ESPE, Zaragoza, Spain. Info: Dr A Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hosp, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

**Sept 10-12, 1992** Int'l Congress on Growth Hormone and Somatomedins During Lifespan, Milan, Italy. Info: Drs D

Cocchi/V Locatelli, Dept of Pharm, Sch of Med, Univ of Milan, Via Vanvitelli, 32, 20129 Milan, Italy.

**Oct 8-9, 1992** Int'l Symp on Growth '92 — 2 Decades of Experience in Growth, Santiago de Compostela, Spain. Dr. S Rossetti, Ares-Serono Symposia, Via Ravenna 8-00161 Rome, Italy. Fax: 39-6-44291324.

**Oct 10-14, 1992** 44th Postgrad Assembly of the Endo Soc, Boston, MA. Info: A Singer, the Endo Soc. Fax: 301-571-1869.

**Nov 9-13, 1992** Ann Mtg of Am Soc of Hum Genetics, San Francisco, CA. Info: M Ryan, ASHG. Fax: 301-530-7079.

**June 3-7, 1993** 4th Joint Mtg of the ESPE/LWPES, San Francisco, CA. Info: Prof M Grumbach, Univ of CA Sch of Med. Fax: 415-476-4009.

**June 9-12, 1993** 75th Ann Mtg of the Endo Soc, Las Vegas, NV. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

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## Genetics & Hormones

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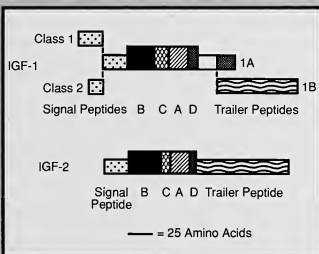
March 1992

## The Insulin-Like Growth Factors and In Utero Growth

**A. Joseph D'Ercole, MD**  
*Professor of Pediatrics*  
*University of North Carolina*  
*Chapel Hill, North Carolina*

The well-described capacity of the insulin-like growth factors (IGFs; IGF-1 and IGF-2) to stimulate cellular proliferation has long made them attractive candidates for a role in the regulation of embryonic and fetal growth (see Figure 1).<sup>1,2</sup> More recent evidence that the IGFs can also stimulate differentiation and/or differentiated cell function in some cultured cells enhanced their potential importance in utero. Claims of a functional role for IGFs in development have been further strengthened by findings that they, as well as their cell surface receptors, are expressed in a variety of tissues early in embryonic life. Despite an impressive body of evidence, derived from many lines of investigation, there remained no direct evidence of a role for the IGFs in utero until the last year. The finding of significant fetal growth retardation in mice with a marked reduction in IGF-2 expression (> 90%) resulting from a hemizygous disruption of the IGF-2 gene (accomplished by homologous recombination of a normal allele and an artificial fusion gene) provides direct confirmation that IGF-2 is a stimulator of fetal growth in mice.<sup>3</sup> Comparable experiments involving the IGF-1 gene have not yet been reported, but a number of in vivo studies have demonstrated the capacity of IGF-1 to stimulate somatic and linear growth in mice postnatally, including during the suckling period, a time that is analogous in many ways to the third trimester of human gestation.<sup>4</sup>

Figure 1  
**Insulin-Like Growth Factor Precursors**



IGF-1 (top) and IGF-2 (bottom) precursor proteins are schematically depicted, with the large blocks representing the mature proteins. Analogous or homologous domains of the precursors are labeled and drawn with the same designs. IGF-1 has at least 2 alternative signal peptides and 2 trailer peptides that differ at their carboxy-terminal ends.

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## Letter From the Editor

Five years ago, Dr. A. Joseph D'Ercole wrote an excellent article for *GGH*, which was entitled "Fetal Growth and Development: A Brief Survey of Cellular Mechanisms." Five years ago, Dr. Joseph Warshaw also wrote an excellent article, which was entitled "Perspectives on Intrauterine Growth Retardation." You may wish to review these articles as you read an update of each of these topics by these authors in this issue of *GGH*. Both updates are excellent.

Terminology regarding intrauterine growth retardation (IUGR) and small-for-gestational age (SGA) infants has been exceedingly confusing. It is time that these terms be clarified. Clarification will enhance our diagnostic and investigative approach to IUGR and SGA babies. Dr. Warshaw has taken a bounding step forward to clarify these terms. You as a reader are invited to write and express your opinion.

Several abstracts in this issue also deal with IUGR and low birth weight. These were selected because of the focus on fetal growth in this, the first issue of the eighth volume of *GGH*.

Robert M. Blizzard, MD

## POTENTIAL ACTIONS OF THE INSULIN-LIKE GROWTH FACTORS IN UTERO

What developmental events might be stimulated by IGFs in utero? Studies of the actions of the IGFs in cultured cells suggest myriad possibilities. Both IGF-1 and IGF-2 are capable of stimulating cellular replication in a variety of cultured cells, including those derived from the fetus. The finding that a near absence of IGF-2 expression in mice with a disrupted IGF-2 gene results in fetal growth retardation without apparent morphologic abnormalities suggests that stimulation of mitosis is a major function of IGF-2 in utero. A number of studies, however, indicate that IGFs, especially IGF-1, also are capable of inducing differentiation and/or differentiated cell function. Examples of the former are the induction of myotubule formation in myoblasts,<sup>5</sup> lens fiber cell formation in chick lens epithelium, and adipocyte differentiation in preadipocyte cell lines by IGF-1. The capacity of IGFs to induce differentiated cell function encompasses the stimulation of glycogen synthesis in fetal rat hepatocytes by IGF-1, type I collagen formation in cartilage by IGF-1 and IGF-2, and prolactin synthesis in human placenta by IGF-1.

The early embryonic expression of IGFs and their receptors lends credence to the possibility that IGFs participate in stimulating a variety of developmental events. But how can such divergent responses be attributed to IGFs? While there is no certain answer to this question, it is likely that different mechanisms mediate and modulate IGF actions in a cell type-specific and developmental stage-specific fashion. In other words, the actions of IGFs may be determined in large part by the nature of the target cell, the receptor it expresses, and the signaling mechanisms triggered by the IGF-receptor interaction. IGF actions will also be influenced by the actions of other regulatory agents that can either alter or determine the cellular response to IGFs, and by the presence of specific IGF-binding proteins (IGFBPs) that can modulate IGF action.<sup>6</sup> Finally, in the case of IGF-1, different precursor forms could confer different biologic responses (see Figure 1, page 1).<sup>7</sup>

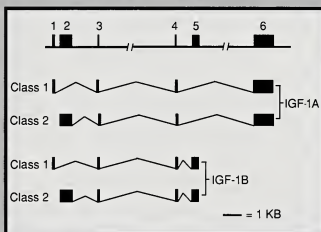
## EXPRESSION OF THE INSULIN-LIKE GROWTH FACTORS IN UTERO

Using the polymerase chain reaction, transcripts for IGF-2 have been detected in 2-cell preimplantation mouse embryos, and have been localized by *in situ* hybridization in placental components as early as day 18 of gestation in humans.<sup>8,15</sup> As gestation progresses, IGF-2 transcripts become more abundant and are expressed in many embryonic and extraembryonic tissues. IGF-2 expression occurs in most tissues of mesodermal origin, especially those that are actively undergoing differentiation, such as somite derivatives, muscle, and head mesenchyme; but it is also strongly expressed in some cells of ectodermal (eg, choroid plexus) and endodermal (eg, liver and bronchi) origin. The onset of IGF-1 expression is later, possibly beginning shortly after implantation at approximately 7 to 8 days of gestation in the mouse, and clearly occurring in early organogenesis. IGF-1 transcripts can be localized in undifferentiated mesenchyme, especially that which surrounds spouting nerves and areas of active remodeling, such as cardiac outflow tracts. Later IGF-1 expression is most marked in a variety of connective tissues, such as those surrounding muscle and cartilage. Because there are limitations in detection of RNA by *in situ* hybridization and apparently rapid developmental changes in IGF expression, it is unlikely that all cell types expressing IGFs have been defined. The widespread expression of IGFs, including the time prior to circulatory system development, strongly supports the concept that during embryogenesis IGFs can act locally on or near their cells of synthesis, ie, in an autocrine or paracrine manner.

## REGULATION OF INSULIN-LIKE GROWTH FACTOR EXPRESSION

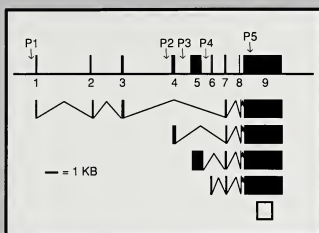
In the embryo and fetus, the factors that regulate IGF expression are for the most part unknown. A number of findings indicate that the control of gene transcription is extraordinarily complex. IGF-1 and IGF-2 are each encoded by single large genes, spanning about 95 and 35 kb of human genomic DNA, respectively.<sup>16,17</sup> Each gene contains multiple exons, at least 6 for IGF-1 and 9 for IGF-2, some of which are used alternatively to generate transcripts of differing composition (see Figures 2 and 3). Genomic DNA 5' to each of the first utilized exons includes multiple putative consensus regulatory sequences (nucleotide sequences that are known to bind transcription factors), but none of these flanking regions contains classic TATA promoters or known response elements. Thus, IGF transcription appears to be regulated by mechanisms that differ from those that have been classically defined. Because IGF transcripts differ in a fashion that depends on the cell

**Figure 2**  
**Human Insulin-Like Growth Factor 1**  
**Gene and Transcripts**



The human IGF-1 gene (top schematic) spans approximately 95 kb of genomic DNA on the long arm of chromosome 12. It contains at least 6 exons (black boxes) that are utilized to transcribe a number of mRNAs (lower 4 schematics). Exons 1 and 2 encode portions of alternative signal peptides. Because these exons may contain several transcription start sites, transcripts in addition to those depicted are likely transcribed. Exon 3 encodes the remaining signal peptide sequence and most of the  $\beta$  domain of mature IGF-1, while exon 4 encodes the remainder of mature IGF-1 and the amino-terminal end of the trailer peptides. Exons 5 and 6 encode the alternatively used segments of the trailer peptide. IGF-1 mRNAs vary dramatically in their length and most of this size difference is due to the length of 3' untranslated RNA encoded on exons 5 and 6. These differences are not depicted.

**Figure 3**  
**Human Insulin-Like Growth Factor 2**  
**Gene and Transcripts**



The human IGF-2 gene (top schematic) lies immediately adjacent to the insulin gene on the short arm of chromosome 11 and spans approximately 35 kb of genomic DNA. It is composed of 9 exons (black boxes) that are alternatively used to generate multiple IGF-2 mRNAs. Exon 7 encodes the signal peptide and most of the domain of the mature protein. Exon 8 encodes the remainder of mature IGF-2 and the beginning of the trailer peptide, and exon 9 encodes the remainder of the trailer sequence. Exons 1 through 6 encode 5' untranslated RNA. Putative promoters for IGF-2 are marked (P1-P4). The open box at the bottom represents an mRNA that does not encode IGF-2 and is presumably regulated by sequences depicted as P5.

of expression and its developmental stage, it seems certain that distinct factors regulate their expression in different tissues. The finding in adult animals that estrogens stimulate the abundance of IGF-1 mRNA in uterus but not in liver, while growth hormone does the opposite, strongly supports this speculation. In several cultured cells derived from fetuses, placental lactogens stimulate IGF production, although the mechanism by which this is accomplished is not known.<sup>18</sup> Cultured fetal adrenal cells dramatically increase their abundance of IGF-2 mRNA after exposure to corticotropin. In vivo IGF-1 expression is reduced in experimentally induced fetal growth retardation; this may be mediated by impaired or inadequate nutrition, which has been shown to decrease fetal liver IGF-1 mRNA. No other specific substances, however, have been definitively implicated in the regulation of IGF expression in the embryo or fetus.

## MECHANISMS OF INSULIN-LIKE GROWTH FACTOR ACTION

The IGFs initiate their actions by binding to cell surface receptors that transduce signals across the cell membrane.<sup>19-22</sup> Three, and



possibly 4, transmembrane proteins are capable of binding IGFs. It seems possible that the interaction of the IGFs with different receptors could result in altered responses to the IGFs and help explain the varied responses stimulated by the IGFs. Most of the known biologic actions of IGF-1 and IGF-2 have been associated with their interaction with the type 1 IGF receptor. It is heterotetrameric, with marked homology to the insulin receptor, and is composed of 2 heterologous pairs of disulfide bond-joined subunits. The  $\alpha$  subunit is extracellular and binds the IGFs, as well as insulin (IGF-1 > IGF-2 > insulin), while the  $\beta$  subunit spans the cell membrane and is phosphorylated rapidly following IGF binding. The autophosphorylation of the  $\beta$  subunit is thought to result in the phosphorylation of cytosol substrates, which in turn sets in motion a cascade of undefined events leading to biologic change. The type 1 IGF receptor is expressed from the 8-cell stage of embryogenesis and its mRNA is widely distributed in mid-gestation rat tissues.

IGFs also bind to the insulin receptor, but because the affinity of the insulin receptor for the IGFs is low, it is unlikely that this receptor interacts with physiologic concentrations of IGFs. Hybrid receptors composed of  $\alpha$  and  $\beta$  subunits of both the type 1 IGF receptor and the insulin receptor have been shown to exist, and it is appealing to speculate that they may mediate specific IGF actions.<sup>19</sup> A gene for another possible receptor, termed the insulin receptor-related protein, has recently been cloned. It is equally homologous with type 1 IGF receptor and insulin receptors.<sup>21</sup> The structure of the insulin receptor-related protein gives it the potential to mediate IGF actions, but it is not known whether this protein is expressed *in utero*.

The type 2 IGF receptor is a single-chain protein that contains multiple repetitive sequences in its extracellular domain and is identical to the mannose-6-phosphate receptor. Interaction of IGFs and this receptor has not been convincingly linked to the growth-promoting actions of the IGFs, but its capacity to translocate enzymes, and possibly the IGFs, to lysosomes may be important in modulating IGF actions by initiating IGF degradation. The type 2 IGF receptor is expressed from the 2-cell stage and is abundant until late in rodent gestation. Its cellular distribution corresponds closely to that of IGF-2 mRNA, suggesting that its function is coupled to that of IGF-2.

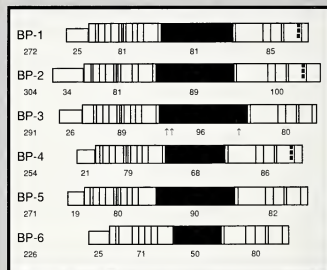
#### MODULATION OF INSULIN-LIKE GROWTH FACTOR ACTION

An additional complexity to understanding the actions of IGFs is the finding that their actions are often exerted in concert with other agents.

Studies of the mitogenic actions of IGF-1 using Balb c/3T3 cells show that these cells traverse the cell cycle only after exposure to IGF-1 and other growth factors, an observation later made in a number of other types of cultured cells. For example, in rat thyroid-derived FRTL5 cells, IGFs stimulate DNA synthesis only in concert with thyroid-stimulating hormone (TSH). Such a scenario may also be relevant to the differentiated actions of IGFs. For example, the stimulation by IGF-1 of progesterone synthesis in cultured granulosa cells is greatly augmented and becomes significant only when follicle-stimulating hormone (FSH) is present.

The actions of the IGFs also are modulated by specific IGFBPs.<sup>6, 23, 24</sup> Six distinct IGFBPs, each bearing a numerical designation, have been identified and their cDNA sequenced (see Figure 4). All the IGFBPs possess conserved sequences of amino acids and numerous

Figure 4  
Precursors of Rat Insulin-Like  
Growth Factor-Binding Proteins



The IGFBPs are a family of proteins with shared structural characteristics, and are conserved among different species. They are synthesized with signal sequences (small blocks). Their mature forms (large blocks) are composed of 3 domains: cysteine-rich conserved amino- and carboxy-terminal domains and nonhomologous domains that lie in the middle of the molecule (black boxes). The thin vertical lines represent the positions of cysteines in amino- and carboxy-terminal portions of the mature proteins. The thick vertical broken lines at the carboxy-terminal ends of IGFBP-1, IGFBP-2, and IGFBP-4 show the location of amino acid sequences having the potential to bind to integrin receptors (Arg-Gly-Asp in IGFBP-1 and IGFBP-2, and Lys-Gly-Glu in IGFBP-4). The arrows below IGFBP-3 indicate potential sites of glycosylation. The numbers below each schematic indicate the number of amino acids in the precursor of the appropriate portion of the molecule.

cysteine residues in similar locations at both the amino- and carboxy-terminal ends. In most experimental situations, IGFBPs have been found to decrease IGF actions, presumably by binding IGFs and, thus, restricting their access to cell surface receptors. Such an inhibitory role is suggested by the increase in IGFBP-1 expression found in experimentally induced fetal growth retardation. Evidence of a role for IGFBPs in augmenting and/or facilitating IGF action, however, also exists. For example, IGFBP-1 and IGFBP-2 can cross rat endothelium and may, therefore, have a role in the delivery of IGFs to tissues. Binding of IGFBPs to the cell surface may be important to IGF actions by facilitating IGF-IGF receptor interactions. The capacity of IGFBP-1 and IGFBP-2 to bind to the cell may depend on the presence of Arg-Gly-Asp (RGD) amino acid sequences that interact with integrin-type receptors on the cell surface. IGFBP-3, also capable of enhancing IGF-1 activity, does not possess RGD sequences but may adhere to cell surfaces through glycosylated moieties. IGFBP-1 and IGFBP-2 are abundant in utero, being expressed in a variety of tissues from at least mid-gestation. Insulin decreases IGFBP-1 and IGFBP-2 expression, while their blood levels rise with fasting. Additional factors, including the IGFs, likely regulate these binding proteins in utero.

## SUMMARY

The IGFs can be viewed as signals for a variety of growth and developmental events. The specific biologic events that the IGFs

stimulate, however, are determined by the signaling mechanisms expressed by target cells and the influence of other agents on these target cells. In addition, these actions appear to be dramatically modulated by the actions of specific binding proteins. A better understanding of the role of IGFs in utero will come from elucidation of the precise mechanisms of IGF action in specific cells.

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# Intrauterine Growth Restriction Revisited

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## INTRODUCTION

Early gestation is characterized by rapid cell division and organ development. These early events occur swiftly, as illustrated by closure of the neural tube between 19 and 29 days of gestation and by development of the heart from the time of the first heart beat at 21 days of gestation to its differentiation as a 4-chambered pumping organ by 56 days. Major malformations that may have profound influences on

subsequent fetal growth and development are already established by the end of the third month. The second trimester is, in large part, a period of growth and functional refinement of those organ systems that must be mature by the time of delivery. The brain undergoes the waves of neural migration and differentiation that will provide the basis for the neural integration and behaviors that are necessary for postnatal survival. In addition, there is both rapid growth and functional differentiation of organ systems such as the lung and gut. By the end of the second trimester the fetus is at the brink of potential survival, as witnessed by infants born at 25 to 26 weeks of gestation and weighing 500 to 600 g who populate our newborn intensive care units with increasing frequency. While survival of such extremely

low-birth-weight infants is possible, it is not a desirable alternative to sustained growth in the intrauterine milieu, which during the last trimester results in a large increase in fetal weight. During this last 3 months of gestation there is deposition of storage fuels such as fat and glycogen, and the fetus more than quadruples in size. It is during this period that the fetus is vulnerable to those genetic and environmental insults that can interfere with normal growth.

## INFLUENCES ON FETAL GROWTH

While fetal growth ultimately is controlled by the genetic endowment, it is nonetheless influenced by diverse factors. Male infants weigh 150 to 200 g more than females at birth, but this confers no survival advantage since infant mortality is greater in males. Size at birth differs greatly between different racial and ethnic groups; for example, the mean birth weight of populations in New Guinea is 2,400 g as compared with 3,880 g in American Indian populations. These weight differences likely reflect variation in maternal size and nutrition as well as genetic factors in different populations.

During fetal development there are important interactions between nutritional state and hormonal and growth factor influences. Insulin, as one of the principal hormones influencing fetal somatic growth, regulates fetal lipogenic activity and has a permissive role in hepatic glycogen deposition and protein synthesis. Fetuses with insulin deficiency secondary to pancreatic agenesis or with a defective insulin receptor, as in the "leprechaun" syndrome, have marked intrauterine growth retardation (IUGR) with decreased adipose tissue and little weight gain during the last trimester of pregnancy. Conversely, fetal hyperinsulinism results in increased adiposity in human infants of diabetic mothers. Other classic hormones, including thyroxine, glucocorticosteroids, and sex hormones, have important influences on specific organ development and on functional and metabolic adaptation but have little influence on somatic growth. For example, thyroid hormones are important for central nervous system (CNS) and skeletal maturation, glucocorticoids modulate lung maturation, and androgens are critical for sex differentiation. Pituitary growth hormone (GH) itself is not of great importance in the regulation of fetal growth and does not influence size at birth. However, a GH-like molecule produced by the placenta, known as placental lactogen or chorionic somatomammotropin, may have a role in modulating fetal growth. In the ovine fetus, maternal malnutrition reduces the number of placental lactogen receptors in fetal liver. The growth-promoting role of placental

lactogen in humans is uncertain, however, since pregnancies in which the gene for placental lactogen is missing result in infants of normal birth weight.

Peptide growth factors that influence fetal growth and maturation include the insulin-like growth factors (IGF-1 and IGF-2, which are discussed by D'Ercole in this issue). In the fetus, these are independent of GH regulation. IGF-1 influences terminal differentiation of a number of tissues, including brain astrocytes, neural outgrowth, and myogenesis. Moreover, even though the influences of IGF-1 appear to be local, serum concentrations of IGF-1 correlate with birth weight.<sup>1</sup> Both IGF-1 and IGF-2 are complexed to binding proteins that modulate their biologic activity. Growth retardation in fetal rats caused by maternal starvation has been associated with decreased expression of IGF-1 and IGF-2 and increased expression of binding proteins in the liver, suggesting that these factors have a role in regulating fetal growth.<sup>2</sup> Epidermal growth factor (EGF) and EGF- $\alpha$ , which may be its fetal form, influence growth and differentiation of epithelial cells, including those in lung and gut. Receptors for EGF are present throughout development and are present in increased numbers in placenta and lung in fetuses with growth restriction induced by uterine artery ligation, suggesting a role for EGF in fetal growth retardation.<sup>3</sup> Additional evidence for an EGF effect on somatic growth is the observation that exogenous EGF administered to rats less than 2 weeks of age decreases growth. This effect of EGF has been related to suppression of IGF-1 concentrations in growth-restricted fetuses.<sup>4</sup> It is likely that the changes in blood flow that characterize the hemodynamic response to fetal nutrient restriction are modulated by endocrine mechanisms. Stressed fetuses have increased circulating levels of arginine vasopressin, which may contribute to the decreased splanchnic blood flow and increased blood flow to the brain that is associated with "brain sparing." Vasoactive prostaglandins also are of likely importance in modulating blood flow to the fetus and the hemodynamic changes resulting in brain sparing.

Maternal constraint of fetal growth and fetal adaptations occur under conditions of decreased nutrient supply or when fetal growth is inappropriate for maternal size. The latter may involve changes in growth factor or hormonal signaling. Mice selected for high plasma IGF-1 concentrations not only were larger but also produced litters with heavier fetuses than mice selected for low IGF-1 concentrations.<sup>5</sup> Maternal constraints on fetal growth also are illustrated by the classic study of Walton and Hammond showing that foals born to shire horses bred with female Shetland ponies are small and, therefore, were appropriate for maternal size. IUGR also may be multigenerational. A large reduction in

### Letter to the Editor

In the December 1991 issue of *GGH*, you cite on page 9 our abstract on the protective effect of GH on steroid damage to bone. Your comment was imprecise, in that the presentation was made by me and not Dr. Raphael Rappaport. I am also not aware of any award to this paper from the ESPE.

Sincerely,  
Zeev Hochberg, MD, DSc  
Department of Pharmacology  
Technion-Israel Institute of Technology  
Haifa, Israel

### Response From the Editor

Dear Dr. Hochberg:

You have learned of the fallibility of editors. Dr. Rappaport also informed me of my fallibility. Please accept my apologies. Your excellent abstract and presentation should have won an award. In that I am correct.

With embarrassment,  
Robert M. Blizzard, MD

maternal weight and newborn size was observed in a marginally nourished rat colony maintained for over 12 generations. More than 1 generation was required to correct the deficit in fetal growth after reinstitution of normal nutrition.<sup>6</sup> Women who themselves experienced IUGR have an increased risk for giving birth to either IUGR or preterm infants. This again emphasizes the importance of maternal factors and the intrauterine milieu.<sup>7</sup>

### DEFINITION

IUGR, or more preferably, intrauterine growth restriction, represents a final common pathway by which genetic and environmental influences result in low birth weight for gestational age. IUGR has been defined most commonly in the United States as a birth weight of less than the 10th percentile for gestational age. This broad definition has resulted in confusion since it is unreasonable to consider 10% of all births as being characterized by pathologic restriction of growth. Small infants in whom there is no evidence that adverse genetic or environmental influences are limiting growth should be spared the IUGR label, which connotes pathology, and should be defined as small-for-gestational age (SGA). Further refinements of these definitions include the following: (1) "small-for-gestational

age" should be applied to all infants <10th percentile; and (2) "intrauterine growth restriction" generally should be reserved for infants <3rd percentile, in recognition of the fact that some infants with growth restriction will fall out of this range if an insult occurs late in gestation. Thus, while all IUGR infants also will be SGA, not all SGA infants will be IUGR.

The confusion is amplified further by the significant differences in 10th percentile birth weights at each gestational age that have been used to define IUGR in different published studies. Differences in published standards of growth have likely been influenced by racial composition, socioeconomic status of the population studied, and elevation above sea level when the standards were developed. The commonly used Lubchenko grids, for example, were developed in Denver, which is approximately 5,000 feet above sea level; IUGR may be underestimated when these charts are used at sea level. What is necessary for an effective comparison between populations is the adoption of a single standard for fetal growth, for example, the standards developed by Brenner based on 30,772 deliveries made at 21 to 44 weeks of gestation in Cleveland.<sup>9</sup> These standards include correction factors for poverty, race, and sex.

### CLINICAL PRESENTATION

Genetic disorders associated with malformations or aneuploid chromosomal defects such as trisomy 18, Down syndrome, or Turner syndrome are obvious causes of IUGR. Infants with decreased growth potential due to structural or genetic defects; congenital infections, including syphilis and HIV infection; and toxic exposures, eg, heroin, cocaine, or alcohol, have a pattern of growth characterized as proportional or symmetric IUGR. That is, head circumference, length, and weight show the same degree of growth restriction and fall within the same percentiles for gestational age. Most infants with this pattern of IUGR born after 36 weeks continue to exhibit sluggish postnatal growth. IUGR also can result from maternal malnutrition or decreased uteroplacental blood flow in maternal disease states such as hypertension and diabetes, in which there may be decreased substrate delivery to the fetus.

Fetuses with growth restriction due to nutritional compromise show greater variability in body proportions and tend to have sparing of head growth.<sup>10</sup> Other conditions in which fetal nutrition is compromised include multiple pregnancies and the smaller of twins in which arteriovenous communications in the chorionic plate limit blood flow to one twin. IUGR also is seen in infants born at high altitudes or to mothers with cyanotic congenital heart disease,



presumably because of decreased oxygen availability. The infants with so-called disproportional or asymmetric IUGR resulting from nutritional compromise can show rapid catch-up growth after birth, but approximately 30% of such infants are still below the 5th percentile at 2 years of age. Infants with disproportional IUGR may be at risk for hypoxic injury, hypoglycemia, hypothermia, and polycythemia due to a chronically hypoxic state. These infants, however, can also be viewed as undergoing an adaptation to an adverse intrauterine environment. For example, constrained fetal growth in the presence of decreased uteroplacental blood flow can be considered an adaptation that decreases oxygen and substrate requirements and contributes to fetal well-being. The sparing of head growth reduction exhibited by IUGR fetuses results from an apparent increase in blood flow to the brain via the carotids when umbilical flow is compromised. IUGR fetuses with head sparing exhibit decreased umbilical blood flow as determined by Doppler flow measurements, and increased carotid flow.<sup>11</sup> An increase in circulating red cell mass, which can increase oxygen transport to fetal tissues, is another adaptation in IUGR. Polycythemia sufficient to result in hyperviscosity at birth can, however, result in neurologic impairment. The IUGR fetus may exhibit functional adaptations to decreased nutrition, such as accelerated lung and neurologic maturation, that would enhance its likelihood of extrauterine survival if born early. Growth-restricted newborns can be neurologically accelerated by 3 to 4 weeks when compared with normal-growth newborns of the same gestational age.<sup>12</sup>

Strategies to treat fetal growth retardation include therapies to decrease the platelet aggregation and uteroplacental circulation abnormalities seen in toxemia of pregnancy. Other treatments have included maternal nutritional supplementation and oxygen therapy. In a promising study of 323 women at risk for fetal growth retardation, administration of 150 mg/d aspirin resulted in a 225-g newborn weight increase over the placebo group and a frequency of growth retardation only 50% of the placebo group.<sup>13</sup> The beneficial effect of low-dose aspirin likely relates to inhibition of the synthesis of thromboxane B<sub>2</sub>, which decreases the platelet aggregation and placental vasocclusion seen in the toxemic state. Although some studies have suggested a benefit to maternal parenteral nutritional supplementation, the long-term results have not been convincing. An adverse influence was observed when short-term administration of glucose to normal patients before delivery resulted in a significant increase in lactic acid and a fall in pH.<sup>14</sup> Direct fetal nutritional supplementation via the amniotic fluid has been

## In Future Issues

**Growth Hormone Deficient-Like Syndromes and Their Etiologies**  
by William H. Daughaday, MD

**The Effect of Irradiation on Endocrine Function in Children: Past, Present, and Future**

by Stephen M. Shalet, MD

**Fragile X Syndrome: Current Status**  
by David Nelson, PhD

successfully carried out in the ovine fetus, but there is little experience in human pregnancy.<sup>15</sup> Philipps et al<sup>16</sup> reported that direct glucose infusion in the fetal sheep raised fetal metabolic rate and increased fetal oxygen consumption. It is likely that these responses would be exaggerated by intra-amniotic infusion of nutrients to fetuses in which there is already compromise of oxygen delivery. When the fetus is "adapted" to decreased nutrient supply, there may be potential risk to increasing nutritional intake without a corresponding increase in fetal oxygenation.

## SUMMARY

Since this topic was presented in *GGH* 5 years ago,<sup>17</sup> we have witnessed an increased understanding of fetal growth influences, particularly the role of fetal growth factors, as well as improved methods of obstetric diagnosis and treatment of intrauterine growth restriction. However, epidemiologic research remains hampered by the lack of a broadly used standard for defining growth. In addition, there is an increasing recognition that some features of IUGR infants, such as low birth weight, polycythemia, and advanced maturation, are actually adaptations to an adverse uterine environment that may enhance fetal survival.

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## Special Report

### Eighth International Congress of Human Genetics October 6-11, 1991, Washington, DC

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Over 5,000 persons attended the Eighth International Congress of Human Genetics, making it by far the largest gathering of human geneticists ever. It is virtually impossible for any one person to provide a complete review of the congress since its size dictated simultaneous sessions for almost the entire meeting. Indeed, after a brief opening session, the congress broke up into 52 concurrent workshops, 16 concurrent symposia, 10 concurrent slide symposia, and 10 concurrent slide sessions, all of which extended over 5 days and covered every conceivable topic related to human genetics. As well, there were nearly 2,500 posters. Nevertheless, there were themes that surfaced recurrently and other items worthy of comment as outlined below.

Developmental genetics was a hot topic. There were a number of papers in which animal models of human disease were described. The use of mice carrying mutant transgenes or mice in whom endogenous genes had been disrupted by gene targeting has eliminated the need for spontaneously arising mutations and provided considerable new insight into early human development and how malformations arise. Imprinting received considerable attention. Although not well understood, the occurrence of imprinting in humans is now well accepted; and its contribution to causing disease was the subject of a symposium.

Genetic and physical mapping of the human genome is occurring at a rapid pace. The recent identification of several disease-associated genes was reviewed. Detection of mutations in the fibrillin gene in Marfan syndrome provides a good example. There was a time when excluding genetic linkage between a disease and a gene locus was considered negative information. However, such data are now being compiled to produce so-called exclusion maps (ie, identifying that part of the genome in which a particular gene locus does not reside) for many conditions. These continue to narrow down the chromosomal regions where disease-associated genes must reside and will eventually permit their localization.

There were many papers addressing the mapping of genes that predispose to common diseases, such as coronary artery disease and Alzheimer's disease, as well as rare diseases.

It has been known for many years that genes are regulated by transcription factors that bind to DNA in a highly specific fashion. Evidence has accumulated that large complexes composed of many proteins that bridge DNA

binding sites are actually responsible for the control. It now appears that the fine-tuning of this control may depend more on the protein-protein interactions among the factors than on the protein-DNA interactions of the factors and the DNA binding sites.

Genetics is the study of variation and, as usual, many new syndromes and variants of established syndromes were described. However, in the case of the chondrodysplasias, a new and simpler nomenclature was unveiled. It groups the disorders into communities and families of chondrodysplasias that share common features and possibly common pathogenic mechanisms.

There was much interest in the technique of fluorescent *in situ* hybridization (FISH). The technique is being used to map genes to chromosomal sites and to determine the number of copies of particular genes or even chromosomes. For example, FISH was employed to detect chromosomal mosaicism in the placentas of infants with intrauterine growth retardation. The technique will probably see many new applications over the next few years.

A wide variety of new "things" were presented. They ranged from new ways to clone and map genes to new approaches for prenatal diagnosis, from new views on human evolution to better ways to set up disease registries and educate professionals and the laity about genetics. For most attendees, including myself, the congress provided many opportunities for information overload. Fortunately, most of the best new knowledge will appear or has recently appeared in the literature.

William A. Horton, MD

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## Does Growth Hormone Treatment Improve Final Height Attainment of Children With Intrauterine Growth Retardation?

Results obtained with growth hormone (GH) treatment in 24 prepubertal (19 males, 5 females) intrauterine growth retarded (IUGR) children (ages 2 to 9 years; mean, 6.3 years) were reported. All patients' heights were less than the 3rd percentile. The mean growth velocity (GV) was  $-0.76$  standard deviation (SD) for age. All had normal levels of GH during pharmacologic testing and/or overnight sampling. Eighteen were considered to have the morphologic features of Russell-Silver syndrome; 6 did not. Subcutaneous GH was given daily for 3 years at doses of either  $15 \text{ IU/m}^2/\text{wk}$  ( $n=11$ ) or  $30 \text{ IU/m}^2/\text{wk}$  ( $n=13$ ) the first year. All received  $30 \text{ IU/m}^2/\text{wk}$  for the following 2 years. Puberty did not occur during the 3 years in any of the patients.

Mean GV (SD for age) increased during the first year to  $+1.4$  with GH  $15 \text{ IU/m}^2/\text{wk}$  and to  $+3.6$  with GH  $30 \text{ IU/m}^2/\text{wk}$ . It remained at  $+1.5$  SD the second year and  $+1.1$  the third year. There was great variability in individual results, with no differences between sexes or between the Russell-Silver patients and the others. However, there was no improvement of height SD score (SDS) for bone age. Therefore, the height prognosis did not increase ( $-1.6$  SD at the onset of treatment and  $-1.5$  at the end). Results were similar regardless of the dose received the first year.

The authors do not offer a clear-cut answer to the question asked in the article's title. They only note that there was no decrease in height for bone age, which usually occurs in IUGR children during the late prepubertal years. Thus, they postulate that these children could possibly reach a better final height than otherwise would have occurred. They also raise the idea that

treatment may be of psychologic benefit because of the advanced tempo of growth.

Stanhope R, Preece MA, Hamill G. *Arch Dis Child* 1991;66:1180.

**Editor's comment:** IUGR is a major cause of significant short stature, with a frequency approximating 1% of the total population. Although the positive short-term effect of GH in many children with abnormal birth lengths has been known for many years, the long-term effect on ultimate stature has not been known. The current study, which employed an excellent methodology, clearly shows that the value of GH in increasing ultimate height at least in Russell-Silver-associated short stature (and probably other types of IUGR) remains uncertain. The question is of great practical importance because of the handicap of severe short stature and the high cost of GH for treatment over many years. This study is not encouraging except in relation to increasing the tempo of growth, which can be important psychologically. Large-scale studies looking at ultimate growth, as well as tempo of growth, are taking place, and we must wait until these are completed to answer the question, "Does GH treatment improve final height attainment of children with IUGR?" Since this question will not be answered immediately, the routine or even frequent use of GH for IUGR patients cannot be encouraged on the basis of published data.

J. C. Job, MD

## Growth Status and Growth Rates of a Varied Sample of Low Birth Weight, Preterm Infants: A Longitudinal Cohort From Birth to Three Years of Age

The Infant Health and Development Program, a collaborative effort by 8 US medical schools, is the basis for this longitudinal study of the growth characteristics of low-birth-weight (LBW), preterm infants. In it, 985 LBW infants were grouped as follows:

| Group | No. | Weight        |
|-------|-----|---------------|
| 1     | 149 | <1,250 g      |
| 2     | 474 | 1,251-2,000 g |
| 3     | 362 | 2,001-2,500 g |

All infants were assessed at 40 weeks postconceptional age and at 4, 8, 12, 18, 24, 30, and 36 months, gestation-corrected age. Growth rates were estimated for 0 to 12, 12 to 24, and 24 to 36 months of age. Each measurement was available for at least 956 children.

Boys in each group differed significantly at all ages for length, weight, and head circumference. Girls differed significantly in head circumference at all visits, in weight until 24 months, and in length until 18 months. In summary, there was evidence of compensatory growth in length for both sexes in the first year of life but none thereafter. However, this is far from complete by age 36 months, gestation-corrected age. The data demonstrate that LBW preterm infants have different patterns of growth during the first years of life, as compared with term infants. Their growth should be monitored on grids developed from similar infants.

Casey P, Kraemer H, Bernbaum J, et al. *J Pediatr* 1991;119:599-605.

**Editor's comment:** These data are the best yet available to compare the growth characteristics of LBW preterm infants with those of term infants. The data do differ from those of other investigators in that others have reported more "catch-up" growth than that reported here. However, as the authors point out, most other studies have been too small and/or have failed to maintain the cohort for long enough intervals to describe adequately the long-term status and patterns of growth in LBW infants. The authors, in total fairness, caution that these data include some on infants who were both preterm, which was a criterion for admission to the study, and LBW for gestational age. The authors plan to use these descriptive growth data to develop comparison standards for monitoring the growth of all LBW preterm infants independent of such clinical characteristics as size (appropriate-for-gestational age versus small-for-gestational age), presence of chronic neurologic disease, and the like. Current National Center for Health Statistics growth charts are not categorized by any such clinical characteristics. The authors are commended for diligently pursuing this complex problem and are encouraged to continue. Further data are very much needed, for example, to chronicle the differences in growth characteristics at all ages until adulthood is attained.

W. L. Clarke, MD

## Parental Imprinting and Fetal Growth

Insulin-like growth factor 2 (IGF-2) has long been implicated as an important fetal growth factor. Three recent reports now suggest that this effect is primarily due to expression of the paternally derived IGF-2 gene (called *Igf2* to distinguish it from the gene product IGF-2).

DeChiara et al used gene targeting to disrupt *Igf2* in embryonic stem cells that were employed to generate mice chimeric for the mutation. Once mice heterozygous for the mutation were established through breeding, transmission of the mutated gene was followed through several generations. The investigators found that when it was transmitted through the mother, there was no effect on the size of the offspring receiving the mutation. However, when transmitted through the father, the progeny receiving the nonfunctional *Igf2* genes were growth-deficient and approximately 60% of normal size. Thus, among heterozygotes for the *Igf2* mutation, only those receiving it from the father were growth-deficient. Using mRNA assays that distinguished between expression of the normal and mutated *Igf2* genes, they further demonstrated that the maternal *Igf2* allele was silent except in the choroid plexus and leptomeninges, where both alleles were expressed. They concluded that the maternal *Igf2* allele is imprinted and therefore inactive in most tissues.

Beckwith-Wiedemann syndrome (BWS) is a fetal overgrowth syndrome in which tumors often arise. The constitutional karyotype is usually normal in the syndrome; however, in several instances DNA studies have demonstrated loss of the maternal contribution of genes that map to chromosome 11p15.5 in the tumors. This has been of considerable interest because it is the chromosomal site to which *Igf2* maps in humans. Suspecting possible uniparental disomy for genes mapping to this region (both sets of genes come from 1 parent rather than 1 set from each parent), Henry and coworkers determined the parental source of several 11p15.5-mapped genes in 21 sporadic cases of BWS with normal karyotypes. The parental source could be determined for at least 1 gene in 8 instances. Three of these 8 had only paternal genes and therefore displayed paternal disomy.

The third report extends this story further. *Igf2* maps to distal chromosome 7 in the mouse. Ferguson-Smith et al introduced cells from very early mouse embryos that carried duplications of either the paternal or maternal distal chromosome 7 into normal mouse blastocysts. The phenotype of the chimeric mice that were generated differed substantially depending upon the

source of the 7p duplication. If the paternal duplication of distal 7 was present, the mice were substantially larger than control mice. In contrast, no size difference was noted when the maternal duplication for distal 7 was present. Comparison of mRNA levels for *Igf2* showed increased *Igf2* expression associated with the paternal distal 7 duplication but very low levels of *Igf2* expression in the mice harboring the maternal distal 7 duplication.

DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 1991;64:849-859.

Henry I, Bonaiti-Pellie C, Chehense V, et al. Uniparental paternal disomy in a genetic cancer-predisposing syndrome. *Nature* 1991;351:665-667.

Ferguson-Smith AC, Cattanach BM, Barton SC, et al. Embryological and molecular investigations of parental imprinting on mouse chromosome 7. *Nature* 1991;351:667-670.

**Editor's comment:** *It is often held that maternal factors contribute more to fetal size than do paternal ones if for no other reason than that the fetus resides in the mother and is exposed to a host of maternally determined physical and chemical factors. However, these 3 investigations, utilizing completely different methods, strongly support the view that at least certain aspects of fetal growth are influenced more by the father than by the mother. The active Igf2 gene appears to be the one inherited from the father, whereas the maternally derived Igf2 gene is inactive in most tissues due to imprinting.*

William A. Horton, MD

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## Prevention of Fetal Growth Retardation (FGR) With Low-Dose Aspirin

The efficacy of low dose aspirin therapy in preventing fetal growth retardation (FGR) was tested in a randomized, placebo-controlled, double-blind trial. The possible beneficial effect of adding dipyridamole to aspirin also was tested. Studied were 323 women (29 to 45 years of age) who had been amenorrheic because of conception for 15 to 18 weeks. All had experienced at least 1 previous pregnancy with FGR and/or fetal death or abruptio placentae. They were randomized into 3 groups, receiving in double-blind fashion either:

**Group 1** (n = 128) aspirin, 150 mg/d

**Group 2** (n = 212) aspirin, 150 mg/d + dipyridamole, 225 mg/d

**Group 3** (n = 74) placebo

Twin pregnancies, uterine malformations, and histories of specific known previous disorders that could affect pregnancy outcome were reasons for exclusion from the study. Of the 323 subjects, 284 satisfied all the criteria and were considered eligible for the epidemiologic analyses.

The birth weight was significantly ( $P=0.029$ ) better in the treated groups ( $2,759 \pm 670$  g) than in the placebo group ( $2,526 \pm 848$  g). The frequency of FGR, evaluated according to Lubchenco's percentiles, was 13% in the treated groups vs 26% in the placebo group ( $P=0.02$ ). The incidence of stillbirths (5% vs 1%) and abruptio placentae (8% vs 5%) was more frequent in the placebo group. The mean duration of pregnancy reached 264  $\pm$  19 days in the treated vs 258  $\pm$  27 days in the untreated women ( $P=0.05$ ).

The frequency of hypertension, proteinuria, hyperuricemia, and thrombocytopenia was similar in all groups. Apart from headache, which occurred in both the placebo and the treatment groups, the incidence of maternal side effects was very low, and no neonatal side effects were observed. In all these respects, no significant differences were found between the group receiving aspirin alone and that receiving aspirin plus dipyridamole.

The authors conclude that their study confirms the efficacy of low-dose aspirin given early in pregnancy in preventing FGR. They suggest that it acts on prostaglandins, and probably inhibits thromboxane production. They do not yet recommend widespread use of aspirin in pregnant women, since they are conscious that much larger scale trials are needed to determine its complete safety. Their conclusion is that low-dose aspirin treatment may be beneficial for any pregnancy considered at high risk of FGR, and they hope that early, reliable, and inexpensive markers of this risk will be found.

Uzan S, Beaufils M, Breart G, et al. *Lancet* 1991;337:1427-1431.

**Editor's comment:** FGR, with its many immediate dangers for the child, is a major concern for neonatologists and obstetricians. It is also of extreme importance for all those who are interested in children's growth, since short stature of intrauterine onset appears to be the main type of severe height insufficiency (with a poor prognosis for adult stature) seen in pediatric and adolescent endocrine clinics. Thus, any attempt to reduce the frequency or degree of FGR may have a great impact on improving this pediatric situation. Although it is on the obstetrical side of fetal medicine, this trial should be of direct interest for all growth specialists.

J. C. Job, MD

## Developmental Genes and Birth Defects

Although mutations of genes involved in early embryologic development have long been suspected as causing birth defects in humans, direct evidence has been lacking. The "suspect" genes are those involved in determining the basic body plan of the embryo. Their products typically regulate expression of other genes and provide position signals that help to control developmental patterns. Several classes of genes have been characterized in lower organisms, particularly in *Drosophila*, based primarily on the patterns of malformation produced when mutations occur. Comparable genes are now being identified in higher organisms, including humans. The 2 papers described below implicate mutations of these genes in 2 human malformation syndromes.

The Greig cephalopolysyndactyly syndrome (GCPS) is an autosomal dominant condition characterized by macrocephaly, unusual facies, and polysyndactyly of the hands and feet. Previous cytogenetic studies in families exhibiting translocations had localized the mutation site to chromosome 7p13. To identify the gene(s) involved Vorkamp et al produced a panel of human-mouse hybrid somatic cell lines from 3 different GCPS patients in whom a chromosomal translocation had produced a small deletion of chromosome 7p. These cell lines contained human chromosome 7 material that bordered the translocation breakpoints both proximally and distally, thus allowing analysis of the deleted or disrupted genes. Hybridization of probes to different portions of a gene called *GLI3*, which was recently mapped to this chromosomal region, showed that in 2 cases the translocation breakpoint disrupted the *GLI3* gene in the first

third of the molecule. Such a mutation would be expected to truncate the *GLI3* protein. The third translocation localized outside the coding region of the gene and was thought to have caused its adverse effect by reducing expression of the adjacent *GLI3* gene. The authors concluded that all 3 translocations led to reduction in the formation of functional *GLI3* protein. Pertinent to this discussion, *GLI3* encodes a so-called "zinc finger" DNA-binding protein that is thought to be a member of the *GLI-Kruppel* gene family. In lower organisms these genes play crucial roles in early development, and mutations thereof produce position-specific malformations.

In a second paper, Chisaka and Capecchi employed the technique of gene targeting to generate mice in which the homeobox gene *Hox-1.5* was disrupted. The *Hox* gene products are transcription factors that regulate development within specific segments of the developing embryo; *Hox-1.5* is expressed roughly in the region corresponding to the branchial arches. They disrupted the gene in pluripotential embryonic stem cells, introduced colonies of cells carrying the disrupted gene into early mouse embryos to generate mice chimeric for the disrupted gene, and then bred the mice to produce offspring heterozygous or homozygous for the mutant *Hox-1.5* gene. The heterozygotes for the mutation were normal. However, the homozygotes died at or shortly after birth and exhibited malformations involving the thymus; parathyroid and thyroid glands; and structures of the throat, heart, arteries, and cranium and facies. This pattern of malformations was remarkably similar to that observed in the DiGeorge syndrome, and the

authors proposed this mouse be used as an experimental model to study the human syndrome. The mechanisms by which such a mutation could produce the observed malformations was discussed at length in the article and in an accompanying editorial by Wright and Hogan.

Vortkamp A, Gessler M, Grzeschik K-H. GLI3 zinc-finger gene interrupted by translocations in Greig syndrome families. *Nature* 1991;352:539-541.

Chisaka O, Capecchi MR. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *Hox-1.5*. *Nature* 1991;350:473-479.

Wright C, Hogan B. Another hit for gene targeting. *Nature* 1991;350:458-459.

**Editor's comment:** *It must be emphasized that the brief descriptions above grossly oversimplify how genes control early embryologic development as well as the nature and interpretation of the experiments reported. Nevertheless, they serve to illustrate that the field of molecular developmental biology is rapidly moving from flies, frogs, and mice to humans, and is finding direct application to the understanding of human developmental abnormalities, ie, birth defects. As pointed out by*

*Wright and Hogan, many other genes involved in early development are currently being studied by gene targeting and related approaches. The results of these investigations should provide a wealth of new information about the causation of human birth defects in the near future.*

William A. Horton, MD

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## Activating Mutations of the Stimulatory G Protein in the McCune-Albright Syndrome

The cause of the clinical symptomatology in McCune-Albright syndrome (MAS), including sexual precocity, multiple hyperfunctional endocrinopathies, polyostotic fibrous dysplasia, and café au lait spots, has been the subject of extensive speculation for many years. The capability of modern genetics to examine mutations of genes has now made it possible to specifically identify an abnormal mutation within exon 8 of the G protein  $\alpha$ -subunit ( $G_{\alpha}$ ) that stimulates cyclic adenosine monophosphate (cAMP) formation. Four patients with severe MAS were studied by the authors and identified as having a mutation of the Arg<sup>201</sup> position in tissue. Two of the 4 had an His mutation and the other 2 a Cys mutation. The abundance of mutations in different tissues was variable. Not only was there evidence determined for mutations in testis, ovary, adrenal, pituitary and thyroid but also in the heart, lung, liver, kidney, thymus, and spleen. Two of the 4 patients died a sudden death, which may have been related to cardiac dysfunction. Unfortunately, the authors were unable to determine whether mutations were present in the polyostotic lesions or in the café au lait hyperpigmentation. Reasonable explanations are given why the mutant was not demonstrable in these 2 tissues as studied. In an editorial in the same issue, Dr. Michael Levine of Johns Hopkins reports finding the same defect in affected skin of 1 patient. Therefore, the defect remains to be identified only in bone, and this will probably be demonstrated in the near future.

The mutant abnormality produces a significant decrease in the guanosine triphosphatase (GTPase) activity of the  $\alpha$ -subunit of the G protein, with the end result of increased adenylyl cyclase activity.

The authors appropriately state that for each case only 1 specific mutation (R201C or R201H) is detected, which is consistent with a monoclonal abnormal cell population. This mutational event occurs prior to development of the trilaminar disc because of the widespread distribution and mutation of tissues derived from all 3 embryologic germ layers, and because of the variable abundance among tissues within a given patient. The absence of mutation in at least 1 tissue from each case is consistent with a somatic rather than germ line mutation. Intriguingly, the  $G_{\alpha}$  mutations were present in virtually all affected

MAS endocrine tissues analyzed. The affected tissues within each organ had a greater proportion of the mutant population than did the unaffected tissues. The presence of activating Arg<sup>201</sup> mutations was first described in sporadic growth hormone-secreting pituitary adenomas, which have autonomous cAMP synthesis. Importantly, the authors clarify the association between Albright's hereditary osteodystrophy (AHO) and MAS. AHO is associated with  $G_{\alpha}$  gene mutations, which lead to a deficiency in G protein. The mutations within MAS are different but of the same gene. Mutations in AHO impair the G protein signal transduction pathway while those found in MAS have the reverse effect, ie, the activation of the G protein pathway, an effect that probably underlies the clinical manifestations of the syndrome.

Weinstein LS, Shenker A, Gejman PV, et al. *N Engl J Med* 1991;325:1688-1695.

**Editor's comment:** *The findings reported in this article are exciting, thoroughly done, and now give us a much better understanding of MAS phenomena. The authors are to be congratulated for their fine work and for their contribution.*

*Clinicians now must be made aware that the symptomatology in severe cases of MAS may be much greater than previously understood. The presence of the mutant gene in multiple tissues can lead to diverse clinical pathophysiology. Incomplete presentations of MAS may represent cases in which there is an even more limited distribution of mutant cells. Liver, cardiac, and renal disease need to be considered in patients who present with MAS. Undoubtedly a much larger group of these patients than previously demonstrated produce excessive growth hormone, which may account for the fact that many patients with MAS do not have the short stature we usually expect in the typical patient with sexual precocity. Unexplained as yet is the significantly higher incidence of this syndrome in females than males.*

*Those interested in this report also will want to read the editorial by Dr. Michael Levine in the same issue of the New England Journal of Medicine, entitled "The McCune-Albright Syndrome: The Whys and Wherefores of Abnormal Signal Transduction."*

Robert M. Blizzard, MD

## Standardized Percentile Curves of Body-Mass Index for Children and Adolescents

The data collected in the First National Health and Nutrition Examination Survey (NHANES I) from 1971 to 1974 were used to construct centile curves for body-mass index (BMI,  $\text{kg}/\text{m}^2$ ) for white US boys and girls (ages 1 to 19 years). The raw means for each age were smoothed by quadratics fitted in 2 sections (males, 1 to 11 years and 8 to 19 years; females, 1 to 13 years and 6 to 19 years) and by splicing the 2 sections together (males at 10 years and females at 7 years).

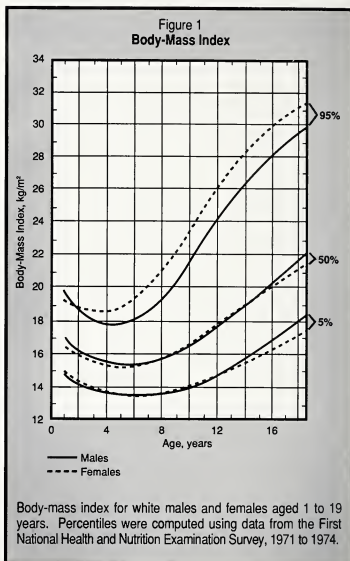
The curves for BMI, by percentiles, for males and females are reproduced in Figure 1. Percentile values in tabular form are presented in the article, and these values will be of importance to those investigators concerned with relating BMI to other growth characteristics.

The authors conclude that these curves may be used to monitor the development of obesity as well as changes in BMI associated with treatment of obesity in childhood and adolescence. The pattern of BMI has been shown to be a predictor of long-term obesity in childhood and to predict morbidity and mortality in adulthood. Therefore, it is recommended that clinicians routinely measure height and weight and monitor BMI in children and adolescents. BMI curves should be developed for other racial groups as well. Further studies also are needed to define appropriate BMI cutoff points to more precisely define obesity in childhood and adolescence.

Hammer LD, Kraemer HC, Wilson DM, et al. *AJDC* 1991;145:259-263.

**Editor's comment:** These curves provide useful cross-sectional standards for BMI for white US children. Admittedly, they relate to the 1970s and values today may be somewhat higher. The NHANES survey oversampled lower socioeconomic groups, but within-age analysis of the data did not show any significant socioeconomic differences. Thus, no adjustment for socioeconomic level was made. These are probably the best childhood population standards for BMI to date.

James M. Tanner, MD



## Effect of Growth Hormone and Resistance Exercise on Muscle Growth in Young Men

Growth hormone (GH) treatment in childhood increases net body protein. GH in adults reportedly increases fat-free mass (FFM). Whether the increase in FFM is due to an increase in muscle protein is unknown. Fiber size of skeletal muscle, whole muscle area, and muscle force-generating capability increase with heavy resistance exercise training (HRET). However, it is unclear how human skeletal muscle and whole body protein turnover are affected by HRET. The purpose of this double-blind, placebo-controlled study was to determine the effects of HRET on FFM, muscle size and strength, the rate of whole body protein turnover, and the rate of protein synthesis in the quadriceps muscle, and to examine whether GH supplementation enhances the anabolic response to HRET.

Sixteen subjects completed a 12-week study in which all underwent a HRET program. Seven received approximately  $40 \mu\text{g}/\text{kg}/\text{d}$  of GH and 9 received placebo. Appropriate and eloquent studies of body composition were carried out to permit answering of the questions asked. As determined by hydrodensitometry, FFM increased significantly in both groups, but the increment was greater in the GH-treated group. Since FFM is principally water, total body water increased in proportion to FFM in the 2 groups. Chest and upper arm circumference increased in both groups, but thigh and mid-thigh circumference increased only in the GH-treated group. Muscle strength improved identically in

both groups, as did increments in concentric force production. Whole body protein synthesis increased more in the GH-treated group, as did body protein balance.

The authors concluded that HRET increased FFM, muscle size, and muscle strength, and tended to increase the fractional rate of quadriceps muscle protein synthesis. GH treatment added to the training regimen (HRET) produced no significant further increase in muscle size, muscle strength, or fractional rate of muscle protein synthesis. The results indicate that pharmacologic doses of GH given to young men with normal GH secretory function do not enhance skeletal muscle protein accretion or muscle function more than resistance training without GH treatment. The greater increase in FFM and whole body protein synthesis rate observed in the GH-treated group indicates that these individuals accumulate additional lean tissue, but it is unlikely that this tissue was skeletal muscle protein. Therefore, the rationale for using GH to amplify exercise-induced muscle growth and thus enhance athletic performance appears to have no foundation in fact.

Yarasheski KE, Campbell JA, Smith K, et al. *Am J Physiol*. 1992;25:261-267.

**Editor's comment:** Amen. A precise, lucid study that all aspiring potentially muscle-bound athletes should read.

Robert M. Blizzard, MD

## Treatment of Children With Down Syndrome and Growth Retardation With Recombinant Human Growth Hormone

Short stature is known to be one of the features of Down syndrome (DS). The authors treated 13 children with DS who were short for age (standard deviation score [SDS] -1.19 to -3.5), microcephalic (-1.58 to 6.60 SDS), and had no heart disease. Before treatment, peak serum growth hormone (GH) concentrations were less than 10 µg/L after levodopa and clonidine stimulation tests in 5 patients, after clonidine in 3 patients, and after levodopa in 3 patients. Three patients had nocturnal integrated GH concentrations of 0.5, 1.5, and 0.65 µg/L, respectively. The endocrine findings before treatment were normal with respect to luteinizing hormone, follicle-stimulating hormone (TSH), thyroxine, and triiodothyronine.

The patients were given recombinant human GH (rhGH), 0.1 mg/kg subcutaneously, 3 days a week for 1 year. The mean growth rate before treatment was  $5.4 \pm 1.6$  cm/yr and increased to  $12.2 \pm 3.2$  cm/yr ( $P < 0.001$ ) after 12 months of rhGH treatment. The mean head circumference SDS before treatment was  $-3.1 \pm 1.3$  and increased to  $-2.3 \pm 1.2$  ( $P < 0.001$ ) at 12 months.

Two patients in whom elevated serum TSH concentrations developed while on rhGH treatment for 6 months were started on levothyroxine treatment. Bone age increment during the year of treatment corresponded to the increment in chronologic age. Plasma hemoglobin A<sub>1c</sub> concentration remained normal. The mean plasma concentrations of insulin-like growth factor 1 at baseline and at 12 months were  $0.54 \pm 0.19$  U/mL and  $1.25 \pm 0.97$  U/mL, respectively ( $P < 0.02$ ). The authors concluded that rhGH therapy can result in a significant increase in annual growth rate and head circumference in children with DS, without significant side effects.

Torrado C, Bastian W, Wisniewski, et al. *J Pediatr* 1991;119:478-483.

**Editor's comment:** This provocative paper offers rhGH as a treatment for short stature and microcephaly in children with DS. The most impressive part of the study is the remarkable response of DS patients to GH treatment. They exhibited catch-up growth with a mean of  $12.2 \pm 3.2$  cm/yr, which is impressive even for patients with hypopituitarism. However, we have to point out several pitfalls of this study, including the lack of data to ascertain the possible causes of GH alterations in DS.

First of all, the height and weight of the patients were compared with growth charts for normal children rather than the standards for children with DS. The growth pattern of these

patients should be compared with children who have the same chromosomal defect.<sup>1</sup> No details were given about the age, sex, and pubertal stage of the patients.

Second, the authors come to the conclusion that neurosecretory problems were the cause of growth retardation. However, only 3 patients had integrated GH studies showing decreased GH levels. The criteria for neurosecretory dysfunction of GH in otherwise normal children are being debated.<sup>2</sup> In patients with problems such as DS, there would be much more debate to establish the criteria for this diagnosis. These patients did not meet the classic criteria of neurosecretory GH dysfunction. The growth velocity before treatment was above normal (5.4 cm/yr) instead of the usual decreased growth rate (below 4 cm/yr). Only 2 of the 13 patients had normal secretion of GH with pharmacologic provocative tests, which differs from the classic criteria and implies a normal response to pharmacologic stimulus and decreased physiologic levels. However, further explanations need to be sought for the excellent response to GH therapy. If this response was not associated with puberty or other factors in medical care that improve growth, it might suggest that DS patients present with a form of GH resistance as seen in other conditions, ie, uremia.

There might be another explanation for the GH unresponsiveness to pharmacologic stimulus. The body weights of these patients were not reported. Nonetheless, the SDS for weight ( $-1.0 \pm 0.7$ ) was higher than the SDS for height ( $-2.2 \pm 0.8$ ). It is a well-known fact that obesity is associated with decreased GH responsiveness.

This paper, despite its deficits, does imply that further double-blind, placebo-controlled studies should be undertaken to clarify the pathogenesis of growth retardation and to confirm the response to rhGH treatment in DS. Moreover, changes in head circumference and its correlation with intelligence must be studied in more detail. Caution should be exercised in initiating GH treatment in DS patients, unless it is undertaken as part of a carefully controlled and well-designed scientific study.

Fima Lifshitz, MD

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**Abstracts:**

Final Height in Turner's Syndrome and Effect  
of Oxandrolone

Mutation in the Gene Encoding the Stimulatory  
G Protein of Adenylate Cyclase in Albright's  
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# GROWTH

## Genetics & Hormones

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Vol. 8 (Supplement 1)

May 1992

### **ACCESS TO TREATMENT WITH HUMAN GROWTH HORMONE: *Medical, Ethical, and Social Issues***

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*A Special Proceedings Supplement*

Dear Colleague:

You as a reader have an exciting opportunity. These presentations and discussions of a symposium that was entitled, "Access To Treatment With Human Growth Hormone: *Medical, Ethical and Social Issues*," represent possibly your first opportunity to learn and evaluate how ethicists, philosophers, and economists regard the endeavors of pediatricians to assist very short children to grow.

This conference, held October 27 and 28, 1991, was limited in attendance to a few pediatric endocrinologists and ethicists because of expenses. You who were not present may be at an advantage. Why, you might say? My reply is based on my personal wish to have been present and involved. I thought being absent would hinder my learning what individuals in different disciplines thought about growth hormone (GH) therapy. Given subsequent conversations with the attendees that identified their significant frustrations at having their secure concepts regarding treatment of growth hormone deficient (GHD) or GHD-like short children questioned, these transcripts were received with some anxiety. There was no need for that. Reading the transcripts exhilarated me because there was so much to learn about the attitudes and concepts of ethicists from the fields of philosophy, economics, and religion.

My absence from the conference spared me the anxiety and frustration of becoming emotionally involved. You also have been spared these initial frustrations and anxieties. Reading the transcripts opened entire new areas of thoughts and information for me to ponder. You, hopefully, will have the same experience.

An editor has multiple responsibilities. One is to assist the reader to gain the maximum return or rewards for the time invested. Because these proceedings are lengthy, and undoubtedly your time is limited, I would like to suggest an approach that may give you the maximum return for your invested time.

Immediately after reading this introduction, scan the "Table Of Contents," the "Presentation Content Outline," and the "List Of Participants" for identification of their qualifications to participate. After reviewing the outline, you may have priorities for further reading. Hopefully, you will decide to invest adequate time to read the proceedings completely. However, you may not have that luxury. Regardless of how much of the contents you intend to read, first read the introduction and preface to each of the 5 sessions by Dr. David Allen which will give you insight into the goals of each session. Then, I suggest that you read the summation papers in Session V by Drs. Lantos and Allen. I believe practicing pediatric endocrinologists may prefer to then

read the presentations and discussions in Session III, "Conceptual and Ethical Issues in Entitlement to GH Treatment," and/or Session IV, "Socioeconomic Issues Relevant to the Treatment of Short Stature." Fellows in pediatric endocrine training, pediatricians, and pediatric residents may wish to focus first on Session I, "Determining GH Insufficiency and the Efficacy and Toxicity of Human GH Therapy" and/or Session II, "Psychologic and Social Issues in GH Therapy." I predict geneticists will not settle for less than reading it all. Nurses will select preferentially on the basis of their own interests and experiences. Ethicists will particularly enjoy Session II, "Psychologic and Social Issues in GH Therapy." All should complete their review by reading the "Concluding Comments" in Session VI by Dr. Alan Weisbard, whose remarks included:

"In contrast to most conferences I attend, at this one I actually learned something. The most fascinating aspect of the conference has been the challenge of communicating across disciplinary divides....As I understand Dr. Stabler, whatever criteria one uses to distinguish GHD from non-GHD, height is not the only issue."

Incidentally, there is a recurrent paradigm that appears in the text that you may find confusing without further explanation. There is a reference to Johnny and Billy – 2 hypothetical patients with short stature – one who has growth hormone deficiency and one who does not. For more information, please see page 46 under the header, "Challenges to the Treatment/Enhancement Distinction."

Before encouraging you to turn to the outline and the participants list, please join me in recognizing and thanking the conference sponsor, the University of Wisconsin School of Medicine, Department of Pediatrics, and the commercial companies and foundations that provided funding for this educational event. These include Genentech, Inc., Eli Lilly, Inc., Ross Laboratories, the Turner Syndrome Society, the Human Growth Foundation, and MAGIC Foundation. Expressions of gratitude and appreciation also are due to Drs. David Allen and Norman Fost, who conceived, organized, and hosted this seminar. They have again proved themselves leaders in their fields.

On behalf of the Editorial Board of *GHH*,



Robert M. Blizzard, MD  
Consulting Editor  
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Special Proceedings Publication  
As A Supplement To  
*GROWTH, Genetics, & Hormones*

# **ACCESS TO TREATMENT WITH HUMAN GROWTH HORMONE: *Medical, Ethical, And Social Issues***

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Sponsored by:  
Department of Pediatrics and  
Program In Medical Ethics  
University of Wisconsin  
Madison, Wisconsin



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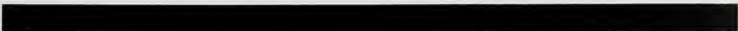
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## INTRODUCTION

Dear Colleague:

Widespread availability of human growth hormone (GH), along with advancing knowledge about its efficacy, are timely and important topics. Ten years ago, pituitary-derived GH was precious, and its rationing a necessity. The advent of recombinant DNA technology has made human GH abundant—abundant, but still expensive. Short children previously denied access to GH theoretically may now be treated. However, for many pediatric endocrinologists, this “feast” of GH has become more frustrating than its “famine.” The luxury of availability has brought uncertainty, controversy, and discomfort to practitioners trying to do the *right thing* with GH. While we have learned much about GH in the past decade, we seem to know less about how best to use it.

One reason for this is that GH therapy has expanded beyond the boundaries of traditional endocrinologic endeavors, where missing hormones are replaced and excessive hormone production suppressed. GH availability has led to the development of GH *augmentation* therapy in addition to GH *replacement* therapy. And future goals of GH therapy appear likely to shift further toward supplementing and “enhancing” individuals’ well-being rather than merely returning them to some physiologic baseline.

“Is it really GH deficiency?” was the perplexing question of the 1980s. Likely to dominate the 1990s, however, is debate among patients, parents, insurance companies, and physician colleagues about “How short is too short?” or “How tall is tall enough?” These are not medical questions; they are philosophic, psychologic, and economic questions. Continuing to do what we endocrinologists do best (eg, finding a better description of GH deficiency [GHD] or exploring new candidates for GH therapy) will not answer them. Today, only endocrinologists should prescribe GH. But in the future, endocrinologists alone will not be able to determine who is and is not entitled to it, especially if GH is effective for non-GHD children.

As a first step in this multidisciplinary process, we have convened physicians, medical ethicists, medical economists, and psychologists to explore ideas and concepts that may help to

guide allocation of human GH. To facilitate discussion, several presenters have been asked to support extreme viewpoints that may not accurately reflect their own beliefs or practice. The current expense of GH obviously invites debate about allocation of health-care resources for the treatment of short stature from any cause. Subjecting children to long-term, invasive treatment requires evaluation of psychologic benefit and harm as well as potential toxicity. Augmenting height potential, perhaps at the expense of others who must do without GH, requires consideration of fairness and justice. The expertise of those who study and write about these aspects of health care will help to clarify these issues.

To those of us who *practice* health care and treat children with short stature, however, entitlement to GH is not an abstract issue. It is hoped that our discussions will also capture the emotional and ethical dilemmas arising from our sincere desire to do the best for every “real life” child, and our uncertainty whether what *can* be done with GH is, in fact, what *should* be done.

The success of this conference depends on an uninhibited, critical, and challenging sharing of ideas. To maintain a small group atmosphere, conference attendance had to be limited. Consequently, many outstanding and deserving individuals have been excluded, and to them I sincerely apologize.

I would like to acknowledge the extremely generous contributions from Genentech, Inc., which are largely underwriting both this conference and the proceedings publication. A great deal of credit is due them for their willingness to support an independent multidisciplinary examination of this issue. Additional support from Eli Lilly, Inc., Ross Laboratories, the Human Growth Foundation, the MAGIC Foundation, and the Turner Syndrome Society is also acknowledged and appreciated.



David B. Allen, MD  
Editor-in-Chief

## Session I:

# DETERMINING GROWTH HORMONE INSUFFICIENCY AND THE EFFICACY AND TOXICITY OF HUMAN GROWTH HORMONE THERAPY

**Editor's comments:** My colleague and co-organizer Norm Fost always says that good ethics begin with good facts. Our first session explores current and emerging information about issues that are critical to the analysis of responsible use of growth hormone (GH): (1) the determination of GH deficiency (GHD), (2) the effectiveness of GH in the treatment of non-GHD short stature, and (3) the possible toxicity of GH therapy. The reader will undoubtedly note that, although our knowledge of GH therapy has advanced greatly in the last decade, *unified interpretations* of the facts we do have regarding definitions of deficiency and assessments of efficacy remain elusive.

David B. Allen, MD

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## METHODS OF ASSESSING GROWTH HORMONE SECRETION AND DETERMINING GROWTH HORMONE DEFICIENCY



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### Introduction

The classic form of hyposomatotropism is a well-characterized clinical disorder due to genetic, structural, congenital, and acquired abnormalities of the hypothalamus and adenohypophysis. It is characterized by:

1. Subnormal secretion of growth hormone (GH) in response to 2 or more standard provocative stimuli in the clinically appropriate patient with:
  - Marked growth retardation,
  - Decreased growth velocity,
  - Delayed skeletal maturation,
2. Known insult (birth or head injury, cranial irradiation, intracranial surgery),
3. An anatomically defined lesion of the hypothalamus, pituitary stalk, or anterior pituitary,

4. An abnormality in the genetic structure of GH or GH-releasing hormone (GHRH), and/or
5. Absence of other explanations for growth retardation.

These criteria distinguish the truly GH-deficient (GHD) child from the child with partial deficiency of GH secretion who may be first evaluated during the evolution of classic hyposomatotropism when stimulated GH secretion is still normal, or who may have sufficient mass of somatotropes to respond to provocative stimulation but who does not release adequate GH on a daily basis to permit normal growth.

This paper will discuss methods of assessing GH secretion and action that help to define GHD. Initially, however, it is important to recognize that the diagnosis of subnormal secretion of GH is *not* the same problem as the selection of the child for GH therapy. These are 2 complementary, but distinct, challenges.

## Measurement of Growth Hormone

Variability in the quantitation of GH levels continues to complicate the definition of deficiency. GH is most often measured by competitive immunologic and receptor binding methods, although bioassays for its determination also have been developed. In serum, GH is present as 22-kd and 20-kd species and in several isoforms that differ in size, charge, and immunogenicity.<sup>1</sup> GH circulates bound to high-affinity and low-affinity binding proteins; the high-affinity binding protein is identical to the extracellular domain of the plasma membrane receptor for GH.<sup>2</sup>

The heterogeneity of circulating GH has resulted in the development of immunoassays with differing specificities and sensitivities. Individual conventional first-generation radioimmunoassays (RIAs) employing polyclonal antibodies to GH often record discrepant GH values for the specimen because each antibody recognizes different epitopes or isoforms of somatotropin.<sup>3,4</sup> With the development of second-generation immunoradiometric assays (IRMAs) employing dual polyclonal and monoclonal antibodies, the specificity and analytic sensitivity of the assays improved, but discrepancies between various assays persisted. GH values as determined by RIA have been reported to be 1.5- to 2.0-fold greater than those determined by IRMA.<sup>4,5</sup> Enzyme-linked immunosorbent assays (ELISAs) and immunofluorescent assays (IFAs) for GH have increased assay sensitivity but the differences between assays remain.<sup>6</sup> Thus, interassay variability in measurements of immunologic GH concentrations is due to the heterogeneity of circulating GH, the specificity and sensitivity of the primary anti-GH serum (and whether it is monoclonal or polyclonal), the form of the immunoassay (RIA, IRMA), the diluent employed, and the standard used. It is essential that each laboratory measuring GH knows the characteristics of its (immuno)assay and the range of values from "normal" to "abnormal."

The sensitivity of radioreceptor assays (RRAs) for GH is often less than that of the conventional RIA.<sup>7</sup> GH measured by RRA has been considered biologically active, but there are no data to support this conclusion.<sup>8</sup> Since there are 12 or more antigenic sites on the GH molecule that are related to the site of interactions with its receptor, correlation between RRA and immunologic assays may be marginal.<sup>9</sup> Nevertheless, most investigators report reasonable correlations between RRAs and RIAs.<sup>7,10</sup> GH concentrations determined by RRA are somewhat less than those measured by RIA in the same specimen, resulting in an RRA:RIA ratio of 0.75:0.90. The ratio in an individual child is usually stable at rest but may vary slightly during episodes of stimulated GH secretion.<sup>10</sup> Rarely, short children may secrete an immunologically intact but biologically inactive GH molecule, as reflected by a very low RRA:RIA ratio.<sup>11</sup>

## Assessment of Growth Hormone Secretion

GH secretion is most often assessed by its secretory response to standard provocative stimuli. Many factors modify the GH

secretory response to stimulation. (1) Endogenous GH secretion is inversely related to body mass and provoked GH secretion is depressed in obese subjects, primarily due to an increase in somatostatinergic tone.<sup>12-14</sup> (2) Feeding or administration of glucose blunts the effect of a GH-releasing stimulus; fasting has the opposite effect. (3) A spontaneous surge in GH secretion prior to administration of a provocative stimulus inhibits the challenge response. (4) Drugs may impair ( $\beta$ -adrenergic agonists or  $\beta$ -adrenergic antagonists) or enhance ( $\beta$ -adrenergic antagonists, sex hormones) the effects of a GH-releasing stimulus. The requirement for 2 or more abnormal responses to GH provocative agents in defining the child with classic GHD recognizes the individual variability of response to any single GH stimulus.

A "normal" response to a GH provocative stimulus has been arbitrarily defined at a level that ranges from 5 to 10 ng/mL (RIA). The number selected will depend on the assay employed for measurement of GH and on the assessment of GH secretory responses in normal children. Most investigators have selected "normal" values employing data from short children in whom no endocrinologic or systemic disease could be identified. Marin et al<sup>15,16</sup> reported that 20% of a normal population ( $n=70$ ) had peak GH responses  $<7$  ng/mL (RIA) in response to all stimulatory tests (see Table 1). Indeed, the lower 95% confidence limit of GH response to exercise, arginine, or insulin was 1.5 ng/mL in prepubertal children and 2.9 ng/mL for children in Tanner stage II (breast or male genital) development. Zadik et al<sup>17</sup> reported that 5% of normal prepubertal and adolescent boys and girls studied had peak GH concentrations less than 3.6, 4.0, and 4.5 ng/mL for the 3 provocative stimuli, respectively. Thus, in children of normal stature provoked GH secretion may be relatively low, and the use of any single uniform cutoff value will yield many false-positive results. Estrogen priming of prepubertal subjects increases the GH secretory response to values greater than 7 ng/mL in prepubertal children, comparable to the 95% confidence limit in normal mid- and late-pubertal subjects.<sup>16</sup>

Endogenous GH secretion also can be assessed by determining spontaneous fluctuations in GH concentrations over 6 to 24 hours. Both repeated withdrawal of discrete blood samples and

**Table 1: Mean Peak Concentrations of Growth Hormone in Normal Children and Adolescents After Provocative Stimulation<sup>14,15</sup>**

| Tanner Stage | I    | II  | III   | IV   | V     |
|--------------|------|-----|-------|------|-------|
| Exercise     | 5.6  | 8.5 | 18.2  | 11.3 | 17.2  |
| AITT         | 4.4  | 7.9 | 10.0  | 19.7 | 26.2  |
| Clonidine    | 3.0  | —   | 7.0—  | —    | 5.0—  |
| GHRH         | 40.0 | —   | 53.0— | —    | 65.0— |

AITT, arginine-insulin tolerance test; GHRH, growth hormone-releasing hormone.

the constant exfusion of blood yield levels of mean GH secretion, but frequent,<sup>18</sup> repetitive sampling provides more information about the pattern of GH secretion.<sup>19</sup> Samples collected at 20- to 30-minute intervals have been analyzed for (1) maximal, minimal, basal, and mean GH concentrations; (2) the amplitude, increment, and frequency of GH secretory pulses; (3) the pulse area under the curve of GH release; (4) the GH pulse width, or the interpulse interval; and (5) the production rate of GH. Elaborate mathematical analyses have been developed to examine the secretory patterns of GH (and other hormones).<sup>20</sup> On a daily basis, serum concentrations of GH increase during fasting; during exercise; in response to a psychologic stress; spontaneously without any provocative event; and reproducibly during sleep stages 3 and 4,<sup>18</sup> when there is enhancement of cholinergic tone and repression of somatostatin release.<sup>21,22</sup> Women have higher mean GH concentrations, greater peak GH secretory bursts, and higher basal GH levels than do men.<sup>23</sup>

In the prepubertal child, the production rate of GH increases slightly after 9 years of age, but is relatively similar to that of the adult.<sup>19,24</sup> There is a marked but transient increase in the integrated concentration of GH, the mean GH concentration and the GH production rate in late prepubertal boys and mid-pubertal girls that coincide with the peak height velocity.<sup>13,14,17,19,24-27</sup> Different investigators have reported correlations between the standard deviation score (SDS) for height and the area under the curve (AUC) of GH secretion or the GH secretory rate for prepubertal children,<sup>19,28</sup> the sum of GH pulse amplitude and growth velocity SDS,<sup>29</sup> height SDS and the sum of GH peak areas, and the growth velocity SDS and the logarithm of the sum of GH pulse amplitudes.<sup>30</sup> The pubertal rise in GH secretion has been attributed to the enhancing effects of sex hormones on GH release, and the physiologic consequence is the contribution of GH to the adolescent growth spurt.

How reproducible is the assessment of the 24-hour (or 12-hour overnight) GH secretory pattern in the identification of the GHD subject? Although Zadik et al<sup>27</sup> suggest that the 24-hour integrated measurement of GH is reasonably reproducible, Donaldson et al<sup>31</sup> report that 12-hour overnight mean GH levels fluctuate by as much as -60% to +160% in individual subjects. Is there any diagnostic utility to spontaneous GH measurements? Rose et al<sup>32</sup> reported that only 57% of classic GHD children were identified by spontaneous GH sampling. Similar observations have been recorded by other investigators.<sup>33,34</sup> Donaldson et al<sup>35</sup> reported that the *maximum overnight GH concentrations* were reliable indicators of GH secretion. Pharmacologic stimulation with L-dopa or clonidine led to underestimations of spontaneous GH secretion in 20% of the short children they studied, and measurement of endogenous GH secretion identified the GH-sufficient child more reliably than did standard GH provocative tests. Thus, measurement of spontaneous GH secretion may be helpful in identifying *GH-sufficient* subjects, but in most instances it adds little to provocative tests in the identification of the GHD child.

An exception to the foregoing statement is slowly growing children who received cranial irradiation for neoplasms of the central nervous system; they may demonstrate normal provoked secretion of GH but subnormal spontaneous GH release. These subjects probably have abnormal neurotransmitter regulation of GH secretion, or "growth hormone neuro-secretory dysfunction,"<sup>36</sup> and either deficient release of GHRH or excessive secretion of somatostatin, or both. In other patients a similar pattern of GH secretion may reflect decreased mass of somatotropes.

## Ancillary Methods for the Assessment of Growth Hormone Secretion

### Urinary Growth Hormone

Immunoreactive GH may be measured in urine after dialysis and concentration. Urinary GH excretion is highest in infancy; growing children excrete more GH than do adults when expressed per unit of body weight.<sup>37</sup> In normal prepubertal children, timed overnight urinary GH concentrations range between 1.1 ng/mL and 5.3 ng/night (IRMA)<sup>38</sup> and correlate with peak GH secretory responses to provocative stimuli and with the mean overnight serum GH concentration.<sup>39</sup> Values are low in hyposomatotropic subjects. The absolute mean daily urinary GH excretion shows modest day-to-day variability,<sup>40</sup> but appears to separate normal children and normal short children from subjects with partial or complete GHD (identified by peak serum GH responses to provocative stimuli). On the other hand, overnight (12-hour) urinary GH excretion when expressed per unit of creatinine, does not separate GHD children from normal or idiopathic short-statured children.<sup>39</sup> Thus, the appropriate method for expression of urinary GH values remains undefined. Currently, urinary GH measurements may be useful as screening tests of GH sufficiency/insufficiency but cannot be considered diagnostic studies.

### Insulin-Like Growth Factors

Insulin-like growth factor 1 (IGF-1) is GH-dependent; concentrations are low in GHD subjects and high in hyper-somatotropic patients. Serum IGF-1 levels are low *in utero* and in infancy, increase with age in both boys and girls, reach maximum values during puberty (earlier and higher in girls than in boys), and decline to adult values as adolescence is completed.<sup>41</sup> Concentrations of IGF-1 correlate more closely with bone age than with chronologic age.<sup>42</sup> However, IGF-1 values are significantly affected by the nutritional status of the individual; they are quite low in malnourished subjects.<sup>43</sup> Furthermore, particularly in very young patients with GHD, IGF-1 values do not reflect the growth-promoting effects of GH; in part, this is likely due to the local secretion and paracrine effects of IGF-1 on cartilage growth.<sup>44</sup> Low values of IGF-1 do not identify the GHD subject with certainty, nor do values within the age- and sex-related normal range exclude the presence of hyposomatotropism. In children with hypopituitarism due to craniopharyngioma, IGF-1 values may often



be normal.<sup>45</sup> Measurement of both IGF-1 and IGF-2 is reported to be more accurate than is the measurement of either alone in the identification of the GHD child.<sup>46</sup>

The urinary excretion of IGF-1 is low in GHD children but values overlap with normal and normal short children.<sup>39</sup> The usefulness of urinary IGF-1 measurements in the identification of GHD children has yet to be determined.

## Insulin-Like Growth Factor-Binding Protein 3

The IGFs circulate bound to high molecular weight carrier proteins.<sup>47</sup> The major IGF-binding protein (IGFBP-3) is a 150-kD protein complex of an acid-stable binding subunit, an acid-labile subunit, and IGF. It is inducible by GH and perhaps by IGF-1 itself. Measured by RIA, IGFBP-3 concentrations are low at birth, rise over the first few weeks of life, and then increase slowly with age.<sup>48</sup> There is a significant increase in IGFBP-3 concentrations during adolescence, when levels rise to adult values.<sup>48,49</sup> Maximal IGFBP-3 concentrations are noted approximately 2 years after the peak height velocity of puberty. Serum IGFBP-3 concentrations are below the normal 5th percentile in 97% of hypsomatotropic children and above the 5th percentile in 95% of short children with normal stimulated secretion of GH. Measurement of IGFBP-3 may prove a useful adjunct to the evaluation of the short child, if its generation is consistently independent of IGF-1. If the synthesis of IGFBP-3 is dependent on IGF-1, its utility may be no better than that of the IGF-1 measurement itself.

## Growth Response to Growth Hormone

Nearly 40% to 60% of healthy short children with normal GH secretory responses to provocative stimuli will respond to the administration of human GH with an increase in growth rate more than 2 cm/year above basal values during the first year of therapy.<sup>50</sup> In the author's experience, the best predictive measurement of subsequent response in such non-GHD children is the pretreatment growth rate — the more slowly a child is growing, the more likely it is that the child will experience a substantial, short-term (1-year) acceleration of growth rate when treated with GH.<sup>51</sup> Thus, the short-term linear growth response to GH cannot be used to identify the GHD subject.

## Conclusion

Classic GHD is a discrete entity, and is characterized by defined clinical, radiographic, and endocrinologic criteria. Children with partial or subtle defects in the secretion of GH are difficult to identify, and no individual assessment of GH secretion or GH-associated biochemical finding unerringly detects such subjects.

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# IS GROWTH HORMONE DEFICIENCY A DISCRETE ENTITY? AGAINST THE NOTION



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## Introduction

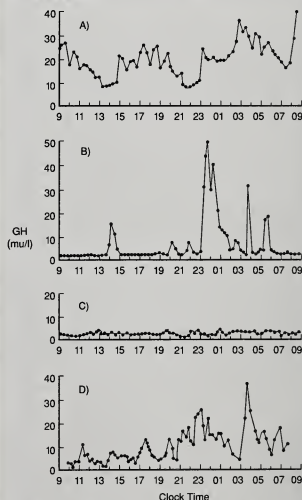
Historically it had been believed that there was a single cutoff level of peak stimulated growth hormone (GH) secretion that could distinguish children with GH deficiency (GHD) from normal children. At a later time it was appreciated that the problem was not as simple as that, and 3 categories of patients could be described by peak GH response to a pharmacologic stimulus: GHD children, children with partial GHD, and normal children. Surprisingly, it was believed that such cutoff levels of GH secretion were independent of age, sex, and puberty. It has become apparent that GHD does exist, but it is a state of absent GH secretion in association with GH gene deletion or abnormalities of gene expression. The more common situation is GH insufficiency, in which there are varying degrees of abnormal GH secretion. It is now appreciated that there is a wide spectrum of abnormalities among children with classic GHD, and that the majority of such children satisfy the criteria for GH insufficiency. Such is this spectrum, and the enormous difficulty of interpreting individual peak GH concentrations, that in countries such as Australia the selection of patients for GH administration is based purely on anthropometric grounds and is totally independent of either pharmacologic or physiologic tests of GH secretion.<sup>1</sup> In other words, the truth be known, I don't know what biochemical GHD is.

## Growth of Normal Children

The centile lines for normal children on a "distance" growth chart are not parallel, but diverge with time. Thus, tall children grow at a higher centile velocity than short children. Interestingly, tall children produce more GH than short children (See Figure 1),<sup>1</sup> and this may explain why the former grow at a faster rate than the latter.<sup>2,3</sup> Does a child growing abnormally and demonstrating abnormal GH pulsatility (with normal peak GH values) have GH insufficiency? I propose that he/she does. The infancy-childhood-puberty model of growth<sup>2</sup> provides a useful mechanistic concept for the control of growth. During the middle childhood years, growth progressively decelerates and is dependent predominantly on GH secretion. It would be surprising if normal children produced the same amount of GH at the age of 3 and 4 years as between 8 and 9 years.

Growth during the pubertal growth spurt depends on both GH and sex steroids; one without the other produces inadequate growth acceleration.<sup>3</sup>

Figure 1: Variability in the Pattern of Physiological GH Secretion



Four serum profiles of 24-hour endogenous GH secretion from four 6-year-old boys. Top (A), child with pituitary gigantism growing at 7 cm/year. (B), normal tall boy growing at 7 cm/year. (C), boy with GH deficiency growing at 3 cm/year. (D), boy with dysfunctional GH secretion growing at 4 cm/year (note lack of return to baseline values between pulses).

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## Patterns of Endogenous Growth Hormone Pulsatility

GH responses to a battery of pharmacologic tests probably represent short-term metabolic changes, rather than demonstrating any relevance about the relationship between growth and GH secretion. In recent years, great interest has been expressed in the interpretation of physiologic GH secretion and growth. There has been considerable controversy about the relative importance of pharmacologic and physiologic tests of GH secretion,<sup>4</sup> especially in relation to the selection of patients who may respond to GH treatment. However, 24-hour patterns of endogenous GH secretion have enabled us to study GH pulsatility, and we have come to realize that there is even greater variation of GH secretion than could be appreciated by the results of pharmacologic tests.

Numerous patterns of endogenous GH secretion have been described, such as the predominantly single pulses seen in children with Turner syndrome and Russell-Silver syndrome.<sup>5</sup> Many children have GH pulses that do not return to immeasurable concentrations between pulses. Persistently high levels of GH secretion and the absence of a characteristic pulsatile pattern have been described in adults with acromegaly and children with pituitary gigantism. (See Figure 1.) Although children with tall stature may have higher peak levels of GH, mean concentrations will be considerably lower than those seen in patients with pituitary gigantism or acromegaly. It has been appreciated for many years that some children with abnormal growth had normal GH concentrations in response to pharmacologic tests. However, physiologic studies have revealed that such children have a dysfunctional pattern, that is, an absence of discrete GH pulses. Such patterns have been described as neurosecretory dysfunction by Spiliotis and colleagues,<sup>6</sup> although this latter term has come to have different meanings.

By the use of such physiologic tests, we have come to realize that the spectrum of GH secretion is far greater than can be appreciated by peak GH concentrations achieved in response to pharmacologic tests. There is a whole range of different patterns of GH pulsatility, although many of these are poorly understood in relation to the pattern of growth observed.

## Growth in Growth Hormone Deficient Children and Response to the Growth Hormone Treatment

The greater the degree of GH insufficiency, the slower the rate of growth and the greater the initial response to GH treatment.<sup>7</sup> There appears to be no distinct cutoff level that can be determined by peak GH levels found in either physiologic or pharmacologic tests.<sup>4</sup> There is very little evidence that GH responses to provocative stimuli of 7, 10, or 15 ng/mL represent distinct clinical conditions. Children with minor degrees of GH insufficiency and short normal children do have increased

growth rates in response to GH treatment, but the response is much less dramatic than in children with classic GHD.

## Growth Hormone Secretion During Puberty

During late prepuberty in both sexes and in early puberty in boys, there is a gradual growth deceleration. This is associated with physiologic GH insufficiency. At the onset of the spontaneous growth spurt, or if exogenous sex steroids are administered, normal GH secretion results.<sup>8</sup> Studies using pulsatile gonadotropin-releasing hormone (GnRH) to treat children with hypogonadotropic hypogonadism have revealed that it is the change in GH secretion, not sex steroid secretion, that correlates with changes in growth rate during puberty in both sexes.<sup>9</sup> In girls, there is a dramatic increase in GH pulse amplitude with the onset of breast development; in boys, the increased pulse amplitude does not occur until a 10-mL testicular volume (genitalia stage 3 to 4) has been attained. Certainly there is no single peak GH level that can be considered normal during the pubertal growth spurt, as the confounding factors of sex and stage of pubertal development need to be defined as well.

## Psychosocial Dwarfism

The characteristic biochemical feature of children with psychosocial dwarfism is hypopituitarism (most commonly GH insufficiency) that reverses with a change of environment.<sup>10</sup> If a child with psychosocial dwarfism is admitted to hospital without parental access, serial physiologic tests of GH secretion will reveal a gradual (but transient) change from organic GHD or GH insufficiency to normal GH secretion during a period of 2 to 3 weeks.<sup>11</sup> Although the patterns of GH secretion are similar between different profiles, there is no sudden change between insufficient and normal GH secretion; there is, however, a gradual increase of pulse amplitude from insufficient to normal GH secretion.

## Low-Dose Cranial Irradiation

The treatment of children with low-dose cranial irradiation—for example, 1,800 or 2,400 cGy administered for prophylaxis of meningeal involvement in acute lymphoblastic leukemia—may induce a dual endocrinopathy in girls.<sup>12</sup> Such girls may have both precocious puberty or early pubertal maturation and GH insufficiency. During the onset of pubertal development, sufficient GH secretion may be present to allow a normal rate of growth for chronologic age, although not allowing for the stage of pubertal development. Such a biochemical lesion of GH secretion has been demonstrated.<sup>13</sup> Thus, a normal growth rate during late prepuberty may be attained, although there is insufficient GH secretion to permit an adequate growth spurt during puberty.



## Neuroradiologic Assessment in Children With Growth Hormone Deficiency

Just as there is a continuous spectrum in biochemical assessment of GH secretion, there is a spectrum of pituitary morphologic lesions in children with GHD. This may range from pituitary aplasia to hypoplasia,<sup>14</sup> although there appears to be no definite relationship between morphologic appearance and endocrine secretion. However, children with pituitary hypoplasia are more likely to have "isolated" GH insufficiency, while those with pituitary aplasia may have evolving multiple pituitary hormone insufficiency.

### Summary

I do not believe GHD to be a distinct entity, but rather a spectrum of disorders of GH pulsatility. The notion of a discrete cutoff level of GH secretion (either pharmacologic or physiologic) to distinguish the GHD from the normal, is purely historical and has no relevance to modern pediatric endocrinology. GH secretion is a continuum.

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## DISCUSSION I: A & B

- A. Methods of Assessing Growth Hormone Secretion and Determining Growth Hormone Deficiency - Allen Root, MD
- B. Is Growth Hormone Deficiency a Discrete Entity? Against the Notion - Richard Stanhope, MD

Moderated by Jo Anne Brasel, MD

FRASIER: We incorrectly use the words "identify," "distinguish," and "define" in describing tests for growth hormone deficiency. The purpose of a laboratory study is to confirm or disprove a clinical perception. When we try to extend the use of a laboratory test beyond confirming the clinical diagnosis, it does not work and then we are disappointed.

LIPPE: I would like to offer an important point: If we do not perform any of these diagnostic studies, we will miss patients with organic diseases that have other consequences. Just because a child is short and slow growing and appears to match the clinical criteria of growth hormone deficiency, it does not mean that you do not want to know that the child has a brain tumor or inflammatory bowel disease, Turner syndrome, or some other disorder which needs to be addressed. If we are not going to evaluate *why* a child is growing slowly and our main goal is only try to treat the "symptom" with growth hormone, we will miss the other pathology. We could do a serious disservice if we choose to ignore the diagnostic relevance of testing procedures and miss other illnesses.

STANHOPE: I totally agree, but I do not do an insulin tolerance test to look at growth hormone secretion. I do it to look at cortisol levels. It is the growth *rate* that tells you about the need for GH, not the GH *level*.

CHARO: Please clarify what a "normal growth hormone" level means. Is it supposed to reflect a global mean and standard deviations from that mean? Or is "normal growth hormone" supposed to be reflective of specific racial groups, nutritional status, sex, age, etc? I am not sure I understand what you are communicating.

ROOT: This has been a major problem. Many studies in the literature have not identified their control group children as normal by various (defined) growth parameters. These data are now appearing in the literature, as we pointed out, and they show extreme variability of growth hormone secretion in normal children.

LANTOS: Dr. Stanhope, if I understand you correctly, your advice to practicing endocrinologists today would be that there is no point in ever measuring growth hormone level. Is that a misunderstanding?

STANHOPE: I do not think that this is far from the truth. That is my personal opinion, but you will hear very different answers from around the table. Measuring the child and tracking their growth rate and velocity is the best thing we can do for them. With many of these children, you will find that you really do not have to do much because they are growing better than you had been informed.

FRASIER: One issue is the practice of good clinical medicine and the making of an appropriate diagnosis. Another issue is deciding what to do about someone who is short. We cannot mix up these 2 issues.

GERTNER: I think it is also rather dangerous to be too much of a "lumper" and merge all cases together. In my view, there certainly is such a thing as growth hormone deficiency, and I found it quite amusing that Dr. Stanhope was showing a slide during his presentation and saying, "This patient clearly has organic growth hormone deficiency." If a person, for example, has a genetic inability to make growth hormone because of a mutation of the growth hormone gene, or if the person's pituitary gland is destroyed, then clearly they are growth hormone deficient. There is no argument about that. The problem that we are facing is that growth hormone deficiency, like hypertension or obesity is a disease of gradation (unlike pregnancy which you either have or you do not), and we need to know where to draw the line.

ALLEN: Dr. Stanhope, you did not show the data describing the relationship between the sum of growth hormone pulse amplitudes and growth velocity in short children. Nor did you say that you believe in the continuum of growth hormone. But I wonder, do you?

STANHOPE: I do believe in the entity, but, of course, we do not have the data to prove it.

BRASEL: I agree with both Drs. Root and Stanhope that there really is an entity of growth hormone deficiency. Cranial radiation studies show us that there is a gradation of growth hormone deficiency with a stage of very low spontaneous GH secretion but normal responses to stimulation tests. When those children are followed further, their provocative tests fail and they have very low secretion of growth hormone. These children are truly growth hormone deficient. The difficulty of using spontaneous secretion studies and urinary growth hormone, or any of several other approaches, is due to the fact that normal children have such low levels from time to time, it is very difficult to distinguish normal from below normal. I could show Dr. Stanhope the growth hormone profiles of perfectly healthy, normally growing children and they would match the profiles that he is interpreting as insufficient growth hormone. Consequently, it is this variability that makes the overnight spontaneous studies less diagnostic than we would like them to be. We wanted them to be definitive, but they have not proven to be so because normal children have similar patterns.

WEISBARD: I find myself continuing to be confused by the notion of growth hormone deficiency as a single discrete entity. Let me ask the question in a slightly different way: Is there a recognized diagnostic status that correlates well with responsiveness to growth hormone therapy—that is, defining the status by responsiveness to the potential intervention? And precisely what relationship does that diagnostic status bear to the discrete entity of growth hormone deficiency?

BRASEL: That just happens to be the next question.

# EFFICACY OF GROWTH HORMONE THERAPY IN PATIENTS WITHOUT CLASSICALLY DEFINED GROWTH HORMONE DEFICIENCY



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## Introduction

The ethical dilemma we are discussing exists because growth hormone (GH) therapy is indeed effective in short children without classically defined GH deficiency (GHD). We think it is reasonable to conclude that endocrinologists would not use an extremely expensive and potentially dangerous treatment in generally healthy children unless it were effective. In this paper, we present data supporting the notion that alternative conclusions are not likely, namely that pediatric endocrinologists are deluded, or simply too hopeful, about GH therapy in non-GHD children.

In our clinic, we often entertain the idea of instituting specific auxologic criteria to treat any prepubertal patient, regardless of stimulated GH levels, by utilizing some combination of low height standard deviation score (SDS), subnormal growth velocity, and predicted adult height (PAH) below a certain level. The last 2 criteria are helpful in distinguishing patients with constitutional delay, who then might receive alternative therapy. Several assumptions are inherent in the suggestion that we initiate therapy in any patient meeting certain criteria. (1) Our decision to institute GH therapy generally rests upon auxologic criteria alone and ignores the results of GH testing, except when the clinical criteria are only marginally met. (2) Neither clinical criteria nor laboratory measures, including GH levels, are particularly helpful in predicting individual response to GH. (3) Sufficient evidence exists that GH therapy of non-GHD patients is effective enough to warrant its use. The role of GH testing in defining GHD is presented in another paper. The third statement is the topic of this paper, with some pertinent data concerning the second statement. We will concentrate on the data from children with idiopathic short stature (ISS, also referred to as normal variant short stature, or NVSS) and with Turner syndrome (TS). These are the 2 populations that we and other investigators have most extensively studied.

Raben<sup>1</sup> was the first to report a short-term trial of pituitary-derived human GH in short children without GHD, demonstrating an increase in growth rate from 3.5 to 8.0 cm/yr.

Tanner et al's report of long-term GH treatment in 55 children included patients with GHD, intrauterine growth retardation, TS, and ISS, among others.<sup>2</sup> The limited success in treatment of non-GHD patients in the Tanner et al study and some of the other early trials has been retrospectively attributed to relatively infrequent GH administration or low doses. By the mid-1980s, however, many studies utilizing pituitary-derived GH in children with normal provocative test results had established a short-term response to GH. For instance, in one 6 month trial, 10 short children unselected for pretreatment growth rate all increased their growth rate in response to GH administered 3 times a week (TIW), with a mean increase from  $4.3 \pm 0.3$  to  $7.4 \pm 0.5$  cm/yr.<sup>3</sup> In a final report from the National Hormone and Pituitary Program Growth Hormone Committee prior to discontinuation of pituitary derived GH, 45 of 48 children with growth rates  $<4$  cm/yr and normal provocative test results had an increase in growth rate during a 6-month trial (mean change, 3.4 to 6.9 cm/yr).<sup>4</sup> Therefore, even before the introduction of recombinant GH, a significant short-term response to GH had been demonstrated in individuals with ISS.

Investigations utilizing recombinant GH have uniformly confirmed the short-term response. The advent of recombinant GH has changed the limiting factor in GH treatment from the GH supply to financial cost, or, rather, to the cost-benefit ratio as defined by the patient's family and physician, and by society in general. It has also allowed for studies of larger, more homogeneous numbers of each class of potential GH candidates and raised the hope that subsets of responders could be delineated, preferably prospectively. Attention in these studies now focuses on the questions of final height, the timing and efficacy of GH therapy in relation to puberty, possible complications, potential predictors of response, and criteria for treatment.

## Growth Hormone Treatment in Children With Idiopathic Short Stature

The US Genentech Collaborative Study Group has enrolled 121 children with ISS and is now in its fifth year.<sup>5</sup> Inclusion criteria

included age >5 yr, height < -2.5 SDS, serum GH >10 ng/mL, and bone age <10 yr for boys and <9 yr for girls. Notably, pretreatment growth rates were not the basis of selection, although most were below the 50th percentile. Patients receiving GH 0.1 mg/kg TIW demonstrated an increase in mean growth velocity in the first year from  $4.6 \pm 1.1$  to  $7.5 \pm 1.2$  cm/yr.<sup>8</sup> There was no detectable difference in growth rate increment between those classified as having familial short stature and those with constitutional delay. A first-year randomized control group exhibited no significant change from the pretreatment growth rate and no change in PAH.

In the second year, both the control and treatment groups were randomized to either TIW or daily treatment groups (both received GH 0.3 mg/kg/wk, with adjustment for weight change). Seventy-five children have now completed 3 years of treatment, and the majority have entered puberty. These results are shown in Table 1, and daily and TIW treatment groups are considered together. However, 3-year cumulative growth response did correlate with dosing frequency, with a higher increment in mean growth velocity SDS and PAH in the daily administration group, consistent with the consensus from other GH studies. Analyzing growth rate for bone age, only 8% of subjects were above the 50th percentile during the pretreatment period. In successive years of treatment, 94%, 70%, and 63% of children were above the 50th percentile for growth rate.

**Table 1: Growth Velocity, Height SDS, and PAH SDS During 3 Years of TIW or Daily GH Therapy (US Genentech Collaborative Study Group)**

|                                | Pretreatment   | Year 1         | Year 2         | Year 3         |
|--------------------------------|----------------|----------------|----------------|----------------|
| <b>n</b>                       | 103            | 103            | 103            | 75             |
| <b>Growth velocity (cm/yr)</b> | $4.6 \pm 1.3$  | $8.0 \pm 1.4$  | $7.5 \pm 1.8$  | $7.0 \pm 1.8$  |
| <b>Height SDS</b>              | $-2.7 \pm 0.5$ | $-2.2 \pm 0.6$ | $-1.9 \pm 0.7$ | $-1.7 \pm 0.7$ |
| <b>PAH SDS</b>                 | $-2.7 \pm 0.9$ | $-2.0 \pm 1.1$ | $-1.8 \pm 1.0$ | $-1.6 \pm 1.2$ |

There was no relationship between response to GH and either baseline 12-hour GH secretion or provocative GH levels, nor did growth response correlate with baseline height, bone age, growth rate, or parental heights. While first-year growth rate SDS was predictive of second-year response, correlation with third-year response was weak. There was no correlation of duration of GH therapy with either the age of onset of puberty or the rate of pubertal advance. Thus, we have concluded from the 3-year data that GH therapy in ISS significantly improves mean SDS for growth rate, height, and predicted height without altering pubertal progression. Unfortunately, we have found no clear predictor of response.

Ongoing European studies of GH treatment of ISS have confirmed many of the US results. Albertsson-Wikland in Sweden has treated 40 prepubertal children for 1 year and 24 children out to 4 years with daily GH.<sup>9</sup> In the 1-year data, mean height velocity increased from 4.6 to 7.5 cm/yr, and 80% exceeded a 2-cm increment. In contrast with the US data, there

was an inverse relationship between either pretreatment growth rate or GH secretion and the growth rate during treatment, suggesting that these baseline factors might determine the likelihood of response. In the multi-year treatment, growth rate improved from a pretreatment 4.2 cm/yr, to 8.1, 6.7, 6.0, and 4.9 cm/yr over successive treatment years. Furthermore, in the period following discontinuation of treatment, mean growth rate was 5.1 cm/yr, contradicting the concern that growth rate off treatment might fall below the pretreatment rate ("catchdown" growth). At a recent meeting, Albertsson-Wikland reported 2-year treatment results in 52 prepubertal children whose mean height SDS increased from -2.9 to -2.0. Her analysis suggested that the final height of 33 children depended upon the timing of treatment prior to puberty, with less improvement (+0.3 height SDS) when therapy was initiated just prior to peak height velocity than with initiation 4 years prior to the peak of puberty (+0.7 height SDS). This stratification in response has not been confirmed in other studies.

The Dutch Growth Hormone Working Group has treated prepubertal children with ISS using criteria of height SDS < -2.5 and height velocity <25th percentile for bone age, ie, slowly growing short children, in a randomized study with controls in the first year and allowing for an increased daily dose in poor responders (<2 cm/yr in the first year or <50th percentile in subsequent years).<sup>7</sup> Mean velocity decreased from 7.6 cm/yr in the first treatment year to 5.1 cm/yr in the second year when the dose remained unchanged (n=11). The majority of 21 children who have completed 4 years of therapy required a doubling in dosage to maintain a growth rate >50th percentile. There were no differences in growth response between prepubertal subjects and those who had entered puberty. After 4 years, mean height SDS was significantly increased, but mean bone age advancement was approximately 1.2 years per treatment year. Mean increase in PAH was thus +0.5 SDS. In another study, Hindmarsh et al<sup>8</sup> in London have treated 16 children, including those with normal pretreatment growth velocities, for 3 years. Height velocity SDS increased from -0.44 to +2.20 (5.3 to 7.4 cm/yr) in the first year, then dropped to +0.74 in year 2, before rising again to +1.96 with an increase in dose during year 3. PAH significantly increased in boys (+6.8 cm) and girls (+4.2 cm).

## Growth Hormone Treatment in Children With Turner Syndrome

Although promising studies of GH treatment of chronic renal failure and intrauterine growth retardation (IUGR) are in progress (reviewed in reference 9), the other group of subjects with short stature in which large and convincing studies have been published is TS. Girls with TS appear to have a skeletal dysplasia rather than GHD or GH secretory dysfunction, although the latter has been reported. Turner girls have a slow growth rate throughout childhood, a continued decline through adolescence, and significantly delayed epiphyseal fusion, presumably attributable to ovarian failure.<sup>10</sup> They are an ideal population for GH trials in that the syndrome is easily verifiable



and that spontaneous puberty does not abbreviate treatment or complicate interpretation of therapeutic success as it does in ISS. The first large prospective clinical trial of GH in TS began in 1983 and is currently in its eighth year.<sup>11,12</sup> In this study, 70 girls ranging in age from 4.7 to 12.4 years, with TS and normal provocative GH testing, were randomly assigned after a pretreatment period to 1 of 4 groups for the first 12 to 20 months: (1) control (no treatment), (2) oxandrolone (0.125 mg/kg/d), (3) methionyl-GH (0.125 mg/kg TIW), and (4) combination of oxandrolone plus methionyl-GH. Subsequently, all groups except group 3 received therapy with combination oxandrolone (0.0625 mg/kg/d) and methionyl-GH; group 3 continued to receive methionyl-GH alone.

Growth data for the first 4 years of treatment are expressed as growth velocity SDS for untreated TS patients (derived from the Lyon European standard curve<sup>13</sup>) in Table 2. The response in height velocity to GH or combination therapy declined over successive years, but in all years was still greater than the pretreatment velocity, as has been the experience with GH use in subjects with GHD or NVSS. Because this decline in growth velocity is partially attributable to the natural age-associated decline characteristic of Turner syndrome, the most telling way of expressing the data is by height velocity SDS relative to an untreated TS population. (See Table 3.) Combination GH and oxandrolone therapy was even more effective than GH alone. However, approximately 30% of subjects on the higher dose of oxandrolone exhibited virilization, and many

**Table 2: Annual Growth Rate of Turner Patients (cm/year)**

| Group                        | Prestudy  | Year 1     | Year 2     | Year 3     | Year 4     |
|------------------------------|-----------|------------|------------|------------|------------|
| 1. Control → Combination     | 4.2 ± 1.1 | 3.8 ± 1.1  | 8.3 ± 1.2* | 6.7 ± 1.4* |            |
| 2. Oxandrolone → Combination | 4.1 ± 1.9 | 7.6 ± 1.5* | 7.1 ± 1.6* | 5.3 ± 2.4* |            |
| 3. Methionyl-hGH             | 4.5 ± 0.8 | 6.6 ± 1.2* | 5.4 ± 1.1* | 4.6 ± 1.4  | 5.5 ± 1.5* |
| 4. Combination               | 4.3 ± 0.9 | 9.8 ± 1.4* | 7.4 ± 1.4* | 6.1 ± 1.5* | 4.9 ± 1.5  |

Data are expressed as mean ± SD. Year 2 represents the first year of phase 2 for groups 1 and 2; this phase began 12-20 months after the beginning of year 1. After year 1, oxandrolone was lowered from 0.125 to 0.0625 mg/kg/day. At year 4, data for all combination therapy groups are grouped together.

\* Significantly greater than the annual growth rate for the control group in year 1 ( $P < 0.05$ ). The annual growth rate for group 4 (combination) is significantly greater than for group 3 (methionyl-hGH) for each of the 3 years ( $P < 0.05$ ). Adapted from Rosenfeld et al.<sup>11,12</sup>

**Table 3: Height Velocity Standard Deviation Scores Of Turner Patients**

| Group                        | Pretreatment | Year 1     | Year 2    | Year 3    | Year 4    |
|------------------------------|--------------|------------|-----------|-----------|-----------|
| 1. Control → Combination     | 0.2 ± 1.2    | -0.1 ± 1.0 | 5.5 ± 1.4 | 4.0 ± 1.7 |           |
| 2. Oxandrolone → Combination | 0.2 ± 1.0    | 4.4 ± 1.8  | 4.2 ± 1.5 | 2.4 ± 2.3 |           |
| 3. Methionyl-hGH             | 0.5 ± 0.8    | 3.1 ± 1.2  | 2.0 ± 1.1 | 1.4 ± 1.5 | 2.9 ± 1.4 |
| 4. Combination               | 0.2 ± 0.9    | 6.6 ± 1.2  | 4.3 ± 1.4 | 3.0 ± 1.4 | 2.7 ± 1.3 |

Growth velocity SD scores are presented as mean ± SD in comparison with untreated Turner patients derived from Ranke.<sup>19</sup>

investigators are wary of the effect of long-term oxandrolone therapy on final height. After 3 years of treatment with GH alone, the increment in bone age was  $2.73 \pm 0.72$  years; 3 years of combination treatment resulted in an increase of  $4.05 \pm 1.23$  years. Nevertheless, the mean PAH of the combination group is greater than that of the GH group, leaving open the question of the usefulness of oxandrolone in the treatment of short stature associated with TS.

The results of this study clearly demonstrate that GH, either alone or in combination with oxandrolone, significantly accelerates growth for a period of at least 4 years. Thirty subjects who have completed therapy (from all groups) have a mean final height of 151.9 cm, compared with initial PAHs (Bayley-Pinnaeu) or projected adult heights (Lyon growth curves) in the range of 143 cm to 144 cm. In spite of this apparent improvement in final height, we feel the study may actually underestimate potential benefit from GH therapy, since the relatively advanced degrees of skeletal maturation at initiation of therapy probably limited the length of potential treatment. Our current policy is to discuss GH therapy with all TS families and to offer treatment when the patient falls below the 5th percentile for normal girls, which usually has occurred by the time a patient is referred to our clinic. Initial treatment is with GH alone, at a dose of 0.05 mg/kg/d. Currently available data are inadequate to assess the most effective dose of GH, although data suggest that a higher growth rate is observed with daily administration, as with therapy of GHD or ISS. These findings from the initial trials of GH in TS have been confirmed in studies from Europe and Japan.<sup>14-18</sup>

## Discussion

The North American and European trials of GH in ISS and TS have provided abundant evidence that GH therapy is effective in significantly improving mean growth velocity for 1 or more years. However, the duration of most published studies has been 2 years or less. In 2 of the largest current studies (United States and Sweden) in ISS, after a dramatic increase in mean height velocity in the first year of treatment, there has been a gradual decline in growth velocity in the second and third years. Nonetheless, the mean growth rates even in the third year were 3.4 cm and 2.8 cm/yr greater than the pretreatment rates in these respective studies. In the Dutch and British experience, growth rates in the later years could be maintained by increasing the GH dose. Thus, it would appear that a significant increase in mean growth velocity increment can be maintained for at least 3 years in ISS patients. In fact, the cumulative growth response is comparable to that seen in GH treatment of GHD, giving rise to the argument that GHD and non-GHD subjects cannot be differentiated on the basis of growth response. One inference of such a conclusion is that the treatment decision should not discriminate between GHD and non-GHD patients, since average treatment outcome is similar.

In TS, only the US study has proceeded several years. The difference between pretreatment and first-year growth velocity

with GH therapy alone (administered TIW) was smaller than those seen in GHD and ISS trials, although several other Turner studies have documented a greater first-year increment. Nonetheless, in the third and fourth year of the Genentech Turner study (daily GH administration), the growth rates were still +1.4 and +2.9 SDS above the standard Turner curve. Therefore, a sustained increase in growth velocity can be induced by GH therapy in either ISS or TS.

Final height data, however, are preliminary in both ISS and Turner trials, and we still must largely rely upon changes in PAH rather than final heights in most studies. In the US collaborative ISS GH study, for instance, only predicted heights are available; mean PAH SDS has improved from -2.9 to -1.6. The Swedish and Dutch groups have reported preliminary final heights in treated ISS, with an improvement of only about +0.5 height SDS over predicted height. Final height data are more impressive in the American Turner study. In this study, 82% of TS subjects still receiving GH and 91% of subjects receiving GH and oxandrolone have already surpassed their PAHs. Those who have completed therapy have added a mean 8 cm to 9 cm over the mean final height in the Turner standard curve.

Several points need to be elaborated here. First, since none of the major studies has an untreated control group throughout the duration of treatment, interpretation of final heights is dependent upon pretreatment PAHs or, in the Turner study, an untreated control outside of the study. We cannot soon look forward to any study resolving the question of final height in a comparison of randomized treated and untreated subjects. Second, therapy historically has been initiated at a relatively advanced age (as in the studies described), which argues that earlier onset of therapy might lead to a substantially greater benefit in final height than demonstrated in studies, particularly since response may be greater at younger ages. Furthermore, even many ongoing studies are utilizing TIW dosing, which is now recognized as inferior to daily administration. Thus, potential treatment response may be underestimated by most studies. Against this, we should add that short-term studies could be biased to overestimate response, in that slow pretreatment growth rates may demonstrate some regression to the mean. Third, final height is not the only measure of treatment success. Some advocates of GH therapy argue that anabolic effects, prevention of further loss of height potential, and the psychologic benefits of improvement in height SDS or growth velocity SDS are great enough to justify therapy. However, few data exist to support such contentions.

Current data are not sufficient to predict the kinds of GH regimens that might be recommended in the future. We have little information on appropriate dosing. If further studies demonstrate that the decline in growth rate in later years of treatment approaches baseline rates, stepwise dose increases may be needed. It is plausible that therapy could be limited to a few years. Similarly, if it is determined that GH therapy during puberty is ineffective, it might be discontinued earlier than is the current practice; on the other hand, substantially higher doses of GH, mimicking the physiologic surge in GH secretion rates,

might be effective during puberty. Alternatively, luteinizing hormone-releasing factor (LRF) analogues to delay puberty may provide the opportunity to extend GH therapy when short stature is severe. It remains to be determined whether oxandrolone or another androgen, certainly less expensive than GH, will play a role in future therapies.

It is important to remember how much individual variability is implicit in changes seen in mean growth velocity or mean height during GH therapy. Clearly, some subjects demonstrate dramatic responses, while others exhibit little change in growth velocity even in the first year of therapy. Obviously, the number of children entered into therapy could be significantly reduced if accurate predeterminants of response were available. Unfortunately, preliminary results of the Genentech Collaborative Study Group study have not established any useful predictor of response. At the moment, the change in height SDS during a 12-month treatment period is the best predictor of longer term response. Although some investigators have reported that pretreatment growth rate is inversely related to treatment response,<sup>9</sup> other studies, including the Genentech Collaborative Study Group, suggest that pretreatment growth rate and height SDS are not helpful. Correlations of height velocity change during treatment with baseline GH secretion rate, baseline serum insulin-like growth factor I (IGF-I), or short-term IGF-I increase<sup>6</sup> have been contraindicated by several studies. Therefore, a predictor of growth response reliable enough to eliminate some patients from treatment consideration remains to be established.

In summary, studies in both ISS and TS demonstrate that GH significantly improves mean growth velocity over several years. Mean PAH is increased, and preliminary data in both populations suggest that final height will be improved. In TS, where the natural history and height outcome are well-documented, GH therapy seems to us clearly justified by the available data. The natural course of the heterogeneous ISS group is more obscure and leads to more tentative conclusions. Assuming that the preliminary final height data in GH treatment of ISS are confirmed, we can restate one aspect of the ethical dilemma:

GH treatment clearly benefits some patients with ISS, but not others. Unfortunately, there are currently no useful criteria other than a treatment trial to predict which patients with normal provocative GH levels will respond to GH.

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# GROWTH HORMONE IS NOT EFFECTIVE IN NON-GROWTH HORMONE DEFICIENT PATIENTS



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## Introduction

I have been assigned the task of presenting the negative position regarding the effectiveness of growth hormone (GH) therapy in patients who are not GH deficient (GHD). I have eliminated from consideration the treatment of children in whom short stature (SS) is associated with any particular syndrome and have chosen to limit this presentation to the effect of GH in children who are short but otherwise normal. Although such children have been grouped under a number of confusing headings, I will ignore this unsatisfactory terminology and refer to them as having simply non-GHD SS. I have attempted to review the literature pertinent to this group of patients and to draw conclusions supported by this information.

I will not argue that there is no initial acceleration of growth in non-GHD patients receiving therapy. The literature is essentially unanimous in demonstrating positive short-term results. There are now several reports of the effect of GH given for 1 year on the height velocity of short children in whom no significant abnormality of GH secretion can be demonstrated.

## Review of Data Regarding Growth Hormone Treatment in Non-Growth Hormone Deficient Children

Hindmarsh and Brook<sup>1</sup> followed 26 short prepubertal children for 2 years; during the second year, 16 were given 2.0 IU GH 6 times each week. When compared with 10 untreated control patients, height standard deviation score (SDS) and growth velocity SDS for both chronologic age (CA) and bone age (BA) were significantly improved. The average growth velocity increased from 5.3 to 7.4 cm/year. Albertsson-Wikland<sup>2</sup> has followed a relatively large number of short children with normal GH function while they received GH 0.1 IU/kg daily. She treated 40 such children for 12 months, during which their height velocity increased from  $4.6 \pm 1.1$  (SD) cm/year to  $7.5 \pm 1.3$  (SD) cm/year. In 80% of those treated, height velocity increased by at least 2.0 cm/year.

Wit and the Dutch Growth Hormone Working Group<sup>3</sup> have reported their first-year experience with GH in short normal

children. These investigators administered GH 2 IU/m<sup>2</sup>/day to 20 prepubertal children who were at least 2.5 SD below the mean height for CA and were growing below the 25th centile for height velocity for BA. BA was <10 in girls, and <11 in boys. Peak GH responses to standard stimuli exceeded their cutoff point for GHD. They also followed a control group of 10 similar children who were untreated. Height velocity during this year of therapy increased from  $4.3 \pm 1.4$  (SD) cm/year to  $7.3 \pm 1.1$  (SD) cm/year. There was no change in the mean height velocity of the control group. The increase in height velocity exceeded 2 cm/year in 11 treated children (55%).

The Genentech Collaborative Study Group<sup>4</sup> reported an analysis of the first 12 months of a study in which 63 short (height below the 3rd percentile for age) children who showed normal GH concentrations in response to standard provocative tests of GH reserve received GH 0.1 mg (0.26 IU)/kg TIW and 58 similar children served as a control group. Children with dysmorphic features, low birth weight, or an illness that could interfere with growth were excluded. Growth velocity was not used as an inclusion criterion. Assignment to the treatment or control group was random. The growth velocity of 50 treated prepubertal children increased from  $4.7 \pm 1.2$  (SD) cm/year to  $7.3 \pm 1.2$  (SD) cm/year while that of 40 prepubertal control children did not change. In 35 treated patients (70%) growth velocity increased by at least 2.0 cm/year. The height SDS of treated children changed from  $-2.7 \pm 0.5$  (SD) to  $-2.2 \pm 0.6$  (SD) after 12 months of GH administration. There were no differences in the responses of children classified as having genetic SS and those classified as having constitutional growth delay. Two additional groups of children also have now received GH for 1 year.<sup>5</sup> Twenty-four children who were given GH 0.1 mg/kg TIW increased their growth velocity from  $5.1 \pm 1.6$  (SD) cm/year to  $8.2 \pm 1.4$  (SD) cm/year and 23 children who were given GH 0.04 mg/kg/d increased their growth velocity from  $4.7 \pm 1.2$  (SD) cm/year to  $9.0 \pm 1.6$  (SD) cm/year.

Cowell and the Australasian Paediatric Endocrine Group<sup>6</sup> have presented the Australian experience in which 37 children receiving GH 0.6 IU/kg/week increased their growth velocity to  $8.7 \pm 1.8$  (SD) cm/year and 40 children given GH 1.2 IU/kg/week increased their growth velocity to  $10.8 \pm 1.8$  (SD) cm/year. Twenty-seven placebo-treated children did not have a significant change in growth velocity. Lesage et al<sup>7</sup> recently



reported that high-dose (0.3 IU/kg/d) GH increased growth velocity in 10 French children from  $4.0 \pm 0.3$  (standard error [SE]) cm/year to  $10.7 \pm 0.6$  (SE) cm/year.

Little data are available that allow conclusions regarding the effect of GH therapy in non-GHD children beyond the first year of treatment. Thus far, they do not point the way to firm conclusions regarding long-term effectiveness. The Genentech Collaborative Study Group<sup>5</sup> has presented data accumulated at the end of 2 years of treatment in their original study patients. During the second year of therapy, half received the same dose of GH (0.1 mg/kg TIW) and half received a weekly dose equivalent to 0.04 mg/kg/d. The growth velocity of patients receiving GH TIW fell from  $7.5 \pm 1.1$  (SD) cm/year to  $6.8 \pm 1.8$  (SD) cm/year. The growth velocity of the patients who were changed to daily treatment was maintained essentially unchanged at  $7.8 \pm 1.4$  (SD) cm/year.

Hindmarsh et al<sup>8</sup> have analyzed the response of their 16 patients after 3 years of therapy. Growth velocity decreased to less than +1 SDS during the second year of GH administration but was restored to that observed during the first year of treatment when the dose of GH was increased to 20 IU/m<sup>2</sup>/week during the third year. Although predicted height was increased over these 3 years, data on final height are not yet available.

The Dutch Growth Hormone Working Group has now analyzed their data over both 2 and 3 years of treatment.<sup>9,10</sup> They have used several different treatment regimens, with small numbers of patients receiving variable GH dosage. After 3 years of GH administration, they concluded that height prediction was improved very little, if at all.

Albertsson-Wikland<sup>2</sup> treated 24 prepubertal patients for 4 years. The average growth velocity of these children was 8.1, 6.7, 6.0, and 4.9 cm/year during each annual treatment period. Thus, maximum catch-up growth occurred during the first year of therapy and catch-up was no longer evident during the fourth year. Height prediction was increased in these children but, again, final height was not yet available in that report. Height prediction was not increased in 13 pubertal patients who were treated with GH.

Data regarding final height in GH-treated non-GHD children are beginning to trickle into the literature in abstracts and poster presentations. The longest running study is that of Van Vliet et al<sup>11</sup> in San Francisco. Although flawed, this work provides useful information. The patients included were both short and growing at a subnormal rate; in addition, only those who showed a positive response over the first 6 months of therapy received long-term GH. An interim report<sup>12</sup> of the progress of these children suggested that they were continuing to respond and that their predicted mature height was improved. However, a more recent report<sup>13</sup> indicates that, although height prediction improved with GH therapy in 50% of these patients, only 13% (3 of 24) had a significant improvement in actual final height.

More recently, Albertsson-Wikland and Karlberg<sup>14</sup> presented preliminary material on the final height achieved by 33 children treated for variable periods of time with GH 0.1 IU/kg/d. The final height of these children varied from +0.1 to +0.7 SDS when compared with their pretreatment height prediction. A large proportion of those who had received GH over several years failed to improve adult stature.

## Summary

What overall conclusions can be drawn at this time?

1. The administration of GH results in a short-term acceleration of growth in a significant proportion of non-GHD children.
2. The positive effect is maximal during the first year of treatment but often wanes with time.
3. Preliminary data on adult height achieved by GH treatment in normal short children is disappointing and suggests that many treated individuals fail to significantly improve adult stature.

Many additional issues remain to be considered. In particular, questions of risk-benefit ratio and cost-effectiveness must be addressed. I hope this presentation fosters further discussion of these areas as well as consideration of the overall effectiveness of GH in non-GHD individuals.

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## DISCUSSION I: C & D

C: Efficacy of Growth Hormone Therapy in Patients Without Classically Defined Growth Hormone Deficiency - Raymond Hintz, MD

D: Growth Hormone Is Not Effective in Non-Growth Hormone Deficient Patients - S. Douglas Frasier, MD

Moderated by Jo Anne Brasel, MD

JOHANSON: Would you care to make a remark about what happens to children with constitutional growth delay in terms of their final height?

FRASIER: We have thought and have taught our students, that children with constitutional growth delay, as generally defined, will reach the height expected for their family, albeit they will get there later as they enter puberty late. However, there is an increasing body of information that says this may not be true, and children with constitutional delay may not have progressed as well as we had once thought.

DANIELS: What exactly is meant by "constitutional"?

FRASIER: These are children who are short, growing at a normal velocity, and have delayed skeletal maturation. I don't utilize "constitutional" to talk about children that are growing at subnormal velocity.

MacGILLIVRAY: We do not have a good historical data base which documents the long range outcomes of short children who have what I call "idiopathic growth failure." They differ from children who are constitutionally delayed in that they are growing at an abnormally low velocity. I think the children referred to by Dr. Hintz who had normal growth velocities prior to entering this study are probably a different population.

HINTZ: Yes, they are different. We wanted to see whether children that have higher growth rates when they enter the study responded less well. In fact, that was not true.

MacGILLIVRAY: Children growing at a pathologic rate are not constitutionally delayed. They have a growth disorder. Without an appropriate historical data base, is it fair to assume that achieving predicted adult height is a success?

BAILY: I'm troubled by the idea that we are using growth data from the 1930s. Some ingenuity should be used to figure out noninvasive ways of studying normal growth to establish a baseline and to determine what happens to children who are not going to be treated.

HINTZ: We have many studies of the growth of normal children. What we are proposing is a need for studies documenting the natural history of growth in abnormally short children. With the exception of Turner syndrome, we do not have outcome measures.

ALLEN: Withholding treatment from abnormally short children, while requiring bone age X-rays every 6 months, would be difficult to defend to human subjects committees.

ROOT: The average height of our society has not changed all that much. If you look at the data from the Boston Children's Hospital anthropometric charts (1930s) and compare them to the National Health Statistics study in the mid-1970s, the third- and tenth-percentiles for heights for boys and girls are almost identical to the first decimal place. We are presumably now achieving our growth potential in this population.

Regarding doses of growth hormone, present low-dose treatment is still twice the amount of GH that "in the old days" was effective in making patients with "classical" growth hormone deficiency grow very well.

WEISBARD: If we examine the effect of GH therapy from the perspective of functional *incapacity*, rather than merely a statistically significant deviation from the mean, perhaps we will discover that we are comparing 8 inches in the "classic GH deficiency" instance with 1 or 2 inches in the non-GH deficient child. Could someone clarify what orders of magnitude we are talking about?

FRASIER: For classical GH deficient children, I think the best treatment now available will allow achievement of the height potential expected in that patients' family. For the non-GH-deficient child, we do not know the long-term result.

LANTOS: Would you agree that your best chance for augmenting the height of a short child is to start them younger?

HINTZ: Yes.

LANTOS: Treat them longer?

HINTZ: Yes.

LANTOS: Seven days a week and at higher dose?

FRASIER: Yes, treat them daily, but I am not sure what is meant by "higher dose." The growth hormone log-dose response curve is not linear; there is a limit to the response achieved at the upper end of the dose-response curve, and toxicity can be expected at some higher GH dosage.

## ADVERSE EFFECTS OF GROWTH HORMONE TREATMENT



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### Introduction

For more than 50 years, growth hormone (GH) has been recognized as a potent chemical messenger, capable of influencing many aspects of metabolism and, of course, stimulating somatic growth. A review of the adverse effects of GH depends on the perspective of the observer. A consideration of childhood acromegaly, in which the previously healthy child is exposed to large quantities of endogenous GH from a pituitary tumor, shows the main adverse effect is gigantism, or excessive growth. In the context of the treatment of short children with GH, however, gigantism is merely an exaggeration of the desired effect. Furthermore, the adverse effects of medications and therapeutic maneuvers have a nasty habit of ambushing us when least expected. Before 1985, only the most farsighted considered the risk that spongiform neuropathies might be transmitted by pituitary GH. Such fears were apparently allayed by experimental studies only to reemerge as cruel reality in a scenario that would credit the best of science fiction writers.<sup>1</sup> GH is currently being given to about 20,000 children in the United States (US) and probably to a smaller total number of patients in the rest of the world. Thus, serious adverse effects occurring with the frequency, for example, of aplastic anemia due to chloramphenicol (approximately 1:10<sup>5</sup>) might well be hidden from us now, only to emerge if GH therapy becomes a much more widely used option. With these cautions, I will review some of the more pressing concerns regarding the adverse effects of therapeutic GH. The listing is not intended to be comprehensive nor, as I have indicated, can our current knowledge be considered an infallible guide to what will be.

### The Oncogenic Potential of Growth Hormone

The theoretic possibility that GH could facilitate the development of cancers has long been discussed. GH is known to favor the development of certain mammary tumors in the mouse,<sup>2</sup> perhaps because of its lactotropic effect. The tendency to develop mammary tumors has recently been observed in transgenic mice constitutionally producing large excesses of GH.<sup>3</sup> A cautionary note regarding a possible stimulatory effect

in childhood leukemia was sounded as far back as 1977.<sup>4</sup> However, the current high level of concern regarding the development of leukemia in treated children arose in 1987 with the publication of reports by Watanabe and colleagues from Japan<sup>5</sup> indicating that the incidence of leukemia in GH-treated children was much higher than expected on an actuarial basis. In a comprehensive review of the situation as it existed 2 years later, Stahnke and Zeisel<sup>6</sup> analyzed the data on 15 cases worldwide of GH-treated children who had developed leukemia. They noted that 7 children had conditions that might predispose to leukemia and that 2 had been on GH for only a very short time. While most reported cases of leukemia associated with GH therapy have been of the acute lymphoblastic variety, at least 2 cases of acute myeloid leukemia have been reported from Japan.<sup>7,8</sup>

In the US and Canada, a total of 9 cases of leukemia in children currently or recently on GH therapy have been recorded.<sup>9</sup> All but 3 had previously received either radiation or chemotherapy, or had surgery for brain tumors without radiation. The classification of these data remains inexact because of the lack of a central data base to which all such cases can be referred. It is, for example, theoretically possible that the same case, reported to various agencies, has been counted twice in this list. There has been no centralized system for the purpose of confirming such data as the type of leukemia, the temporal relationship to GH therapy, and the eventual outcome. Efforts are now in progress to set up and maintain a central reporting system. In a recent review from the United Kingdom,<sup>10</sup> 16 patients (out of 1,901 treated) developed tumors during or after GH therapy. Four of these had had diagnosed brain tumors before GH therapy was started. Thirteen of these new tumors were intracranial, the others being Hodgkin's lymphoma, osteosarcoma, carcinoma of the colon, and basal cell carcinoma. The authors considered that their survey provided no evidence of an increased risk of malignancy as a result of GH therapy.

The issues raised by the GH/leukemia relationship can be only summarized here. They are:

1. Does GH deficiency (GHD), *per se*, predispose to leukemia?
2. Are children who develop brain tumors intrinsically more likely to develop leukemia?

3. Does chemotherapy, therapeutic irradiation, or a combination of the 2 predispose to leukemia?

4. Does GH increase the incidence of leukemia among patients known to be predisposed to hematologic malignancy, such as those with Fanconi syndrome, and in those patients previously treated for leukemia?

I believe we are a long way from authoritatively answering any of these questions. Nearly all the patients reported as having developed leukemia during or after GH therapy have been GHD. In addition, 1 or 2 cases of leukemia have been reported of developing in GHD, nontumorous children before GH therapy could be started. The annual incidence of leukemia in US children aged 5 to 12 years is about  $1.4 \times 10^{-4}$ . The low number of GHD children and the universality of therapy for GHD children render it unlikely that this question will ever receive an answer.

US data establishing a 3:1 preponderance of brain tumor patients among GH-treated leukemia victims (set against a 3.5:1 preponderance of nontumorous patients in one large survey of GH treatment<sup>11</sup>) strongly suggest that with or without GH treatment, children who have had brain tumors are more likely than the general population to develop leukemia. It will be necessary to obtain good follow-up data on children treated for brain tumors who did not receive GH before the question of whether GH poses a special risk for this population can be addressed.

When the connection between leukemia and GH therapy was first raised, most oncologic and neurosurgical opinion was that neither brain tumors, per se, nor the standard therapies for these disorders were associated with an increased incidence of leukemia. Since then, however, Blatt and colleagues<sup>12</sup> have provided evidence for such a link. Their paper reviews the literature on 10 subjects who developed leukemia after treatment for brain tumors. In an accompanying editorial,<sup>13</sup> Dr. Anna Meadows considers that GH may act as a promoter of expansion of a group of cells that have already sustained a "first event" that set them on the path to malignancy.

Given the pitfalls and limitations of the epidemiologic approach, it is not surprising that experimentalists have tried to discover whether GH might have a direct effect on the transformation of hematologic cells in vitro. Human lymphocytes possess GH receptors and there has been considerable interest in any role that GH might play in modulating these cells' proliferative response to antigen. GH also is capable of influencing the proliferation and differentiation of hematopoietic stem cells,<sup>14</sup> a property that has been used to assess patients' cellular sensitivity to the hormone.<sup>15</sup> Both GH and insulin-like growth factor 1 (IGF-1) have been reported to stimulate the proliferation of cells derived from human hematopoietic malignancies. Physiologic concentrations of both peptides separately increased proliferation of laboratory cell lines derived from human leukemia and lymphoma cells and had the same effect on blast cells freshly obtained from the

marrow of children with acute lymphoblastic leukemia and acute myeloid leukemia.<sup>16</sup> The results of this sort of experiment support views such as the one attributed above to Meadows. However, they do not provide any evidence that GH or IGF-1 can induce the transformation of nonmalignant cells into malignant ones. Attempts to show such changes in vitro have been made, but to date none have been successful.

In summary, GH treatment is associated with an increased risk of hematologic malignancy. In the US, this is confined to patients who have been treated for brain tumors, who might well be predisposed to the development of leukemia. In Japan, even children with idiopathic GHD appear to have an increased risk of leukemia when they receive GH. GH might enhance the proliferation of existing clones of transformed cells, but there is no evidence that the transformation event itself can be induced by GH. Much careful epidemiologic and laboratory research remains to be done in this heavily charged area.

## Growth Hormone and Metabolic Derangements

### A) Hyperinsulinemia

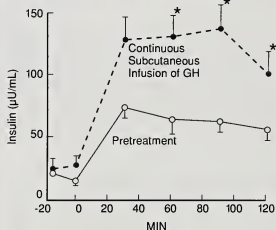
The relationship between GH and insulin has fascinated diabetologists for many years. Acromegaly is associated with diabetes of the insulin-resistant type and there are reports that glucose tolerance may revert rapidly to normal when a growth hormone-secreting adenoma is resected. In experimental work both insulin-like and anti-insulin actions, probably mediated via different receptors,<sup>17</sup> have been attributed to GH. However, the anti-insulin effect predominates during the long-term administration of exogenous GH. Serum insulin levels rise because GH induces resistance to the glucose-lowering effects of insulin. The reported extent to which exogenous GH induces hyperinsulinemia has varied widely and may depend on such factors as the dose and duration of therapy, the age and body habitus of the recipient, and the mode of administration of the GH. In our study of the effects of continuous low-dose subcutaneous GH infusions in GH deficient children, we observed significant increases in the insulin response to an oral glucose tolerance curve after only 3 days at  $0.8 \mu\text{g/kg/hr}$ <sup>18</sup> (see Figure 1). More recently the Yale group, using a hyperglycemic clamp technique, have demonstrated a sharp increase in insulin resistance when girls with Turner syndrome were started on GH<sup>19</sup> (see Figure 2). Other studies of GH treated children have not demonstrated much in the way of insulin resistance during chronic therapy.

Even if we accept that most children on GH develop a degree of insulin resistance, it is far from clear whether this leads to any harmful consequences. Insulin resistant diabetes as a complication of GH therapy is virtually unknown. Insulin resistance is associated with obesity, hyperlipidemia, atherosclerosis, and ovarian hyperandrogenism. However, none of these conditions are likely to present in the patient population that receives exogenous GH, and they have not been reported as



adverse effects of treatment. Nevertheless, we should remain vigilant, especially as regards any possible delayed incidence of macrovascular disease.

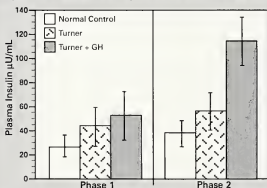
**Figure 1: Effects of Low-Dose Subcutaneous GH on Insulin Response in GHD Children**



\* $P < 0.05$

Mean plasma immunoreactive insulin levels from oral glucose tolerance tests performed before and during continuous subcutaneous growth hormone infusion.<sup>18</sup>

**Figure 2: Effects of GH Therapy on Insulin Response in Turner Syndrome Patients**



Mean plasma immunoreactive insulin levels during the Phase 1 and Phase 2 responses to intravenous glucose tolerance tests. The normal subjects were prepubertal girls. Girls with Turner syndrome were studied before and again during daily subcutaneous therapy with growth hormone.<sup>19</sup>

## B) Sodium retention

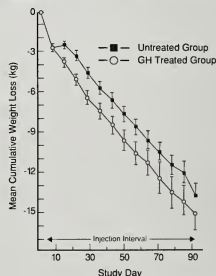
Edema and sodium retention were among the first physiologic consequences of treatment noted by the early GH pioneers. GH-induced sodium retention is dose dependent and often appears to wane in intensity as therapy is continued. The cause is unknown but may reflect an antinatriuretic effect on the renal tubule attributable to GH itself or to IGF-1 or insulin. Mild sodium retention is best detected by careful weighing of the patient. This point is well illustrated in Figure 3 which is taken from Snyder et al<sup>20</sup> and shows parallel declines in mean body weight in obese and nonobese subjects. At every time point during treatment the mean weight is greater in the GH-treated

than in the untreated subjects, presumably because the former show a degree of GH-induced salt and water retention. Apart from some discomfort at the lower extremity, and some anxiety when parents observe a symptom known to be associated with serious illness, GH-induced salt and water retention do not pose a clinically significant problem. This statement may, however, lose some validity if GH ever comes into widespread use as an anabolic agent in critical care settings or in the preterm newborn.

## C) Increased energy expenditure

One reason for including this subject in a review of the critical adverse effects of GH might be sought in the copyediting practices of some of our leading medical journals, perhaps with the concurrence of investigators who publish therein. The fact that GH induces a marked increase (up to 25%) in resting metabolic rate was first reported by Henneman et al in 1960.<sup>21</sup> At the same time, those authors described a marked fall in appetite which, they said, had originally been observed by Beck et al in the first patient ever to receive human GH.<sup>22</sup> The rise in basal metabolic rate coupled with the fall in appetite and the lipolytic effect of GH all contribute in part to the loss of fat tissue in treated patients, as exemplified by common observation of the results of treatment in severely deficient children.<sup>23</sup> Is this an adverse effect? Certainly so, if the title of a recent *Lancet* paper is to be taken seriously.<sup>24</sup> These authors found a 12% increase in resting energy expenditure after 6 months of GH treatment, together with a considerable decrease in body fat. Even as modified by the terminal interrogative, the title "Treatment of Short Normal Children and Growth Hormone—A Cautionary Tale?" sounds quite provocative. And so it proved, with plenty of attention from the serious press and heightening of general concern about GH.

**Figure 3: Effects of GH Therapy on Sodium Retention**



Mean cumulative weight loss in two groups of adult volunteers on a calorie restricted diet. The initial delay in weight loss observed in the growth hormone-treated group (—○—) relative to the untreated group (—■—) is attributable to sodium and water retention in the former group.

From Snyder et al.<sup>20</sup>

Others have interpreted similar data in a very different light. For example, the article presenting the findings that fat mass was reduced, muscle increased, and heart rate increased was entitled, "Beneficial Effects of Growth Hormone Treatment in GH-Deficient Adults."<sup>25</sup> The statement of Rudman et al that "the effects of 6 months of human growth hormone on lean body mass and adipose tissue mass were equivalent in magnitude to the changes incurred during 10 to 20 years of aging"<sup>26</sup> was soon transformed by the lay press into statements implying that treatment with GH could make individuals look and feel 20 years younger. Like previous authors who had looked at GHD children and adults, these authors found that GH could increase lean body mass (by a mean of 8.8%) and decreased fat mass (by 14.4%). Successive investigators, therefore, going back since the first human use of GH over 30 years ago, agree that GH can profoundly alter the metabolism of both fat and lean tissue.

These changes inevitably accompany somatic growth or any other primary therapeutic action for which GH is given. Perhaps the adverse effect against which we must guard is any tendency to sensationalize observations on the effects of GH and the attendant false hopes and fears that can flow from such handling of our data.

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**Table 1: Review of Literature on Patients With Leukemia Following Treatment of Brain Tumors<sup>11</sup>**

| Patient | Age* | Sex | Brain Tumor                          | Therapy                             |              |               | Interval Between Dx of BT and L | Reference <sup>†</sup> |
|---------|------|-----|--------------------------------------|-------------------------------------|--------------|---------------|---------------------------------|------------------------|
|         |      |     |                                      | Chemotherapy                        | Radiotherapy | Leukemia      |                                 |                        |
| 1       | 4.2  | M   | Astrocytoma, pilocytic <sup>††</sup> | -                                   | +            | ALL           | 36 mo                           | 4                      |
| 2       | 5    | M   | Glioblastoma multiforme              | BCNU<br>V<br>Mtx<br>Dec<br>VP16     | +            | AMML          | 15 mo                           | 10                     |
| 3       | 6.6  | M   | Medulloblastoma                      | CCNU<br>V<br>P                      | +            | Mixed lineage | 83 mo                           | Present case           |
| 4       | 12   | M   | Malignant ependymoma                 | -                                   | +            | APL           | 1.5 yr                          | 8                      |
| 5       | 18   | M   | Germ cell tumor (CNS) <sup>††</sup>  | -                                   | +            | ALL           | 6 yr                            | 3,5                    |
| 6       | 30   | F   | "Malignant tumor" occiput            | CCNU<br>VM26                        | +            | APL           | 38 mo                           | 11                     |
| 7       | 39   | M   | Astrocytoma, grade IV                | CCNU<br>VP16<br>DDMP<br>Dec         | +            | ANLL          | 51 mo                           | 12                     |
| 8       | 42   | F   | Anaplastic astrocytoma               | CCNU<br>Procarbazine<br>VM26<br>Adr | -            | AMML<br>RAEB  | 43 mo                           | 11                     |
| 9       | 56   | F   | Meningioma, angioblastic             | CCNU<br>VM26                        | +            | AMML          | 81 mo                           | 9                      |
| 10      | 60   | F   | Oligodendroglioma                    | CCNU                                | -            | AMML          | 51 mo                           | 9                      |

\* Age given in years, months, at time of development of leukemia.

† Reference numbers refer to references given in the paper quoted.

†† GH administered following diagnosis of brain tumor.

NOTE: mo, months; yr, years; BT, brain tumor; L, leukemia; CCNU, lomustine; BCNU, carmustine; V, vincristine; Mtx, methotrexate; Dec, decadron; VP16, etoposide; P, prednisone; VM26, teniposide; DDMP, 2-4diamino-5-4 dichlorophenyl-6-methylpyrimidine; -, not given; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; APL, acute promyelocytic leukemia; CNS, central nervous system; AMML, acute myelomonocytic leukemia; RAEB, refractory anemia with excess blasts; Adr, Adriamycin.

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## DISCUSSION I: E

### E. Adverse Effects of Growth Hormone Treatment - Joseph Gertner, MD

Moderated by Jo Anne Brasel, MD

**DANIELS:** I was curious to hear about the interaction between sex hormones and growth hormone and the ways in which they may partly affect each other. Does the presence of large doses of growth hormone have a long-term effect on sexual development in puberty or later?

**GERTNER:** I do not think there is evidence of a long-term effect, and even the effects at the time of treatment are disputed. Charles Brook and collaborators find that puberty is accelerated when growth hormone is given. However, the Collaborative Genentech Study shows no evidence of acceleration of puberty during growth hormone treatment. Sex hormones appear to increase GH secretion at the time of puberty. Yet when one reaches 21 years of age, sex hormones are still made but growth hormone secretion goes down. I do not think there is evidence that growth hormone adversely affects reproductive capacity or sexual function.

**FRASIER:** Are there clinical circumstances under which you would recommend that growth hormone not be given to a deficient individual because of the possibility that the incidence of leukemia might be increased?

**GERTNER:** My reading of the Wilkins Society statement and the worldwide literature is that there is an increased incidence of leukemia in patients with a prior brain tumor who have previously had brain tumors and who are now being given growth hormone. This applies whether they had radiation or chemotherapy or even surgery. We have a dilemma and it is not known whether this increased

incidence of leukemia is related to or independent of growth hormone therapy.

**BRASEL:** The Wilkins Drugs and Therapeutic Committee is to meet again in 1993 with cancer and epidemiology experts to reassess the data on this issue. One important question to resolve is, "Is there a subgroup of patients for whom we should recommend no growth hormone treatment under any circumstances?"

**FOST:** I wanted to raise two questions about efficacy, if I could. This conference is focused obviously on growth hormone treatment for children, but there are other clinical uses. There are recent studies on the effect of growth hormone on aging. Do you think there is anything to that? Is it promising? Is it expanding? And, secondly, what is its effect upon athletes for anabolic purpose and for performance enhancement? Could someone give a brief statement of what they think the state of knowledge is in those areas?

**HINTZ:** In terms of experiments with growth hormone in aging, there are short-term metabolic effects, some of which may be useful in terms of enhanced protein synthesis. At the other end of the spectrum is the fact that acromegals do not live forever nor are they particularly strong. None of us has any data about the use of growth hormone therapy in athletes. From what I know about short-term effects in adults, it might be useful in the short term, particularly along with a weight-building program and perhaps androgens. In the long term it is unlikely to be very useful.

**LIPPE:** There is a third group—the hypopituitary adult.

**HINTZ:** Right. Well, that is another issue. There you are treating a disease.

JOHANSON: In reference to Dr. Fost's question about athletes or adults, there either has been or will be published in the March issue of *Journal of American Physiology*, a study by Yarashevski and Bier on giving young men growth hormone and also exposing them to training exercises. The growth hormone-treated group showed some changes in metabolic parameters, but did not improve any kind of functional measurements — that is, strength or whatever, in all the things those folks do.

MacGILLIVRAY: I want to ask a question about one of my patients who is a boy of almost 13. He has had total body irradiation and a bone-marrow transplant; he is doing beautifully except he stopped growing after the total body radiation and transplant. He now wants to grow again and to go through puberty. What should I do if I find he is growth hormone-deficient?

GERTNER: Oncologists are adamant in saying, "There is no risk in giving growth hormone." But I do not know how they can be so confident. One can look for clonality in the marrow-derived cells. If someone is going to have a relapse of leukemia, there may already be a clonal element in the present bone marrow. If a relapse were predicted, you would not treat them. I do not know how to respond to your question except to say that we do treat such children with growth hormone if they are deficient.

STANHOPE: I treat them, as well; it is always easy to generalize, but with an individual patient it is much more difficult. With the hematologist or the oncologist, I actually talk to them and tell them what the evidence is before actually letting them make the decision.

UNDERWOOD: One thing I think we are doing right is to focus on these at-risk groups for the possibility of leukemia. I was particularly disturbed by an article in the *Journal of Pediatrics* (1991;119:478-483) that presents the results of a small trial of growth hormone therapy in patients with Down's syndrome who have a known predisposition to leukemia. I think we need to keep focusing on the at-risk groups.

ALLEN: Several of the adverse effects, including psychological effects, of treatment and perhaps even tumor development could possibly be minimized by reducing the duration of exposure to the growth hormone. A French group has reduced exposure time by using high doses of growth hormone for a couple of years. Do you think that is a direction we should be moving in?

GERTNER: I like the idea of short-term trials with high doses, but I do not know the side effects of such a program. I do not know anyone who can tell you that hyperinsulinism of a certain degree for 6 years is worse than hyperinsulinism of a higher degree for 2 years; or that a shorter exposure to a burst of high dose growth hormone is more likely or less likely to cause mitotic disease. There is no data or information to answer those questions.

MACKLIN: One of the hardest things, I find, is to assess risk/benefit ratios. You have now told us about the presumed risks, the perceived risks, the hypothetical risks, the side effects, and the possible long-term but unknown risks. But will the information you provided ultimately help us? Even if we had much better data and much more experience than your report this morning showed, we still might have difficulty with the risk/benefit ratio. So what, then, would you advise, given the uncertainty? Is there a provisional assessment that you can make about these kinds of risks when we are thinking about the risk/benefit ratio?

GERTNER: Well, I will stick my neck out; this is a very, important question. I would say that the risks are extremely small in nondeficient short children, apart from the psychological aspects, which Dr. Stabler may address at some more length. I believe the risk of physical illness and potential harm to children treated with growth hormone is really small.

FRASIER: I do not disagree with Dr. Gertner, but I must add "at presently used doses," because there is this tendency that if a little is good, then more might be better. We need to guard against that.



## Session II:

### PSYCHOLOGIC AND SOCIAL ISSUES IN GROWTH HORMONE THERAPY

**Editor's comments:** It is often assumed that a short-statured individual will be socially and emotionally unfulfilled, and concern about psychologic harm is invoked as a primary rationale for treating short stature. Yet, data confirming this assumption and, perhaps more importantly, the efficacy of growth hormone (GH) therapy in alleviating the psychosocial consequences of short stature are scarce. In this session, psychologic risks of short stature and the emotional risks and benefits of its treatment are discussed. The degree to which socioeconomic advantage and gender-related pressures of "heightism" have shaped current allocation of GH is also addressed.

David B. Allen, MD

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### GENERAL AND SPECIFIC PSYCHOLOGIC CONSEQUENCES OF GROWTH IMPAIRMENT IN CHILDREN AND ADULTS



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#### Introduction

Growth hormone deficiency (GHD) affects more than somatic growth: those who treat GHD patients are aware of the many social and interpersonal difficulties and problems faced by these individuals. Short stature (SS) has been the focus of explanations for such problems. Only recently has it become clear that lack of stature cannot be solely responsible for the unsatisfying life quality of many GHD patients. This paper documents some of the deficiencies experienced by patients and suggests that an ethical approach to treatment must focus not only on issues of growth and stature but also on issues of social and developmental life quality.

#### Psychologic Aspects of Height

The principal question governing the therapeutic use of GH is whether the patient qualifies for treatment by virtue of

demonstrable absence or insufficiency of GH secretory capacity.<sup>1</sup> Even from this seemingly straightforward viewpoint there is much debate over the criteria for defining GHD. Confounding the issue is a discussion of the concept of "heightism," which alludes to the presumed effect of SS on self-image and hence self-esteem.<sup>2</sup> Arguments are made, as the basis for this belief, that GH treatment be available to non-GHD patients with the expectation that increased growth velocity will improve self-esteem. Much has been written about the adjustment and psychologic functioning of short children,<sup>3</sup> although little has been incorporated into the formulation of ethical guidelines for GH treatment.<sup>4</sup> Allen and Fost<sup>5</sup> present a balanced review of biologic and psychosocial rationales for treating non-GHD "entitled" children under certain circumstances. These authors are concerned that GH therapy be available only to those for whom height is a serious handicap. They insist there is no clear line of demarcation as to what constitutes such a serious handicap, but suggest a height below the 1st percentile. Important to this discussion is the point that height alone is not a reliable predictor of happiness and self-

esteem.<sup>6</sup> Indeed, they suggest that an adult height at the 5th percentile should be considered as a target for GH therapy. Recent behavioral studies of short men suggest that significant psychologic stress is associated with lack of height, even within the range of what is considered "normal-short."<sup>7</sup> Thus, it seems premature to propose absolute height standards for treatment goals, but rather the focus should be on adverse biologic, psychologic, behavioral, and social effects of SS, and how can they be rectified.

## Recent Psychologic Research Findings

Several well-controlled studies of GHD children have begun to clarify the nature and extent of the learning difficulties associated with GHD.<sup>8</sup> Siegel<sup>9</sup> has suggested that the academic achievement profiles of GHD children closely resemble those of children with specific learning disabilities and attention deficit disorders. These findings are supported by those of others.<sup>10</sup> Interestingly, a British study examining nonreferred short children (<3rd percentile) found no evidence of school-related difficulties except those explained by socioeconomic status.<sup>11</sup> This finding illuminates the fact that SS, per se, is not correlated with academic difficulties and strengthens the belief that a pituitary disorder is most likely the cause of such problems. Taken together, most recent studies strongly suggest that neuropsychologic functioning is fundamentally impaired in many GHD children. The underlying mechanisms are not clear but the outcome in terms of school performance deficits is well-documented.

Other studies have shown that many GHD children have behavior and social problems.<sup>12</sup> In a recent report from the National Cooperative Growth Study, we found a high incidence of problems related to inhibited-disinhibited behaviors.<sup>13</sup> In a group of patients of various diagnoses about to begin GH therapy, some 27% were reported as having problems ranging from withdrawal and anxiety to distractibility. Others have observed similar traits, although generally in much smaller groups of patients.<sup>14</sup> In considering these findings, it is important to bear in mind that patients with less severe GHD, ie, constitutional SS, as a group tend to demonstrate fewer behavioral deficiencies. Patients with greater GHD, ie, panhypopituitarism, seem to experience more cognitive and behavioral difficulties.<sup>14</sup> This suggests a relationship between psychosocial functioning and degree of endocrine deficiency, a notion not entirely novel in the psychiatric literature.<sup>15</sup>

## Comprehensive Care of Short Children

Stature, and our concern with it, is in some ways an obstacle to gaining truly comprehensive care for short children. There is no question that for those children who fit the medical criteria, GH therapy has much to offer as a growth-promoting agent. However, what about the children who do not fit the criteria? And are growth velocity and stature the only focus of treatment? Little is known about the fate of referred-untreated children, since

there is no effective follow-up mechanism. However, available studies on children with constitutional delay of growth (so-called normal-short children) suggest they experience a degree of difficulty in their schoolwork and in interpersonal relationships with family and peers.<sup>16</sup> Thus, an argument can be made for considering nonclassic GHD short children as legitimate candidates for treatment since they exhibit many of the characteristics of classic GHD children. Although on standard provocative tests these children may secrete GH at levels above traditional diagnostic criterion, nevertheless they show other signs of neuroendocrine or neuropsychologic dysfunction.<sup>17</sup> If one adopts a comprehensive approach to treatment, these areas of difficulty, ie, inhibited behavior, learning disabilities, difficulty maintaining attention, may all be considered part of the diagnostic syndrome. Therefore, it is possible that patients will present with few or several facets of the syndrome—some with clearly deficient pituitary function and growth delay, others with more subtle signs such as SS and learning disability. If the only criteria applied are biologic measures of GH secretory capacity, many patients will fail detection.

## Who Can Assist in Diagnosis and Treatment?

Comprehensive therapy for patients undergoing GH treatment includes attention to behavioral, psychologic, and educational concerns.<sup>18,19</sup> To fully evaluate a patient's needs requires an assessment of cognitive and mental status, social and emotional maturity, educational achievement, and quality of life. Endocrinologists are not trained to do these things, and so consultation from pediatric or clinical child psychologists should be sought. Formal standardized psychometric testing together with individual and family interviews, and school consultation as indicated, will provide much useful data. This comprehensive approach will reveal hidden deficits in many cases, regardless of the presumed etiology of SS.<sup>13</sup> Combining the skills of endocrinologist, psychologist, social worker, and educator permits multiple interventions to occur simultaneously. For example, short children frequently require special educational services at school.<sup>20,21</sup> In many cases these learning problems are overlooked by teachers either because they view the child as generally immature because of stature or because the child is quiet and withdrawn in class, hence never attracting adult attention. Left unattended, these problems gradually impair a child's quality of life and lead to unsatisfactory social and vocational adjustment in adulthood.<sup>22</sup> Recognizing the potential for such problems and initiating early intervention contributes greatly to improved social functioning and better overall treatment outcome.<sup>18</sup>

## Growth Hormone Patients as Adults

The need for GH persists through life, albeit in diminishing levels.<sup>23</sup> Treatment of GHD patients with GH replacement therapy is usually discontinued when epiphyseal fusion occurs

and/or stature approaches normalcy.<sup>1</sup> Recent research implies that this approach may end treatment prematurely and leave GHD patients with an unacceptable quality of life.<sup>24</sup> GHD adults fare poorly in the educational, vocational, and social domains.<sup>25</sup> They live with their parents longer,<sup>26</sup> marry less frequently,<sup>27</sup> and achieve little academic success<sup>22</sup> compared with their normal peers. Importantly, GHD adults report experiencing a diminished quality of life, they particularly lack energy, and experience social isolation.

Although much has been written about the presumed effects of lack of stature on self-esteem, there is little empiric evidence for such claims. Martel and Biller<sup>6</sup> found short males reported being stigmatized for their stature and experienced increased anxiety in many social situations. Hensley<sup>7</sup> correlated height, gender, and self-esteem in a group of 210 undergraduates. He found no clear relationship between SS and low self-esteem. We recently compared 25 adult hypopituitary patients with an age-, sex-, and education-matched group of normal short individuals.<sup>28</sup> Psychometric tests showed the hypopituitary patients to be more introverted, less open, and less assertive than normals. Their physiologic response to behavioral stress was also much less marked than normals, suggesting their social behavior is as much influenced by endocrine deficiencies as by height. McGauley<sup>29</sup> tested the quality of life of 24 GHD adults before initiating GH treatment in a double-blind, placebo-controlled study. After a 6-month trial, GH-treated individuals reported significant increases in factors associated with improved quality of life, increased energy, and less perceived illness. Other studies have shown that GH treatment given to GHD adults increases certain cognitive functions such as short-term memory.<sup>30</sup> Salomon et al<sup>31</sup> demonstrated the metabolic effect of GH replacement in GHD adults, noting that increases in lean body mass and reduction in fat mass occurred.<sup>31</sup>

Evidence is mounting that supports the view that GHD adults on continued GH treatment benefit in many ways unrelated to their height status. Many of these individuals are living unsatisfying and unproductive lives. These deficiencies in life quality cannot be attributed solely to the deleterious effects of SS and inappropriate socialization during childhood. Long-term comprehensive treatment utilizing hormone supplementation, vocational rehabilitation, and psychologic counseling is necessary to ameliorate the myriad difficulties faced by GHD adults.

## Summary

GHD is a lifelong condition that requires treatment consistent with specific individual needs on a continuing basis. GHD is not a unitary condition affecting only growth rate and stature. Beginning in childhood it is known that GHD individuals experience a number of learning and behavioral difficulties not associated with growth rate or stature.<sup>8</sup> Unfortunately, treatment is directed only toward the problems associated with growth. Moreover, this treatment regimen usually ceases when epiphyseal fusion occurs, thus never addressing many important symptoms nor considering a patient's long-term needs. These patients grow up to be adults with poor educational preparation,

few friends, and inadequate job skills. In addition, because of their endocrine deficiencies, GHD adults may lack the assertiveness and competitive drive necessary for success in the modern workplace. Proper treatment of these problems requires a comprehensive, multidisciplinary approach.<sup>19</sup> Through such methods, we may expect more satisfactory treatment outcomes for these patients and their quality of life.

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# SHORT STATURE, STIGMA, AND BEHAVIORAL ADJUSTMENT



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## Introduction

Whether growth hormone (GH) therapy enhances self-esteem and behavioral adjustment is at the heart of the current debate about treating short children with GH.<sup>1</sup> The assumptions underlying the rationale for treatment appear to be that: (1) short stature (SS) is a stigma that can compromise behavioral development; and (2) improved growth rate and/or increased stature relative to peers is likely to result in improved self-esteem and behavioral adjustment in the patient. The few studies of self-esteem in children treated for SS provide contradictory evidence of improvements,<sup>2</sup> and the larger literature on stigma and self-esteem indicates that stigmatization does not automatically lead to poorer self-esteem and adjustment.<sup>3</sup> This report will review available data on self-esteem in children treated for SS and the likely connections between stigma and self-esteem that are seen in the social psychology literature. The present focus is limited to normally proportioned SS individuals for whom the principal observable difference is one of height deficit relative to the individual's age and sex peers. The reader is referred to other sources<sup>2,4,5</sup> for reviews of methodologic problems such as lack of control subjects, small samples, varying measurement strategies, and pooled diagnostic groups that have complicated the interpretation of existing data on clinical samples.

## Definitions

Self-esteem refers to a person's global sense of self-worth or satisfaction with one's self.<sup>6,7</sup> Stigma is a characteristic that engenders discriminatory behavior on the part of others. Stigmatization refers to the sociopsychologic process of holding negative opinions of others based upon the belief that they are different from the observer's reference group. Typically, stigmatization involves formulating judgments about other people based upon attributes they are perceived to possess and acting upon those judgments in a way that undesirably restricts their opportunities for growth and development. It is closely related to prejudice, discrimination, and negative stereotyping. The process has been studied in such diverse groups as blacks,<sup>8,9</sup> women,<sup>10,11</sup> the obese,<sup>12</sup> the mentally ill,<sup>13,14</sup> the physically disabled,<sup>15</sup> persons judged to be unattractive,<sup>16</sup> and short men.<sup>17</sup>

SS is a *relative* concept, and invariable definitions of who is "short" are not possible. In practical terms, this means that there will always be short individuals even though a short person in one population may have the height of a tall person in a different population. Given the ubiquitous nature of SS, what can be said about the personal and psychosocial correlates of SS?

## Height and Social Interactions

Extreme SS is a handicapping disadvantage, especially in negotiating a physical world designed for average-statured individuals. Physical size and height, in particular, also play a role in shaping interpersonal interactions. Height is positively correlated in adults with perceived levels of authority<sup>18</sup>; potential threat<sup>19</sup>; social status<sup>20</sup>; salary, promotions, and hiring<sup>21</sup>; and interpersonal spacing.<sup>22,23</sup> Outcome studies of groups of former GH recipients have associated low rates of employment and marriage with SS.<sup>24</sup>

Clinical studies of GH-deficient (GHD) children have noted a tendency for these children to be juvenilized by other people.<sup>4,25,26</sup> Juvenilization results from the incorrect belief that, for example, a 10-year-old child with the height age of 6 years *really is 6 years old*. Lack of pubertal development is likely to compound the misperception. Juvenilization is particularly common during interactions with strangers, but parents and health-care professionals are not immune from the natural tendency to "believe one's eyes."<sup>27</sup> A common example, which most SS teenagers can report, occurs when the server in a restaurant automatically hands the teenager a child's menu, unwittingly restricting the opportunities of the teenager. GHD young adults are clearly aware of having been treated younger than their chronologic age.<sup>27</sup> The impact of this phenomenon on psychosocial development has not been systematically studied beyond documenting its occurrence.

Juvenilization has been cited as a major factor in the adjustment difficulties of short children. While no clear pattern of adjustment problems has been identified, behavioral immaturity, social withdrawal, depressive symptoms, and low self-esteem have been suggested as outcomes of juvenilization in various clinical samples of SS children.<sup>2</sup> However, the extent to which



the adjustment difficulties seen in clinical syndromes can be attributed to the process of stigmatization rather than syndrome-related psychophysiologic features has been questioned. Turner syndrome (TS) girls have low self-esteem compared with test norms and more adjustment problems than a matched sample of girls with familial SS.<sup>28,29</sup> TS girls also showed poorer skills decoding the affective meaning of facial expressions. Thus, specific neuropsychologic differences between TS and non-TS girls may be more important than SS in explaining the observed differences in adjustment. Similarly, school difficulties shown by some GHD children may be related more to atypicalities in their intellectual and attentional skills than to psychosocial reactions to SS.<sup>30</sup> Low rates of dating, marriage, and sexual activity reported for young adult male hypopituitary patients also may be related to both the stigma of SS and neuroendocrine deficits.<sup>31-33</sup>

The fact that some but not all SS patients evidence adjustment difficulties argues that SS, per se, cannot completely explain the adjustment difficulties seen in clinical groups of SS children. A better understanding of SS children with successful behavioral adjustment, as well as multivariate comparisons of clinical samples with matched normal control subjects, is needed to clarify these questions.

## The Short Stature Stereotype

Both adults<sup>17</sup> and children<sup>34,35</sup> ascribe more negative attributes to short persons than to tall persons. For example, typical attributes assigned by 5- to 13-year-old schoolchildren to the shortest of 3 human silhouettes were "weak," "scared," "follower," "no friends," and "unsuccessful." In contrast, the same group of 229 children assigned "strong," "leader," "brave," "smart," and "awesome" to the tallest silhouette.<sup>36</sup> The attributions of the youngest children (5 to 7 years) did not differ from those of the oldest group (11 to 13 years) even though the youngest children had a much less accurate knowledge of their own height and relative height position among their peers.<sup>37</sup> Undergraduates of both sexes rate short men more negatively than tall men. This effect was more pronounced for men who were themselves short (62 inches to 65.5 inches) than for men who were average (68 inches to 70.5 inches) or tall (72 inches to 76 inches).<sup>17</sup> Similar ratings of men were given by undergraduate women. Also, undergraduate men (especially short men)<sup>17</sup> tended to overestimate their own height.

There also appears to be a sex difference in the way males and female value their body and in the value that Western society places on shortness in men and women. In general, men form a global evaluation of their entire physique in which larger is better (except obesity). Women develop a more detailed evaluation based upon various body parts; thus, smaller (except breasts) is more highly rated.<sup>38</sup> In terms of society's expectations, small women are generally viewed as being cute or petite, whereas short men are seen as less competent, withdrawn, and less desirable than taller men as dating partners.<sup>17</sup> Mothers<sup>39</sup> and teachers<sup>40</sup> of preschoolers rate taller,

more muscular children as more competent and socially influential. The effect seems to be more pronounced for boys than girls and may help explain why fewer girls are treated for SS. These and similar data<sup>38</sup> suggest that, all other things being equal, SS tends to be associated in the minds of others with less desirable personal attributes, especially for males. Furthermore, this perception is established by middle childhood and continues into adulthood.

## Self-Image, Self-Esteem, and Behavioral Adjustment

Self-image (one's multidimensional representation of oneself) is associated with one's self-evaluation of personal worth (self-esteem).<sup>38,41</sup> Several theories about the development of self-concept predict poorer self-esteem and adjustment difficulties as an outcome of exposure to social stigma generally.<sup>42,43</sup> and the stigma related to SS particularly.<sup>17,44</sup> The data are not clear, however, whether social stigma *automatically* results in poor self-esteem in the general population<sup>3</sup> or for SS individuals.<sup>2,45</sup>

Early work<sup>46</sup> establishing a positive correlation between satisfaction with one's body and high self-esteem has been replicated in various ways.<sup>47-49</sup> However, this relationship is not necessarily a causal one. The importance to an individual of any particular dimension of self-image (eg, physique, achievement, social skills) appears to have an important influence on the dimension's association with ratings of global self-esteem.<sup>3,50</sup> For example, some children with high self-esteem concurrently rate their physical appearance, academic competence, and/or behavioral conduct poorly. The importance of the negatively rated dimensions appears to be "discounted" relative to children's feelings of global self-worth.<sup>50</sup> Focusing on one's strengths may provide an avenue to high self-esteem in the face of social stigmatization. Consequently, it appears possible for children to have a poor image of their physique due to SS while maintaining high levels of overall self-esteem and self-worth.<sup>2</sup> This phenomenon provides a rationale for psychologic intervention to manage an insult due to stigmatization.

A further complication in interpreting the relationship between a negative stereotype of SS and self-esteem can be inferred from studies of self-esteem and stigmatization due to race<sup>51</sup> and sex<sup>52</sup> that found no decrement in self-esteem following a negative social appraisal when the source of the appraisal could be identified as prejudiced. The overall self-esteem of a SS child, therefore, might *not* be adversely affected when the child perceives the source of the stigmatization as prejudiced against or unfair treatment of himself/herself.

A third complicating factor for understanding the impact of stigmatization due to SS on self-esteem derives from the importance of knowing to which group the social comparison is being made. Specifically, insults to the self-esteem of short children may not be potent if the child identifies primarily with other short children. Mainstreamed mentally retarded (MR) children rate their scholastic competence as highly as classmates

with normal intelligence while mainstreamed students with learning disabilities (LDs) but normal intelligence rate themselves more poorly than regular students.<sup>7</sup> Examination of the primary reference group for the MR and LD children showed that the MR children routinely compared themselves to other MR children while LD children compared themselves to non-LD children. Thus, the stereotype associated with SS may not contribute to negative self-esteem when the SS child perceives his/her competence in a specific area as being better than the competence of other SS children.

## Treatment and Self-Esteem

The available data provide information on the behavioral correlates of treatment rather than demonstrations of treatment effects. There have been no placebo-controlled evaluations of the behavioral effects of GH treatment. The challenges of conducting such a study are significant.<sup>5</sup> Available reports involve patients treated with pituitary-derived GH and with treatment schedules and expectations that were quite different from the options made possible by biosynthetic GH. Consequently, the effects of modern GH therapy on behavioral functioning have yet to be demonstrated.

Early clinical reports suggested that GH treatment was associated with a "readjustment syndrome" in which patients were forced to reexamine their own behavioral expectations as the expectations of other people changed.<sup>53</sup> Increased depression, unrealistic treatment expectations, and behavioral inhibition have been reported in patients receiving intermittent<sup>54</sup> and continuous<sup>55</sup> GH treatment. About half of such children also exhibited low self-esteem. Parents, as well as children, have unrealistic expectations of the effect of GH treatment.<sup>56</sup>

Only 1 of 4 published prospective studies<sup>5</sup> using well-standardized behavioral measures assessed self-esteem. Siegel<sup>30</sup> found no differences in the self-esteem of GHD children who were underachieving in school compared with those who were achieving adequately. Contradictory comparisons of self-esteem in GHD and control patients have been reported at professional meetings.<sup>37,58</sup> Rosenfeld et al<sup>45</sup> presented data on the effect of a brief course of testosterone on the self-esteem and social activities of teenage boys with constitutionally delayed growth and puberty. Teenagers showed poor self-esteem ratings at baseline and improvement in self-esteem 1 year later, regardless of whether they received the testosterone treatment. However, only the treated group reported increases in the frequency of socializing at the 1-year mark.

## Summary

Several important links are missing in our understanding of the effect of the stigmatization on the psychologic and social development of SS children. As noted above, there is ample reason to believe that SS is associated with a negative stereotype,

at least in males, and that stature plays a subtle but important role in determining how one is treated by other people. However, the relationship between these 2 phenomena and the self-esteem and adjustment of SS individuals has yet to be clearly drawn. Some SS children have self-esteem and/or adjustment problems while others do not. How are the poorly adjusted children different from the well-adjusted ones? The existing studies of stigma present cross-sectional data on groups of stigmatized persons rather than prospective analyses of the course of development of stigmatized individuals. We, therefore, know little about how a SS child develops a reference group for comparison, or under what circumstances a SS child can devalue the stigma of SS and focus upon his/her strengths.

There have been no comparisons of clinical and nonclinical samples of SS children. It is not known, therefore, if the adjustment and self-esteem characteristics of the 2 groups are the same or different. Perhaps the SS cases that come to medical clinics are more likely to be experiencing behavioral and emotional difficulties.

Lastly, psychosocial phenomena are inherently multivariate. At the very least, sets of physiologic and psychosocial factors interact to produce a given behavioral outcome. Focusing predominantly on 1 or 2 specific factors is likely to produce an understanding of only limited utility.

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## DISCUSSION II: A & B

A. General and Specific Psychologic Consequences of Growth Impairment in Children and Adults - Brian Stabler, PhD

B. Short Stature, Stigma, and Behavioral Adjustment - Richard Clopper, ScD

Moderated by Louis Underwood, MD

CALLAHAN: It seems to me that the older one gets, the less important stature is. By the time one is 50 or so, people do not seem so big. The big people seem to be diminished. It would be interesting to do a longitudinal study to see how perceptions of height change over the course of a lifetime.

CLOPPER: That study has not been done; however, it seems that as people grow older, they successfully adapt to the insults that come with being short or tall or colorblind. I think that the aging process has the effect of smoothing over many of these social interactions—in helping individuals to turn a situation to their advantage.

STABLER: Individuals who do not have neuroendocrine impairment have many things going for them that allow them to compensate. In my experience, individuals who are not endocrinologically compromised can adapt to almost anything, through acceptance, modification of their behavior, and development of personal style. The condition, and adaptation to it, becomes part of their persona.

LANTOS: Toward the end of your talk, Brian, you indicated that there is a relationship between depression, other psychosocial problems, poor school performance, and short stature. We know from existing study data that certain extreme psychological problems can lead to neuroendocrine problems which, in turn, lead to short stature. When we look at people who are short and have low growth hormone levels and poor school performance, we are accustomed to deducing that the low growth hormone level is the cause and the poor school performance is the effect. But it is possible that the depression is the primary factor in both the growth hormone level and the poor school performance?

STABLER: Yes, I think such a biobehavioral connection is entirely possible. "Growth" is a term that we use in referring to the vast range of all types of growth, including our emotional growth. "Development" however, is a more

STABLER: germane and meaningful term that perhaps more accurately connotes the complexities we are addressing here.

HINTZ: What is more effective for treating these children, psychotherapy or growth hormone therapy?

STABLER: I am not sure that I know, but you would never want to treat anyone with growth hormone without some type of counseling or psychotherapy. I think this work can be done by nurses, social workers, school counselors, or school nurses to mention a few resources. The key is to build ego strength in the child as growth is accelerated.

## GROWTH HORMONE TREATMENT: DOES ASCERTAINMENT BIAS DETERMINE TREATMENT PRACTICES?

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### Introduction

This report will focus on the question, "Does ascertainment bias determine the patient population currently being treated with growth hormone (GH) in the United States (US)?" As the basis for our analysis we will use the data base provided by the National Cooperative Growth Study (NCGS), a postmarketing surveillance program established by Genentech, Inc at the time Protropin® was introduced in October 1985. Currently, over 12,000 children treated with GH have been enrolled in this program. This sample is estimated to represent more than 50% of patients who have ever been treated with GH in the US and more than 70% of those treated with biosynthetic GH; it is the largest collection of data ever assembled to assess GH treatment practices. It includes data on a cohort of patients previously treated with pituitary GH whose treatment was stopped when pituitary GH was withdrawn from the market due to potential contamination with the agent that causes Creutzfeldt-Jakob disease; a cohort with no prior treatment who enrolled between 1985 and 1987; and another cohort with no prior treatment who enrolled from 1988 to the present. These cohorts will be referred to as groups 1, 2, and 3, respectively. Some of the data from the

first 2 cohorts have been previously reported<sup>1</sup> and the current data have been submitted in abstract form.<sup>2</sup> The data in this report represent the diagnostic categories and demographics of all patients enrolled in the study, regardless of whether they have stopped receiving treatment. The analysis demonstrates that while diagnostic criteria may be changing, treatment is still largely restricted to white males, while females and blacks continue to be underrepresented.

### Methods

Over 350 pediatric endocrine centers currently participate in NCGS. The pediatric endocrinologist or designated staff member fills out a 2-page enrollment form designed by the NCGS coordinating endocrinologists. Patient data are coded by initials and date of birth, and patient (family) permission to participate is obtained whenever required by the local institutional review board (IRB).

Enrollment data include: gender; race/ethnic origin; height at diagnosis (if patient is in group 1) or at enrollment (if patient is



in group 2 or 3); GH test results; and clinical diagnosis. The diagnosis of GH deficiency (GHD) is subdivided into idiopathic GHD (IGHD) and organic GHD (which is further subdivided into craniopharyngioma, other central nervous system (CNS) tumors, irradiation, infection, trauma, CNS defects, histiocytosis X); septo-optic dysplasia (SOD) is listed separately. Patients enrolled with a medical condition not defined as GHD were classified either by their identified diagnosis, ie, leukemia or a chromosomal defect such as Turner syndrome (TS), or placed in a separate category called "Other." Enrollment information also included associated disorders such as hypoglycemia.

Height standard deviation scores (SDSs) for patients were calculated using mean heights and standard deviations (SD) for normal subjects derived from the National Center for Health Statistics<sup>3</sup> using the following formula:  $SDS = (height - mean height \text{ for normal subjects of the same sex at given age}) / SD \text{ of height for normal subjects of the same sex at this age}$ . In the GHD groups, the peak GH concentrations were not normally distributed and were truncated (since we arbitrarily excluded patients from these groups with values in excess of 10 ng/mL) so that medians were computed and nonparametric tests were used to test for significance among groups. Data entry and management were performed by Biometric Research Institute, Inc., in Arlington, Virginia. Data were analyzed by the Biostatistics Department at Genentech, Inc.

## Results

Table 1 shows the demographic characteristics of all 12,046 patients by sex, race/ethnic origin, diagnostic category, and history of previous GH treatment. Overall, males outnumber females by a 2:1 ratio. When the TS category is subtracted from the female group, females are outnumbered by a ratio of 2.6:1. Whites account for almost 87% of treated children, with blacks representing 5%, and Hispanics, Asians, and Other constituting the remaining 8%. The actual percentage of blacks, 5.3%, is only one third of what might be expected given that it is estimated that 15.5% of the US population 19 years of age or younger is black.<sup>4</sup>

Table 2 subdivides the different diagnostic categories by sex. The male to female ratio of the IGHD group is 2.9:1, which is the same ratio as in the Other group, 2.9:1. While overall the organic GHD group was less skewed in sex distribution (1.7:1), only the smaller groups of CNS defects, histiocytosis X, and SOD approached a 1:1 sex ratio.

Height SDSs were examined for the IGHD, organic GHD, SOD, and Other groups. For both the previously treated patients (group 1) and the never-treated patients (groups 2 and 3), there were significant differences between males and females, with females being statistically shorter than males in the IGHD and Other groups. There were no significant differences in height SDS between males and females in the organic GHD and SOD groups. When whites were compared with blacks, there were no significant differences in height SDS in group 1 in any of the diagnostic categories. However, in groups 2 and 3 blacks were

shorter than whites in the IGHD and Other groups. In addition, while we note that the actual height SDS at which patients with IGHD were being enrolled has become less negative (that is, the patients are less short relative to children of the same age and sex), since 1988 the overall sex and racial differences have remained.

The median maximum GH concentrations achieved during diagnostic testing was evaluated over the course of this study. For group 1 patients (who had been previously treated with GH prior to initial enrollment), there was a significant sex and racial difference in the concentrations, with median maximum GH concentrations being lower for females than for males and lower for black males than for white males. The maximum GH concentration was noted to increase over time for patients enrolled with no previous treatment, so that the difference in median maximum GH concentration between males and females was not as significant, but both black males and females had lower values than white males and females.

## Discussion and Conclusions

The 12,046 patients reported in this study represent a majority of the children who have been treated with GH in the US since 1985. Thus, an analysis of their demographics provides a basis for the discussion of treatment access. We will focus on several aspects of the question of access and hope that additional insights and perspectives will emerge from the discussion.

**Table 1: Genentech National Cooperative Growth Study Demographics of All Participants**

n=12,046\*  
1985-1991

| Demographics              | n      | Percent |
|---------------------------|--------|---------|
| <b>Sex</b>                |        |         |
| Male                      | 7,939  | 65.9    |
| Female                    | 4,107  | 34.1    |
| <b>Race/Ethnic Origin</b> |        |         |
| White                     | 10,389 | 86.25   |
| Black                     | 639    | 5.3     |
| Hispanic                  | 299    | 2.5     |
| Asian                     | 232    | 1.9     |
| Other                     | 429    | 3.6     |
| <b>Etiology</b>           |        |         |
| Idiopathic GHD            | 5,051  | 41.95   |
| Organic GHD               | 1,303  | 10.8    |
| Septo-optic dysplasia     | 281    | 2.3     |
| Turner syndrome           | 1,110  | 9.2     |
| Other                     | 4,243  | 35.2    |
| <b>Previous Treatment</b> |        |         |
| Yes                       | 2,018  | 16.8    |
| No                        | 9,970  | 82.8    |

GHD, growth hormone deficiency.

\* Small differences in subtotal reflect missing data.

Table 2: Genentech National Cooperative Group Study Participant Etiology by Sex

| Etiology                       | Sex   |         |        |         | Ratio<br>Male:Female |
|--------------------------------|-------|---------|--------|---------|----------------------|
|                                | Male  |         | Female |         |                      |
|                                | n     | Percent | n      | Percent |                      |
| Idiopathic GHD                 | 3,752 | 74.3    | 1,299  | 25.7    | 2.9:1                |
| Organic GHD                    | 813   | 62.4    | 490    | 37.6    | 1.7:1                |
| Infection                      |       | 11      |        | 0       | 11:0                 |
| Craniopharyngioma              |       | 251     |        | 148     | 1.7:1                |
| CNS Tumor                      |       | 315     |        | 191     | 1.6:1                |
| Trauma                         |       | 49      |        | 24      | 2.0:1                |
| Irradiation only               |       | 90      |        | 51      | 1.8:1                |
| CNS defects                    |       | 82      |        | 61      | 1.3:1                |
| Histiocytosis X                |       | 15      |        | 15      | 1:1                  |
| Septo-optic dysplasia          | 165   | 58.7    | 116    | 41.3    | 1.4:1                |
| Turner syndrome                |       |         | 1,110  |         |                      |
| Other                          | 3,163 | 74.5    | 1,080  | 25.5    | 2.9:1                |
| Total                          | 7,893 | 65.8    | 4,095  | 34.2    | 1.9:1                |
| CNS, central nervous system    |       |         |        |         |                      |
| GHD, growth hormone deficiency |       |         |        |         |                      |

CNS, central nervous system

GHD, growth hormone deficiency

The first is the issue of the sex difference found in the IGHD group. The predominance of males is not a finding new to this study, having been previously reviewed by us<sup>1</sup> and reported in virtually all studies of GHD around the world, including the surveillance study parallel to NCGS currently being conducted in Europe.<sup>5</sup> However, in almost all cases, the diagnosis is made at a tertiary center and therefore is largely dependent on the demographics of the patients referred for evaluation. Thus, one has to ask if the predominance of males is due solely to biologic differences or whether social factors play a role. That there are biologic differences which account for some degree of male predominance is suggested by the higher incidence of breech delivery and/or perinatal asphyxia in male infants, and the reports of these complications occurring frequently in males with GHD. Other biologic or suggestive causative factors have not emerged. Conversely, the first report of GHD detected by population screening of schoolchildren (using a height SDS of  $-2.5$  for initial ascertainment) noted that "none of the children with previously diagnosed GHD were girls, although 5 of the 9 children found with GHD during the study were girls."<sup>6</sup> This group later went on to study much larger numbers of schoolchildren and to note a male to female ratio of 1.5:1, which is not nearly as great as the 2.9:1 ratio in this data base. In addition, when one looks at the height SDS of the children detected by population screening, there are no significant differences between the males and females. Finally, our initial examination of the NCGS data base in October 1987 noted a lesser sex ratio (2.5:1) than we find now. All these data suggest that as awareness of available treatment for GHD increases, there is an even greater tendency to refer or test males as compared with females. Our data show the same 2.9:1 sex ratio in the Other group, comprised largely of children who do not have classic GHD, to further support the hypothesis that referral may be biased toward males.

While it is beyond the scope of this paper to provide an in-depth discussion of the possible reasons for sex differences in the organic GHD patients, we will include this group in the discussion of a second issue, namely, the height SDS at which the diagnosis of GHD is made. Females are statistically shorter than males at the time the diagnosis of IGHD is made, but not at the time the diagnosis of organic GHD is made. When height SDS data were analyzed over the years of this study for the IGHD group, it was noted that the SDS at the time of entry is increasing for both males and females, but the differences between them remain. Since we are unable to develop a biologically based hypothesis for the height differences occurring between males and females with IGHD as we did in the organic GHD groups, we can only conclude that short stature in the otherwise healthy child is perceived differently by the family and/or the physician depending on the sex of the child. Females who are short may be perceived as "cute" but not pathologically short until their SDSs are more adversely affected than those of males.

A third, interrelated, issue is the absolute peak GH concentration reported for the patients diagnosed as having IGHD. Notwithstanding the fact that these measurements were done in numerous laboratories by many methods, there were statistically significant differences in the concentrations for males and females, with females having lower values. This tends to support the finding that not only are females shorter at the time of diagnosis than males, but they also have a more severe degree of GHD.

Finally, we will briefly discuss the racial demographics of our patient population as it pertains to whites and blacks (since the number of patients in the other racial/ethnic categories is currently too small to assess meaningfully). We are not aware of any studies in the literature that discuss the prevalence of GHD

among blacks. Thus, the fact that we are considering blacks to be underrepresented in NCGS is based entirely on the 15.5% figure that has been reported as the percentage of blacks in the US 19 years of age or younger.<sup>4</sup> The data for the patients in NCGS, however, can be assessed independently of the issue of proportionate representation. We note that among the IGHD group, black males overall are shorter than white males, and black females are shorter than white females. When mean maximum GH concentrations were assessed, blacks had significantly lower concentrations than whites. Finally, a disproportionate number of blacks in NCGS had the concomitant pretreatment diagnosis of hypoglycemia as compared with whites. Taken together, these data suggest that referral for evaluation may be delayed in blacks unless there is a medical condition, such as hypoglycemia, that prompts ascertainment.

We have not discussed whether patients actually being treated with GH represent an unbiased sample of the patients referred for evaluation or even of those for whom treatment has been prescribed. That dimension includes not only the economic aspects of treatment access but also the medical criteria used by the physician to initiate an evaluation and the medical and social criteria used to recommend therapy. These questions can be answered only by prospective data collection.

## DISCUSSION II: C

### C. Growth Hormone Treatment: Does Ascertainment Bias Determine Treatment Practice? - Barbara Lippe, MD

Moderated by Louis Underwood, MD

UNDERWOOD: I guess we can conclude that ascertainment bias is not primarily the fault of the pediatric endocrinologist.

LIPPE: That is my conclusion.

BAILY: There is an underlying implication that somehow it is bad that women are shorter. But it seems to me that if you had a treatment to make people shorter, interested girls would overwhelmingly outnumber boys. The fact is that it is much more of a social handicap for boys to be short, and therefore, not surprising that short girls are not often identified or selected for treatment.

LIPPE: I would differ with you because black children and girls with brain tumors are *also* not being identified. Secondly, I do not think it is fair to say that just because people have not paid as much attention to girls and blacks that they should be denied a look. We used to treat tall girls to make them shorter, but they are not asking for that treatment anymore.

HINTZ: If your hypothesis is correct, Barbara, there should be a large number of untreated adult females. Has anybody looked?

LIPPE: I think not.

In conclusion, we feel that the NCGS data strongly suggest that nonbiologic ascertainment bias has played and continues to play a role in determining the demographics of patients being treated with GH. While one could take the position that, as a part of this bias, there are groups of children for whom the treatment criteria might be arguable, we must strongly stress that clearly there are groups of children who are not being treated, or are not being treated in a timely fashion. The ethical and social conclusions that emerge from this conference must embrace this concern.

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BAILY: The preceding 2 speakers were talking about the bad effects of being short from a psychological and psychosocial point of view. If, in fact, this is the main benefit of treatment and short stature is less of a social handicap for girls, it is not surprising that fewer girls are identified and referred for evaluation. You also seem to be suggesting that there are medical implications associated with short stature as well, so that if I have a short daughter, I ought to bring her in even though I am not bothered by her shortness. Can you please clarify this for me?

LIPPE: The message is that we ought to look at individuals equally first and make the judgments later. I do not think we should deny them access first.

CHARO: Based upon information provided in the first presentation, I understood that specific objective problems associated with growth hormone deficiency are *not* the result of the way the world interacts with the person with the deficiency. I thought that was the point of the comments about attention deficit, hyperkinetic activity, and similar conditions. Thus, it seems we are considering two categories of patients: those who need to be identified because they have a condition that should be addressed with some kind of medical treatment, and those for whom stature is a cosmetic issue akin to having a complexion blemish.

The fact that women are shorter, in general, may be a very significant factor in explaining the phenomenon of gender relation. It would seem dangerous to dismiss the need to identify girls who are shorter than their genetic potential as not being in need of treatment because the *cosmetic* impact is not perceived as a significant disability.

**STABLER:** Part of the problem may be that many pediatricians have a perspective that contributes to referral bias. Many pediatricians have difficulty arriving at the decision to refer a patient to the pediatric endocrinologist. Given that bias, there is reason to suspect that pediatricians may be biased in respect to gender, too.

**LANTOS:** Your presentation seems to assert that short women are underrepresented rather than short men being overrepresented, and that ascertainment of growth hormone deficiency and the provision of treatment is a good thing. I am not sure I am ready to go along with that. Consider the historical treatment of tonsillar irradiation in Chicago as

another therapy that was subject to class bias when it was first used. A presentation like yours would have shown that blacks and poor people were being discriminated *against*, when, in fact, these people were favored by not receiving a treatment that was later determined to be linked to the development of thyroid cancer. You could argue that middle class white males are being tortured with a shot a day for 5 years that offers them little benefit, all because their parents believe that the therapy is good for them.

**LIPPE:** Well, *you* could argue that! (laughter)

### *Session III:*

## **CONCEPTUAL AND ETHICAL ISSUES IN ENTITLEMENT TO GROWTH HORMONE TREATMENT**

**Editor's comments:** Traditional clinical endocrinology is firmly rooted in a disease-oriented model of medicine, ie, the replacement of deficient hormones and suppression of hormonal excess. With the advent of growth hormone (GH) treatment of non-GH-deficient children (eg, Turner syndrome), GH enhancement therapy has been added to GH replacement therapy. Is short stature now considered a "disease?" Is the disease label still relevant to the issue of entitlement to GH therapy? Is GH enhancement therapy a justifiable medical endeavor? These questions, addressed in this session, illustrate the complex ethical dilemmas that emerge from expanding GH efficacy and availability.

David B. Allen, MD



# THEORETICAL AND POLICY DEBATES OVER THE STATUS OF SHORT STATURE AS A DISEASE



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## Introduction

Is short stature (SS) a disease, and does its designation as such really matter? The debate over these questions can be carried out at 3 levels, which I will label the "clinical," the "policy," and the "theoretical," respectively.

### *The Clinical Level*

By "clinical," I refer to the debate among clinicians and clinical scientists over the physical and psychologic risks and benefits of human growth hormone (GH), administered both to GH-deficient (GHD) and to normally short, non-GHD children. I also include in this category the technical disputes over how GHD can be measured, and the difficulty of agreeing on a cutoff point for defining it.

### *The Policy Level*

I will use the term "policy" as shorthand for a number of social, moral, economic, and political issues that play a role in determining the proper course for public policy on the allocation of GH: its cost, its effect on other elements of health care, and questions of entitlement and justice.

### *The Theoretical Level*

By "theoretical" I refer to questions about the status of GHD and SS as diseases, when we consider these questions in abstraction and divorced from policy debates — assuming that one can do so.

I will have little to say about the clinical issues. Their resolution does, however, have important bearing on the other 2 categories of questions. They become most interesting only if GHD can be well-defined, and only if GH provides more benefit than risk for normally short as well as for GHD children. If these claims are untrue, then the policy and theoretical issues are relatively moot.

My aim in this paper is to sketch some of the logical relationships that hold among these levels of debate. Drawing on the insights of some philosophical colleagues, I will argue that the policy questions are not entirely independent of the theoretical ones, although the political process might ignore these entailments. This directs us to look to theoretical accounts

of the concept of disease to help in settling our policy disputes. However, I will suggest that the issues of GHD and SS may be especially difficult cases for the best of these accounts, and that we may be frustrated in looking to them for guidance.

## Is the Policy Level Sufficient?

Assuming the outcomes stated above in the clinical debates, do we need to ask whether SS is really a disease, ie, to enter the theoretical debate at all? Could we not determine the issue like any other question of public policy, referring in the main to the positive and negative consequences of providing GH to normally short children? Indeed, much of the debate so far has remained at this level. We are told of the staggering potential cost: up to \$10 billion annually—greater than the entire health-care budget of many countries. And we are reminded of the distress and stigma caused by extreme SS among United States (US) children and adults. Why not proceed to balance cost and benefit and be done with the matter? What does it matter if SS, or even GHD, is really a disease or not?

I would like to present some ideas on this question in dialectic fashion, first stating an argument that declares the theoretical question irrelevant. This argument urges that we carry out the debate entirely on the policy level. I will then present an argument for the opposite position, of the need to consider the theoretical issues in their own right.

## The Policy Argument

According to the argument that the policy level is the only relevant one (let me call this the "policy argument"), the belief that we must first decide whether SS and GHD are diseases stems from a misunderstanding of the nature of disease classifications. This mistaken view attributes to disease classifications an objective, scientific status that in actuality is only an illusion. The policy argument holds that deciding what a disease is and what it is not is a moral, political, or social question like any other. In support of this contention, observers have noted the wide variation among cultures in how diagnosis and classification are carried out. Lynn Payer, in her insightful book, *Medicine and Culture*,<sup>1</sup> notes:

"Often, all one must do to acquire a disease is to enter a country where that disease is recognized — leaving the country will either cure the malady or turn it into something else. The American schizophrenic of a few years ago might well have found his disease called manic-depressive disease or even neurosis had he sought a second opinion in Britain; in France he likely would have been diagnosed as having a delusional psychosis. Blood pressure considered treatably high in the United States might have been considered normal in England; and the low blood pressure treated with eighty-five drugs as well as hydrotherapy and spa treatments in Germany would entitle its sufferer to lower life insurance rates in the United States."

Moreover, it is easy to find diagnostic categories that today are seen clearly to be instruments of social policy, whether or not they were recognized as such by clinicians of the past. The stock example is "drapetomania," a disease of slaves that impelled them to try to run away,<sup>2</sup> and there are many others.

One event in modern history seemed to clinch this view of disease classification: the decision in 1974 by the American Psychiatric Association to decide whether homosexuality should be deemed a disease by putting the matter to its membership for a vote. This step seemed to confirm the claim, pressed by the gay community, that the classification of homosexuality as a disease was an exercise of social control, a purely political phenomenon. The fact that it was settled by political means seemed appropriate as well as revealing.<sup>3</sup>

The precise sense in which disease classifications are to be considered social rather than scientific varies according to the theorist. At a minimum, diseases are said to be those variations in physiologic functions that deny people what they or society value; as values differ across society and societies, so do disease classifications. As with homosexuality, the classifications can be means of social control. The act of diagnosis can be a "performative" act, which means that it not only describes but also prescribes. Someone deemed sick by a doctor has an excuse not to go to work, for example, or, in the extreme case, may escape punishment for a crime.

Dr. John Lantos is quoted in the *New York Times Magazine* story on GH<sup>4</sup> as saying that shortness "has become a disease only because a manipulation has become available, and because doctors and insurance companies, in order to rationalize their actions, have had to perceive it as one." The policy argument would reply that some such story can be told for every disease classification. From this point of view, it makes little sense to complain that shortness is "wrongly" (unscientifically?) being called a disease. We must sit back and watch these social processes define diseases, as they usually do, even though it is open to us to join in and affect the process ourselves.

## The Theoretical Argument

The view that disease classification is best understood as a purely social phenomenon, and that it is always "value-laden,"

dominated much of the debate over the concept of health until quite recently. However, the opposite point of view, one that insists diseases are objectively defined through medical science, has been made more plausible by the recent work of a few philosophers, most prominently Christopher Boorse.<sup>5,6</sup> Boorse and the others agree that many disease classifications have a value element, but they insist that these are embellishments (or, in some cases, distortions) of an underlying objective, scientific understanding of natural phenomena. What makes drapetomania so ludicrous is that it obviously is *not* a disease in any objective sense of the term; that it stands out among disease classifications is testimony to the lack of value elements in most of the others. Similarly, Boorse agrees that corresponding to the scientific concept of disease, there is a social role, the sick role, which involves value elements. Boorse wrote:

A disease is an *illness* only if it is serious enough to be incapacitating, and therefore is:

- (i) Undesirable for its bearer;
- (ii) Entitled to special treatment; and
- (iii) A valid excuse for normally criticizable behavior.<sup>5</sup>

Boorse has since withdrawn even these concessions.<sup>7</sup>

Nevertheless, underlying these value or social elements is a scheme of classifications within the science of medicine that can be determined from an understanding of physiological function without regard to social attitudes. The function of the heart is to pump blood; this is a scientific fact, not a social convention. A heart that fails to pump blood well is a diseased heart; this too is fact, not a decision, performance, or value judgment. It may be that in our society an incapacitating disease excuses one from work; but the social fact here is the excuse, not the disease classification. When diseases are properly defined, they are scientific categories.

Thus, Boorse's view suggests that to pretend to decide whether SS is a disease purely on the basis of the social advantages and costs of doing so is quite wrongheaded. Either it is a disease, or it is not; let the chips fall where they may.

## The Policy Importance of the Theoretical Considerations

If we accept Boorse's view of the objective nature of disease classifications, then in which direction are we pointed for policy purposes? It might seem at first that by moving diagnosis from the social to the scientific realm, Boorse has robbed it of any particular policy significance. This view would be congenial to that expressed by several authors in this debate, such as Allen and Fost,<sup>8</sup> ie, the etiology of a child's SS may be of secondary importance in determining the proper use of GH.

However, Boorse's analysis has proven to have considerable policy relevance, at least in the view of Norman Daniels, whose

book, *Just Health Care*,<sup>9</sup> has become the most influential theory of justice in health-care delivery. Daniels was drawn to Boorse's view on its own merits, but it turns out to have some favorable implications for those who, like Daniels, believe that society owes its citizens a basic minimum of health care. Perhaps surprisingly, Boorse's denial that disease is a value-laden concept is what makes his view congenial to these theories of justice. One might suppose that very broad, value-laden definitions of health and disease, such as the World Health Organization's definition equating health with overall well-being, would be favored by those who argue for a government role in ensuring access to care. However, the opposite is the case. If the concept of disease is purely social and value-laden, anything could be properly called a disease if social conventions so decided. But it is absurd to argue that societies owe their citizens everything. A more restricted view of what counts as a disease, such as that provided by Boorse, suggests a package of benefits we can credibly demand that governments deliver. Daniels's argument, which I will not repeat here, begins with Boorse's account, upon which he bases his view of social entitlements.

What conclusion could we draw from Boorse's writing, and that of Daniels, on the use of GH? This is a complicated question, and I will not be able to state a careful answer here. However, the answer most in the spirit of these analyses would seem to be that children who are short because their parents were short and who are otherwise healthy are not diseased: social values aside, there is no pathology. They are no more diseased than would be an Inca of similar stature who happened to be adopted by an American couple. And from this it would seem to follow, in the spirit of Daniels's theory, that no social entitlement exists for GH for short-statured children. However, there may be compelling social policy reasons apart from justice for providing GH to healthy short children.

This result may please some readers. Common sense seems to tell us that short people can be fully healthy, and that the social disadvantages of shortness are no more indicative of disease than were Jewish looks in Nazi Germany or the appearance of African ancestry in the Old South. And we may applaud the view that would deny any moral imperative to providing GH as a health-care entitlement, given the range of truly serious ailments that the same money could remedy. Thus, what may seem to be the commonsense views on both the policy and the theoretical levels are in a nice harmony.

Before ending, however, I would like to point to a possible source of trouble in this account. I do not believe that I am the only reader of Boorse's account of disease classifications who, while quite impressed and nearly convinced, has retained the

suspicion that physiology is simply inadequate to define proper functioning, and that at some level the designation of a condition as a disease or pathology must make reference to social norms or conventions. GHD, in fact, seems to be a particularly revealing case. Allen and Fost<sup>8</sup> challenge their readers to make a meaningful distinction between 2 children destined to measure 5 feet, 3 inches: one whose shortness stems from GHD, the other whose shortness stems from inherited SS. With the first, it is true, we may find a particular organ that is not pumping out GH at expected rates; we may designate this a pathology, and the child as diseased. But why do we not designate the genetic inheritance of the second child as also pathologic? Those genes express themselves through some chemical pathway, perhaps not yet understood, which in a child expected to attain normal height might be regarded as pathological. Indeed, we might speak of a genetic disease if a child with tall parents suffered SS as a result of an independent mutation that produced precisely the genes that yield SS in children born to short parents.

Children of short parents, however, are regarded as "healthy" and their SS as "natural." One looks for an objective explanation for these disease classifications, but lacking that it seems plausible to attribute our classification behavior to social convention or tradition. We notice, and brand as pathological, that which attracts our attention as anomalous (and undesirable). Given our Danielsonian instincts, which direct us to fulfill entitlements when we see real diseases, the person with anomalous distress may receive help while the person with anomalous distress we expect is called unlucky. Is this a fact about medical science or about human psychology and society? This line of questioning may, if successfully pursued, upset the comfortable harmony that seemed to hold between our best policy and theoretical views on SS and the allocation of GH. I suspect that they pose some difficulties for our theories of disease and of a just health care system as well.

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## IS SHORT STATURE A DISEASE AND DOES THAT MATTER?



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### Introduction

The question to be addressed is whether short stature (SS) is a disease and does its classification as such matter. In formulating an answer, we need to ask: Does it matter for what purpose SS is being classified as a disease? I will argue here that it is not necessary to conceptualize a condition as a disease in order to entitle its bearer to treatment, and also that there is no good reason to consider SS a disease. It is obvious that whether SS is a disease is not an issue to be determined by scientific discovery, but rather one to be decided on the basis of cogent reasons. Depending on which criteria are selected for what is to count as a disease, a case could conceivably (but not plausibly) be made for classifying SS as a disease. That classification would entail stretching the boundaries of the concept of disease beyond its usual limits. So it is important first to examine the reasons *why* it might be argued that SS should be considered a disease.

### The Disease Label: Benefit or Burden?

According to one view, it matters a great deal whether a condition is classified as a disease:

What hinges on the decision to refer to a process or state . . . by the word *disease* rather than be some other term? Obviously, a great deal. Medical attention, medical support, medical treatment, and medical research are devoted to the treatment, care, amelioration, and prevention of disease. . . . Some groups have actively proselytized for the acceptance of certain conditions, such as alcoholism or gambling, as diseases. Other groups have worked to remove the label of disease from behavior such as homosexuality, masturbation, and schizophrenia.<sup>1</sup>

Although these observations about disease labels are true, they fail to indicate why those groups have sought to either embrace or shed the label of disease. A disease label can constitute a benefit or a burden. First, it can be a benefit if it results in the sorts of attention and treatment cited in the above quotation. Second, it can be a benefit if it serves to excuse an individual from behavior that would otherwise be blameworthy or

culpable, as in the case of coprolalia exhibited by a person suffering from Gilles de la Tourette's syndrome. The desire to have alcoholism and gambling classified as diseases stems from these 2 reasons.

Yet diseases can also be stigmatizing. Perhaps the most extreme case is leprosy, and somewhat less so, epilepsy. A current example is AIDS. Until quite recently, cancer was a stigmatizing disease and people were ashamed to admit to a diagnosis of cancer. And in some social and cultural groups even today, psychiatric diseases remain highly stigmatizing.

But SS differs from all of these in one key respect. It is the physical condition itself that is the stigmatizing factor, regardless of whether it bears a disease label. A decision that SS deserves to be considered a disease would not contribute an additional stigma, nor could it serve to act as an "excusing" condition in any way similar to the other diseases or disorders just mentioned. Socially undesirable behavior is different from aesthetically undesirable body build because people are blamed for the former but not for the latter.

But is that always true? Consider obesity. In years past (perhaps today as well) it was not uncommon to hear people "excuse" their obesity by saying it stemmed from a thyroid condition. There is surely an etiologic difference between obesity that stems from an underactive thyroid and obesity that results from overeating. Consequently, the former may be a more "excusable" body build than the latter. Obesity itself is not a disease, although it is correlated with all sorts of diseases. Yet children as well as adults may be just as handicapped by being obese as they are by being abnormally short. I will return to this comparison later in addressing the question of whether it matters if SS is a disease.

### Why Consider Short Stature a Disease?

If a condition has to be conceptualized as a disease in order to entitle its bearer to medical treatment, then that would be a reason to argue that SS is a disease. The presupposition is that a "yes" answer to the question whether SS is a disease entitles an individual to medical treatment that could ameliorate the condition and that a "no" answer would carry no such entitlement. I will try to show why this presupposition is wrong.



Specific reasons that might be used to support the claim that SS is a disease fall into different categories. For ease of reference, I use the following arbitrary, shorthand labels for these categories: (1) medical intervention, (2) insurance and other entitlements, (3) social policy, and (4) psychosocial consequences.

*1. Medical intervention.* Growth hormone (GH) therapy is undeniably a medical intervention. Hormones can have side effects, possible toxicity, and other complications. Their administration requires medical expertise and for that reason should remain in the hands of medical professionals. Because medical professionals are qualified and authorized to treat diseases, SS should therefore be considered a disease.

This argument embodies several different presuppositions. First, it assumes that if SS is not a disease, then physicians might not be permitted to offer GH to patients. Conversely, if SS is a disease, then control over decision making about the use of GH should be left in the hands of medical professionals.

The first reply to this argument is that physicians can and should be involved in prescribing and monitoring the use of GH whether or not SS is a disease. GH is a pharmaceutical product that might pose risks to users and may be contraindicated for use in some patients. Therefore, it is reasonable to vest control over its use in the hands of physicians, whose expertise is required for excluding medically unsuitable candidates and whose ethical obligation is to seek the best outcome for each individual patient.

A second reply is that a large number of different conditions treated by physicians are not diseases and there is no call for classifying them as diseases simply because physicians are or must be involved in treatment programs. One example is obesity. Overweight people go to doctors for weight loss programs, for medications that can aid them in dieting, and for surgical procedures such as liposuction. Although obesity places people at elevated risk for a wide variety of diseases, being fat is not a disease. It does, however, carry many of the same social consequences as being short. For another example, plastic and reconstructive surgeons perform many procedures, ranging from cosmetic enhancements to correction of disfiguring facial anomalies. Although some of those conditions may be associated with underlying diseases, they are not themselves diseases. Nevertheless, surgery must be performed by members of the medical profession.

*2. Insurance and other entitlements.* Since 2 other presentations at this conference address the question of insurance reimbursement and whether GH should be an entitlement in the allocation of health-care resources, I can safely dodge those issues here. However, it is worth emphasizing that entitlements to insurance or health-care resources often have little or nothing to do with how a particular condition is classified and whether the classification constitutes a disease.

Not everything that is a disease gives rise to a particular entitlement, and many things that are not diseases do give rise to

entitlements. For example, there is no controversy over whether the numerous malfunctions, impairments, and deteriorations that comprise heart disease are really *diseases*. Yet there is no entitlement to a heart transplant or (in the days when Jarvik and De Vries were very busy) to an artificial heart.

Or, to take some examples on the opposite side, pregnancy is not a disease, despite the fact that it has been "medicalized" and medical complications can arise. Insurance pays for prenatal care, and the call for better allocation of prenatal services to poor women and teenagers does not rely on an assumption that pregnancy is a disease. Additional examples abound in the sphere of mental health. Clinical psychologists use the diagnostic category "adjustment reaction to stress" to obtain insurance reimbursement for clients, but there is typically nothing about those individuals' feelings or behavior that would warrant a diagnosis of psychiatric disease. Finally, ignorance is not a disease yet an entitlement exists to education in societies that provide free, public education to children. So it is clear that there may be entitlements to some social goods whether or not the human need for those goods stems from a condition that can properly be called a disease.

*3. Social policy.* To classify a condition as a disease would appear to place its bearers in a position to benefit from certain social policies, for example, antidiscrimination clauses that prohibit barring individuals from school or employment based on their disease. Alternatively, having a disease might qualify an individual for certain positive benefits, such as sick leave, home care, or other special services.

There are several different replies to this argument. First, an individual need not be placed in a disease category in order to qualify for protection against discrimination, as the civil rights and women's movements clearly demonstrate. Second, there are useful conceptual categories other than disease on which social policies can rest, for example, impairment, disability, and handicap. If SS does, in fact, constitute a handicap or disability to individuals, it is not necessary to classify it as a disease in order for benefits to accrue to such individuals. Third, social policy can authorize paid leave or other employee benefits for circumstances that have nothing to do with disease, disability, or handicap. An example is maternity leave and, in its expanded version, paternity leave following the birth of a child. Whether a social policy serves to prevent discrimination or provide benefits, a condition need not be shoehorned into the category of disease in order to qualify.

*4. Psychosocial consequences.* Children and adults who are abnormally short can suffer unhappy psychologic consequences as a result of their condition. If they are socially unpopular, fail to attain positions of leadership, or otherwise perceive barriers to their advancement that might be attributable to SS, they might become chronically depressed or anxious. Similarly, these barriers close off opportunities that would be open to individuals who are of average or taller than average height. This argument would contend that suffering such social and psychologic consequences should qualify abnormally short individuals as having a disease.

Many things cause people to become depressed or chronically unhappy that clearly are not diseases: unrequited love, financial loss, the death of a loved one, and failure to make the football team, to mention only a few. The fact that a condition or circumstance gives rise to psychologic consequences in the individual who experiences it has nothing whatever to do with whether the condition is a disease.

As for the social consequences of being abnormally short, appropriate concepts are already in use and are more suited to SS than is the concept of disease. Ones already mentioned are impairment, disability, and handicap. In addition, the concept of malady has been proposed<sup>2</sup> as a general term, broader than that of disease, dysfunction, handicap, or disability, and encompassing all of these. It is defined as follows:

"A person has a malady if and only if he or she has a condition, other than a rational belief or desire, such that he or she is suffering or at increased risk of suffering, an evil (death, pain, disability, loss of freedom or opportunity, or loss of pleasure) in the absence of a distinct sustaining cause."<sup>2</sup>

## Does It Matter?

There are numerous examples of statistical deviations from normalcy that are not considered diseases, such as intelligence. In the case of Down syndrome and other genetic conditions, lower than average intelligence has a biologic substrate. If it were possible to intervene in utero to correct this chromosomal anomaly or others like it, it would be irrelevant whether the condition were termed a disease. Whether lower than average intelligence ought to be "fixed" (were it possible to do so) does not depend on whether it is termed a disease, or even whether its etiology can be considered a disease. Even in cases where no genetic or other biologic underpinning can be ascertained, now or in the future, if low intelligence could be improved by medical intervention, what argument could be given to show that it would be ethically wrong to do so? Only if the potential medical risks of the intervention outweighed the expected benefits of achieving normal intelligence could an ethical argument be mounted.

Suppose there was a successful medical treatment for obesity. Would we have to ask, first, whether obesity is a disease before we are prepared to offer it to parents for their children? If not, it is probably because obesity places an individual at risk for so many serious or life-threatening conditions (even if they mostly occur later in life). Like obesity, SS often places people in our culture at a distinct social disadvantage. But unlike obesity, SS is not correlated with other potentially harmful diseases. Obesity fits more comfortably within a medical model than does SS, but that fact alone does not show that it is ethically inappropriate for physicians to offer GH to parents of abnormally short children.

I can think of only one compelling reason why it might matter whether SS is considered a disease. That reason relates to the

professional duty of physicians to mention treatment options and to offer therapy from which patients could benefit. If SS were to be considered a disease, a standard of care would evolve obligating physicians to offer GH to patients (or the parents of patients) who have the "disease."

However, what physicians mention among available options for patients should not be a function of whether the patient's condition is classified as a disease. The risk-benefit ratio is one of the factors that should determine what physicians are obligated to offer their patients. Assessing that ratio, along with the other factors, may not be an easy task. But whether the patient's condition falls into the "disease" category is irrelevant to the physician's obligation.

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## DISCUSSION III: A & B

- A. Theoretical and Policy Debates Over the Status of Short Stature as a Disease - Daniel Wikler, PhD
- B. Is Short Stature a Disease and Does That Matter? - Ruth Macklin, PhD

Moderated by Norman Fost, MD

WIKLER: What is fairness in society? Most of us carry our ideas about this around in our heads, but do not listen very well. That leads to some very strange and arbitrary judgments. For example, we agree that it is perfectly fine for workers to compete for jobs even though there is unemployment. One is smarter than another, but the smarter one does not always get the job. However, if a person is mentally retarded, he or she may be *guaranteed* a job based upon the handicapped status, with certain jobs being reserved for this group of individuals. Our social perspectives set forth that it is unfair for an individual to be denied or to receive certain privileges and status based upon intellect. An underlying societal conflict is that we want to live within a competitive system, based upon some guarantees or provisions for certain intrinsic benefits, yet at the same time, we have less appreciation for some of the effects of this system. So we selectively participate in and perpetuate an arbitrary system in which some physical attributes are perceived as handicapping—and thus warrant protective measures—while other conditions are perceived as less severe and are not supported by the system in the same manner. We label one condition a handicap, which

WIKLER: translates into a protected status and societal management attempts to ameliorate some of the effects of this competitive system.

Both this question and Dr. Macklin's response imply that growth hormone-deficient and GH-responsive children should be treated in the same manner. We may not yet be able to decide what constitutes a handicap, but it would be the same for both children assuming there are no medical consequences due to growth hormone deficiency other than short stature.

FOST: Thus, a person who is growth hormone deficient and destined to achieve a final stature of four feet six inches would be no more or less entitled to treatment than a nongrowth hormone-deficient individual with an equivalent projected final height. Either *both* individuals are entitled to treatment or neither is. Do you agree with this?

MACKLIN: Yes. In fact, I apologize because I did not draw that conclusion explicitly in my paper.

CALLAHAN: We have conventionally defined many things, as physicians do, by way of offering treatment. Society permits and supports this approach. However, if we look ahead to the entitlement debate, our crisis at this point is deciding when and where to draw a line on some of these very casual conventions we have allowed to develop. Can we find better standards than societal conventions for assessing entitlement to treatments physicians may provide? Utilizing a strict risk/benefit standard, we could, for example, have physicians presenting a valid position for addressing and managing the threat of nuclear war with the equivalent decision-making power as the State Department, and so forth. Can physicians provide more substantive criteria? And if our decisions are to be based upon a risk/benefit ratio, there should be a relationship to actual physiological status, as opposed to cultural or societal conventions.

MACKLIN: One reason that physicians should be involved in this decision-making process is that an expertise is required for both the clinical examination of patients and in administration of the treatment. Here I am specifically addressing the *clinical* aspects of services that physicians offer to patients. A distinction needs to be made between whether physicians are *obligated* to offer these services or whether physicians may be *permitted* to offer these services. In resolving the entitlement issue, I think we have to grapple with the question of *obligation*.

CALLAHAN: One of my reasons for supporting a fairly rigid medical standard (namely, some kind of physiological deficiency), is that this affords one the basis to make some consistent policy decisions.

MACKLIN: My argument is that a condition does not have to be defined as a disease in order for a physician's expertise to be relevant to patient management.

CALLAHAN: Yet consider that several of the cases you mentioned have some ultimate biological consequences.

FOST: Pregnancy is covered because of the importance of preventing medical complications, such as hypertension, diabetes or prematurity, when, in fact, one does not need a physician to manage a *normal* pregnancy.

MACKLIN: But you do need physicians, I presume, if you are going to be administering hormones that have physiologic effects that need to be monitored. We do not want the disability expert to be prescribing and treating with growth hormone.

DANIELS: Many technologies that slowly become the province of physicians (eg, cosmetic surgery) involve very sophisticated techniques that only physicians are qualified to perform; yet there is a widespread perception that despite the advantages that meeting some cultural standard of attractiveness might confer on individuals, it is *not* a social responsibility to make sure that this sort of service is an *entitlement*. Most healthcare systems are inclined to deny coverage for cosmetic surgery, whereas *reconstructive* plastic surgery is frequently an entitlement. By what criteria is this distinction made? *This* is an important question to answer. The issue is not solely what falls in the purview of the expertise of physicians. I would suggest that the key moral and policy question is this: which inequalities; that is, which individual differences, create obligations (or entitlements) on others to correct for the inequality? If we get away from what physicians actually *do* and what insurance companies have historically covered, and focus on resolving the underlying question, what gives use to a social obligation to medical services, then maybe we can arrive at some reconstruction of what is appropriate for inclusion as an entitlement in our healthcare system.

WIKLER: I think both Drs. Daniels and Callahan are hoping for an objective, physiologic definition of disease which will elucidate what this healthcare system owes each of us. Suppose that I (who is very tall) had a son who was born very, very short, due to a new genetic mutation. Does my child have a new genetic disease or a physiological defect? Given that short stature is not expected in my family, my guess is that it would be considered a failure of physiological function and therefore, a disease entity. Yet, if I adopt an Incan child, my short son might grow to be taller than the Incan child, yet the Incan child would, in fact, be considered perfectly normal.

CALLAHAN: If you consider it a disability to be short, then demonstrate the cause (eg, the mutant gene). If you determine that the etiology is due to a mutant gene, then insurance companies will provide coverage for the treatment.

WIKLER: Suppose the mutant gene turns out to be identical with the Inca's natural genes. Now what? (laughter)

HINTZ: Look for an Incan father. (laughter)

WIKLER: The Incas are fine. They are all happy and healthy. They are short and they are healthy. My kid has got a mutant gene and he is sick. They have the same gene, however. My claim is that ultimately this is a cultural determination or stipulation.

MENZEL: I want to pursue whether we should be treating the *social* prejudice or the *physiological* state. I agree entirely with your analysis of intelligence as an example. There is a good reason why intelligence is considered in a job situation and why society may want to pursue that track and support people with low intelligence. But what can you say for making judgments on the basis of stature once you are off the basketball court? Very little. Therefore, it is debatable whether medicine should be the proper avenue for addressing the problem.

LUSTIG: What we label a disease evolves over time. Even a decade ago, gambling and alcoholism were not considered diseases, but now they are. Two criteria, define diseases. Firstly, there must be a biological cause. Today, we are considering genes for alcoholism. With gambling, we are examining the change in CNS dopamine receptors. Homosexuality is not far behind with a new focus on the structure of the hypothalamus (reported in *Science* recently). Secondly, whether or not something gets labeled a disease by society is whether there is stigmatization associated with it. Short stature has a biological cause and stigma associated with it; so doesn't that qualify it as a disease?

WIKLER: Actually, I disagree with most of your examples. Suppose we discover that there is a part of the brain that is different in homosexuals—or a physical correlate of the behavioral difference. This does not qualify it as a disease rather than a variation.

LUSTIG: No, absolutely not. But there are a lot of people who are actually starting to think about it in that light. In fact, the homosexual community may be happy to be relieved of the burden of origin. We can also ponder that if society started calling homosexuality a disease, would we then start looking for therapies?

DANIELS: It is really not enough to simply say that there is a physical correlate, competency to treat, or a stigma for a condition to be labeled a disease. Much of cosmetic surgery is done to correct conditions that have physical correlates of impairment and some degree of social stigma, but we do not, by any means, consider them diseases.

BAILY: I heard this morning that there is a definite difference in the benefits of GH treatment for GH-deficient versus idiopathic short children. Now to me, as an economist, that is a very solid reason for distinguishing between these two groups and for determining entitlement to treatment. But how do you handle the issue of short stature as a "handicap?" Prescribing growth hormone for "handicapping" short stature is extremely expensive. If my child had a birthmark, it would not be reasonable to attempt to have everyone "forget" or ignore birthmarks. I would seek laser treatment for the child. The therapy would be relatively inexpensive to achieve the effect. I do not care whether it is a disease or not. The question is, what are the benefits relative to the cost of using an entitlement program?

HINTZ: You (Dr. Baily) make the assumption that the most GH-responsive children should be distinguished by GH secretion testing. I do not think that's true. Groups of children we call classically growth hormone deficient may or may not respond better in the first year. There is a huge overlap, and we cannot accurately, prospectively pick out those children that are going to respond.

FRASIER: However, there are groups of children, that should be treated, such as children with craniopharyngioma—children who do not grow and have no growth hormone response.

GERTNER: The child (patient) is usually not the individual who is being asked what they want and how they see the future. This comes from the parents, which makes it difficult to know how much is really decided by the patient and how much is, as it were, forced on them through social factors and social conformity.

LANTOS: I have just been writing down some of the diseases that people have been bringing up as analogies to short stature — obesity, homosexuality, minor facial abnormalities, and gambling. And it does not seem like such a strong argument to say that because doctors have dealt with these or called them diseases, that short stature should be managed in the same manner. There is another category of diseases (like heart disease, cancer, diabetes) that are unambiguous and so do not require consideration for this policy. It seems like what we are doing is acknowledging that short stature is more like gambling than cancer.

ALLEN: We erroneously use the term "short stature" as a diagnosis that has fairly uniform meaning. I suggest that



ALLEN: entitlement to growth hormone therapy should not be dependent on diagnosis, but rather upon disability. Entitlement to a variety of medical treatments should be based on an individual's inability to take advantage of opportunities within a normal range regardless of the degree of, eg, facial disfigurement or other handicapping feature. What is the degree of disability that results from extreme short stature, and under what circumstances can we consider someone "entitled" to help for this disability, regardless of the etiology? When we talk about the basic healthcare needs that our society is responsible for providing, at some point we have to make this type of decision, (eg, determine where "handicap" begins). Is this a concept that can be applied to short stature?

WIKLER: Americans talk about equal opportunity. How seriously do we mean that? Do we *really* want to equalize opportunity for everyone? Well, that is preposterous because we would have to intervene in every aspect of our private lives! I am the tallest person here. So if that is an advantage, should I be handicapped in some other way so that the shortest person here would begin at an equal level as I? If we really mean equal opportunity, the intervention required would be so enormous that it would require an interventionist state like we have never seen before. A fallback position is this — there are certain features that have natural advantages. Thus, we select characteristics and differences between people and designate certain ones as unfair. Other characteristics will not be considered. Once we have created ways to level some of these inequities, we will have achieved equal opportunity or close to it.

MacGILLIVRAY: Some of the confusion about short stature is related to the terms we use. Dr. Wikler used the phrase normally short, nongrowth hormone deficient children. Children who are growing along the 3rd, 10th, and 25th centiles have a normal growth rate and can be appropriately classified as "normally short." However, another name needs to be applied to pathologically short children with subnormal growth rates. Currently they are described as having idiopathic short stature and this causes confusion because the term short stature is being used for children with innocent short stature as well as for children with pathologic growth. I prefer to refer to the latter group as idiopathic growth failure and consider GH therapy appropriate for some of them because their abnormal growth velocities will prevent them from achieving their genetic endowment. If we continue to use the term normally short child for children with pathologic growth, we are conveying to the public, to the insurance companies, to philosophers and to economists that these children are normally short when they are not.

WEISBARD: Our definitions of disease seem to be driven by the availability of a potentially efficacious medical intervention and the existence of a group of physicians who can and would like to use that intervention, with the hope of

benefitting some "patients." I recently attended a conference considering whether infertility is a disease, and what sorts of medical and societal responses are appropriate. There was widespread consensus that surgical "plumbing" repairs for both men and women should be an entitlement. There was disagreement as to whether IVF and more exotic modes of assisted conception should also be covered. Interestingly, there was near consensus regarding another potential response to biological infertility. Most participants felt that adoption-related services should *not* be covered, even though adoption might represent a less expensive, more certain, and socially quite appropriate, way of responding to the desire for a child when a couple cannot conceive naturally. I disagreed with that conclusion, and would argue here that availability of medical treatment is almost morally irrelevant to our social judgment about what priority should be given to the treatment of short stature, compared to other social needs. I would urge further consideration of Dr. Menzel's suggestion of a trust fund to support a variety of approaches, not necessarily all pharmacological, to the range of psychosocial problems associated with short stature. Of course, this implies rethinking the way we define and utilize what we now call "health" insurance.

DIEKEMA: We should not minimize the importance of classifying problems as diseases, since such a categorization tends to lead to entitlement for insurance benefits. Insurance companies often require a medical diagnosis before they will pay for services. Short stature does not fit nicely into the category of disease, and so we may need to look for other reasons that support entitlement to growth hormone therapy.

MACKLIN: There are some entities that do not fall into the same category because we take them for granted—at least in this society (and always have), in contrast to medical care, that are an entitlement. One of these entities is education. Education is a social good to which everyone is entitled, unlike healthcare, which is still not viewed as a right or social good. It is not necessary to call ignorance a disease in order for there to be a social entitlement to education. This represents a peculiar historical aspect of the United States of America, where it has never been questioned whether education is an entitlement (although this is not the case in many other cultures). Oddly enough, however, we remain one of the few societies who do not consider medical care an entitlement. Should there be free public education for everyone? If you had fewer people educated, you might have more people performing unskilled labor and perhaps a better distribution of jobs in the society that would run more efficiently.

CHARO: I agree with the comment that a lot of this has to do with the simple creation of a new tool. Furthermore, I think this is really a debate not about disease or entitlement, but about the locus of control for this new tool and the implications of its use to circumvent social prejudice. We have all agreed that extreme shortness can be a disability

CHARO: due more to societal prejudice than to functional impairment. Because physicians understand the techniques, they can evaluate the risks and the benefits on a physical level (or the likely effectiveness of growth hormone therapy for this particular person), and, therefore, they may be the most appropriate locus of control. But that may not be the case. The victims of prejudice are in the best position to balance for themselves what risks they are willing to take on in order to circumvent this prejudice. True, victims often make stupid decisions on their own behalf. But physicians do not view themselves simply as the people with tools to be put to use for other people. Most physicians think of themselves as healers or caregivers to the whole person and do not view themselves in this more humble, restricted fashion, whether it is to cure a plumbing problem or to circumvent or overcome a social disability with a medical tool.

FRASIER: In looking at alternative approaches to the problem of short stature, there are other therapeutic agents that may be effective, safe, and less costly (eg, anabolic steroids). These have been used for years in the management of short children with a variety of conditions.

GERTNER: The point I would like to address is related to the question: Does it matter whether short stature is a disease? As pointed out beautifully before, disease is relativistic in time and space. If, in this time and in this space, people think that short stature does matter, regardless of its being a disease or not, is that enough to make it matter?

MACKLIN: Could we just simply go ahead and call it a disease? There are a lot of things that people think matter. Being a philosopher, I can talk in "oughts." The question is, ought it to matter as much to people as it does? And here we have to look at the social barriers, the psychological consequences, and how people have lost opportunities caused by being abnormally short, regardless of whether they have sufficient secretion of growth hormone. I saw a TV program about cosmetic surgery in southern California where extremely attractive teenagers were going in to have

chin clefts and tucks and the like. To these "Valley Girls" and their moms, it matters, but is that to say it ought to matter? No. If we had more evidence regarding social barriers, the stigma leading to actual discrimination, handicap, and the loss of opportunity, then I think that could provide objective evidence that it does matter.

FRASIER: I must object to the stereotyping of southern California teenagers. (laughter)

TESCH: As a person with Turner syndrome who has to deal with extreme short stature and complete infertility, I would like to point out that it is not just the social perception and psychological discrimination that create the desires and hopes for GH treatment. It is also the fact that there are physical opportunities that are completely lost, closing off certain opportunities to these individuals from normal functioning in society. Whether it is driving a car, buying clothes, or finding appropriate marital opportunities, you know that you have these physical handicaps, and lack some capabilities that almost every other person in the world has.

MACKLIN: Well, this goes back to the discussion that we had a moment ago regarding whether or not there ought to be other kinds of entitlements that society should provide. Think of all of the legislation and the entitlements that people with various disabilities have, whether it is the hearing impaired, the visually impaired, or others. There are ways in which social decisions have been made to modify the environment for people who have these various handicaps to enable them to do things more easily. We can probably think of several examples. Perhaps the clothing manufacturers might be one of the first places to look. That is, women of extreme short stature should not have to buy their clothes in the little girls' department. Society might make certain provisions for people with extreme short stature in the same way that we have made adaptations for the hearing impaired or vision impaired.

POST: Thank you very much.

## GROWTH HORMONE THERAPY FOR SHORT STATURE: CAN WE SUPPORT THE TREATMENT/ ENHANCEMENT DISTINCTION?



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### Medical Need and the Scope of Obligations to Treat

Many medical technologies, new and old, can alter people in ways they desire to be changed. When do we have a social obligation to ensure that such preferences are met? Do rights to health care include entitlements to have those preferences met, resources permitting? What should insurance cover?

The most inclusive answer to these questions is that we have such obligations whenever someone desires to eliminate an unwanted physical or mental condition. This would allow subjective preferences to place enormous demands on resources, holding us hostage to the extravagant tastes of others.<sup>1,2</sup> Since we do not believe it is medicine's task to make everyone equally happy, we reject this view and its implication that we should have to pay for liposuction or face lifts. Instead, we think obligations arise only when medical treatments address more important problems.

A less inclusive answer is that we have such obligations whenever people desire to eliminate conditions that put them at some disadvantage. The notion of disadvantage is meant to be objective, including some forms of suffering as well as the competitive disadvantages that result from lack of capabilities, such as marketable talents or skills. When disadvantages are not the result of prior choices or personal fault, the view has some initial grip on us. Our egalitarian inclinations may lead us to think we owe something toward eliminating them.<sup>3,4</sup> But this still assigns medicine too great a role as a social equalizer. It is not medicine's task to make everyone an equal competitor, eliminating wherever possible all inequalities in the distribution of talents and skills or other capabilities.<sup>5</sup>

A more modest answer that tends to match a wide range of our practices, including our insurance practices, is that we have obligations to provide services whenever someone desires that a medical *need* be met. Generally, this is taken to mean that the service involves *treatment of a disease or disability*, when disease and disability are seen as departures from species-typical normal functional organization or functioning.<sup>6,7</sup> Characterizing medical need in this way implies a contrast between uses of medical services that *treat* disease (or disability) conditions and uses that merely *enhance* human

performance or appearance. Enhancement does not meet a medical need even when the service may correct a competitive disadvantage that does not result from prior choices. Accordingly, medicine has the role of making people *normal* competitors, not *equal* competitors.

### Challenges to the Treatment/ Enhancement Distinction

Despite its wide appeal, the distinction between treatment and enhancement seems arbitrary in light of hard cases like these:

Johnny is a short 11-year-old boy with documented growth hormone deficiency resulting from a brain tumor. His parents are of average height. His predicted adult height without GH treatment is approximately 160 cm (5 feet 3 inches).

Billy is a short 11-year-old boy with normal GH secretion according to current testing methods. However, his parents are extremely short, and he has a predicted adult height of 160 cm (5 feet 3 inches).<sup>8</sup>

These cases make the distinction seem arbitrary for several reasons. First, Johnny and Billy will suffer disadvantage equally if they are not treated. There is no reason to think the difference in the underlying causes of their shortness will lead people to treat them in ways that make one happier or more advantaged than the other. Second, although Johnny is short because of dysfunction whereas Billy is short because of his (normal) genotype, both are short through no choice or fault of their own. The shortness is in both cases the result of a biologic, "natural lottery."<sup>9</sup> Thus, both seem to be undeserved disadvantages. Third, Billy's preference for greater height, just like Johnny's, is a preference that most people hold; it is not peculiar, idiosyncratic, or extravagant. Indeed, it is a response to a social prejudice. The prejudice is what we should condemn, not the fact that they both form an "expensive taste" in reaction to it.

Other hard cases not involving GH treatment also challenge the treatment/enhancement distinction. Consider gynecomastia:

Ben has significantly enlarged breasts. The enlargement is the result of a prostate tumor. Bob has significantly enlarged breasts. The enlargement is a side effect of hormone treatment he is receiving for another medical condition. Bert has significantly enlarged breasts. The enlargement is the result of his taking anabolic steroids to promote muscle development. Bruce has significantly enlarged breasts. The enlargement is not the result of any diagnosable disease condition.<sup>10</sup>

Medical insurance usually covers Ben and Bob for the surgery involved in treating their conditions. Bert may have coverage as well, though his "responsibility" for assuming the risks of a prior enhancement therapy raises questions not faced by Ben and Bob. Bruce, however, will generally have to pay for his own breast reduction. Still, all of them will suffer equally if not treated, and their preference for breast reduction is not idiosyncratic but socially conditioned. It is also clear that Bruce, like Ben and Bob, is not in any way responsible for his condition.

These hard cases raise the following questions: Does the concept of disease underlying the treatment/enhancement distinction force us to treat relevantly similar cases in dissimilar ways? Are we violating the old Aristotelian requirement that justice requires treating like cases similarly? Is dissimilar treatment unfair or unjust? This moral question must be addressed separately from another question: even if it is not obligatory to provide GH therapy that merely counts as enhancement, is it permissible to do so? Some argue that GH for short but otherwise normal children violates ethical norms because of the uncertainties concerning risks and benefits.<sup>11</sup> In a sense, this is a prior question: if it is not permissible to enhance the height of short normal children with GH, then it can hardly be obligatory to do so. Still, I shall not address this question here. I focus on the philosophic issues involved in the treatment/enhancement distinction because they are of general importance and are independent of our current uncertainty about the risks and benefits of GH therapy.

## The Treatment/Enhancement Distinction and Equality of Opportunity

Despite the challenge of hard cases, the treatment/enhancement distinction should play a role in deciding when we are obligated to provide medical services. To show that this distinction is not arbitrary from the point of view of justice, despite the hard cases, I shall argue that it fits better than alternatives with what I shall call the *standard model* for thinking about equality of opportunity. Of course, the standard model may be indefensible. That is a much broader question, one I cannot adequately address here. Still, I can show the standard model helps specify a reasonable limit on the central task of health care.

Disease and disability restrict the range of opportunities open to an individual. Health-care services maintain, restore, and compensate for losses of function that result from disease and disability. They thus restore people to the range of capabilities

they *would have had* without disease or disability, given their allotment of talents and skills. Our *standard model* for thinking about equality of opportunity thus depends on taking as a given the fact that talents and skills and other capabilities are not distributed equally among people. Some people are better at some things than others. Accordingly, we assure people *fair* equality of opportunity if we judge them by their capabilities while ignoring "morally irrelevant" traits like sex or race when we place people in schools, jobs, and offices. Often, however, we must correct for cases in which capabilities have been misdeveloped through racist, sexist, or other discriminatory practices. Similarly, by preventing or treating disease and disability, we can correct for impairment of the capabilities people would otherwise have. The standard model does not call for our eliminating all differences in capabilities through medical enhancement.

This limitation of the standard model can appear arbitrary. As I noted earlier, our capabilities are themselves the result of a natural and social lottery, and we do not "deserve" them. We just are fortunate or unfortunate in having them. We can mitigate this underlying arbitrariness *somewhat* as follows. Those who are better endowed with marketable capabilities are likely to enjoy more goods such as income, wealth, and power. If we constrain inequalities in these goods so that those who are worse off do as well as possible, considering all alternatives, then social cooperation will work to the benefit of all.<sup>9</sup> Still, this constraint does not eliminate all inequalities in the capabilities people have and thus in the opportunities individuals enjoy, especially since we enjoined to judge people in light of their capabilities. If our egalitarian concerns require that we strive to give people equal capabilities, wherever technologically feasible, then we should not settle for mitigating the effects of this reliance on equality of opportunity as standardly understood.<sup>12</sup> Rejecting the standard model pushes us toward leveling all differences in capabilities; from that perspective, the distinction between treatment and enhancement has no point.

From the perspective of the standard model of fair equality of opportunity, however, it is reasonable to limit the task of medical services to restoring people to normal functioning and thus the range of opportunities they would have had absent disease or disability. In the standard model, the treatment/enhancement distinction retains its point. For purposes of justice, it is enough that the line between disease or disability and its absence is *uncontroversial and ascertainable through publicly acceptable methods*, such as those of the biomedical sciences, for the general run of cases. Being able to draw a line in this way allows us to refer counterfactually in a relatively clear and objective way to the range of opportunities a person *would have had* in the absence of disease and disability; it facilitates public agreement. My claim that we have obligations to provide health-care services that meet people's medical needs, within resource limitations, thus derives from accepting the standard model for thinking about fair equality of opportunity. Abandoning the treatment/enhancement distinction would push us toward a much more radical form of egalitarianism. I can here neither defend nor criticize such a view, except to point out that dropping the distinction does not



just open the door to GH therapy for short normal children, eliminating one anomaly. It begins a cascade of changes in the scope of medicine that would forever change its face and might threaten the social consensus that gives medicine the strong moral grip it has on us and our resources.

It might be thought that we do not need to adopt such an extreme position if we abandon the notion of disease or disability. If extreme shortness could be considered a "handicapping" condition, then we might still be able to appeal to the standard model of equality of opportunity.<sup>8</sup> GH therapy would simply move people into the range of capabilities they would have had were they not "handicapped." This "compromise" approach does not seek full equality in capabilities, only the end of handicapping disadvantages.

There are serious objections to dropping the reference to disease in drawing this version of the treatment/enhancement distinction. First, we need a clear notion of "handicap." Specifying the shortest 1% of individuals as "handicapped" will itself seem arbitrary after the first cycle of therapies creates a new group of shortest people. Not treating the newest group would then seem arbitrary in light of a new set of hard cases. Second, it will now be medicine's task to eliminate all comparable handicapping conditions. In our racist society, this means black or brown or red skin. Should we eliminate the melanin or oppose discrimination? Although the compromise approach does not seek equality of capabilities, it vastly expands the function of medicine and, by medicalizing social problems, risks losing whatever consensus exists on the moral importance of meeting health-care needs.

Two further points should be made in defense of the treatment/enhancement distinction. First, the problem raised for it by our hard cases is similar to the kind of problem all rules face when their justification derives from the fact that general conformity to them is on the whole either better or fairer. We can almost always describe hard cases in which the very reasons that lead us to adopt a rule to cover the general case also lead us to think the rule is nonoptimal or unfair when applied to them. Though

troubling, hard cases do not always count as counterexamples that force us to reject the rule. Sometimes we must swallow the discrepancy between the particular case and the general run of things if we want a generally better or fairer distributive scheme.

Second, not all of our social obligations to provide treatment for people derive from the central considerations of justice to which I appealed in my account. For example, we have compelling reasons for providing public funding for nontherapeutic abortions, which do not count as meeting a medical need (prevention aside). The social obligations that derive from these reasons may be as compelling as the considerations of justice I claim are central. It is important, however, when considering public policy to keep our lines of argument distinct. We may well develop reasons for thinking certain enhancements are as morally important to provide as some treatments. Still, these reasons will differ from the concerns about justice that provide the central justification for rights to health care.

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## *Session IV:*

# **SOCIOECONOMIC ISSUES RELEVANT TO THE TREATMENT OF SHORT STATURE**

**Editor's comments:** While market forces may eventually reduce costs, the present reality is that growth hormone (GH) therapy is extraordinarily expensive. Questions of entitlement, therefore, depend not only on considerations of efficacy and risk-benefit analysis but also justification of the cost-benefit analysis. What would it cost to expand the population eligible for GH therapy? Should private insurance financing be expected or public financing supported? What health-care resources should be allocated to the treatment of short stature? In this session, these questions are examined from insurance, economic, and philosophic perspectives.

David B. Allen, MD

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## **GROWTH HORMONE THERAPY FOR CHILDREN WHO ARE NOT GROWTH HORMONE DEFICIENT: SHOULD INSURANCE COMPANIES PAY FOR THE TREATMENT?**



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### **Introduction**

Deciding what services ought to be reimbursed by third-party payers in this country is a complicated process. It asks us to consider our social priorities, while calling into play information from medical science and economics. Criteria must be identified to determine to which services individuals should have access; then it must be decided whether a given service satisfies those criteria. Determining whether children of short stature (SS) who are not growth hormone deficient (GHD) should have human growth hormone (GH) therapy reimbursed by insurance companies is a prototypical decision in this process.

Once GH could be produced synthetically and was no longer in short supply, a new group of individuals, comprised predominantly of parents of non-GHD short children, started to inquire about gaining access to the drug. Although certain reasons offered for their requests concerned potential functional limitations (eg, inability to reach the pedals of a car), more

frequently psychosocial justifications were provided, including that the child would have better opportunities, increased social acceptance, and, ultimately, greater self-esteem, if he or she could receive injections of GH.

This paper will discuss whether insurance companies ought to reimburse families for GH treatment of a non-GHD child through analysis of: (1) how companies generally make reimbursement decisions, (2) which issues ought to be considered in the context of GH therapy specifically, and (3) the main arguments for and against insurance reimbursement in this context.

### **How Are Reimbursement Decisions Made?**

Reimbursement decisions are not made by a uniform process: every insurance company itself makes the decision about whether to reimburse a particular service. The Health Insurance Association of America (HIAA), the trade organization of

commercial health insurance companies in this country, does not make specific recommendations concerning what services should be covered. Rather, it researches some subset of procedures and therapies and disperses this information to member companies in the form of "technology assessments." Each company itself then makes the decision regarding coverage. It is assumed that there are many medically appropriate ways to respond to various conditions, and different insurance "products" at different prices are offered to consumers that reflect this assumption.

Generally, if a service is part of established medical practice, it will be reimbursed. If it is not part of established practice — as is the case with GH therapy for non-GHD children — there is no predicting whether insurance companies will cover the therapy. Nor is it known what grounds companies will use to define a therapy as experimental or conventional. In making the decision, the medical director of a company may consult a variety of sources, including the relevant medical literature, the "technology assessments" available from HIAA, studies conducted by other organizations, medical experts in the relevant field, or medical directors of other insurance companies.

In addition to the lack of uniformity of coverage among companies, coverage is not necessarily static *within* companies, either. That is, if a certain treatment or procedure is submitted as a claim and the decision is made by the medical director that it is "experimental" or otherwise not reimbursable, the policy holder may appeal and, in certain cases, the company may decide to reverse its decision.

## Issues to Consider

In considering whether to reimburse GH therapy for non-GHD children, 2 general issues arise. The first is how experimental the therapy is considered to be, that is, what is known about its efficacy. The second is whether SS is considered to be a "disease" comparable to other diseases for whose treatment companies generally provide reimbursement.

Efficacy considers whether synthetic GH will improve either the growth rate or the final expected height of non-GHD short children. Until the efficacy of providing GH therapy in this context is firmly established, most companies are likely to consider the treatment experimental. Data from studies examining changes in expected height following this type of treatment yield mixed results. One study reported a doubling of the growth rates of 6 of the 7 children studied,<sup>1</sup> while another trial found a 50% increase in growth rate in only 2 of the 10 children studied.<sup>2</sup> Similarly, some investigators suggest that certain identifiable groups of children (for example, those who had lower baseline growth rates) were more likely to respond to treatment,<sup>3,4</sup> while other investigators have not found clinical or biochemical predictors indicating which children are most likely to respond.<sup>5</sup> In one study that followed children longer term, predicted adult height of subjects increased by a mean of 2.8 cm after a mean of 3.6 years, but only 13% of children had final

adult heights that were more than 2.5 cm greater than their baseline predicted adult heights.<sup>5</sup> For insurance companies to be expected to reimburse GH treatment for non-GHD children, it is likely that there will need to be greater consensus in the medical community that GH therapy is efficacious.

A second key piece to efficacy in this context, however, is the degree to which the child's emotional well-being improves following treatment. Given that the reason parents typically request this treatment is because they believe their child is experiencing less social acceptance and/or fewer opportunities than his or her taller peers, the appropriate test of efficacy is not simply whether *height* changes following treatment, but whether there is less rejection or enhanced self-esteem as a result of therapy. This, of course, is a saliently different question from whether taller children or adults *generally* have greater opportunities and/or acceptance than their shorter peers. Efficacy defined in this way has not been well studied, although one survey of adults who had been treated with GH when they were children found that the adults still had greater psychosocial difficulties than their peers.<sup>6</sup>

The other consideration is whether this type of treatment is consistent with other types of services that companies do reimburse. That is, if GH therapy were demonstrated to be efficacious and therefore no longer considered experimental, is it the "type" of therapy a company would reimburse? As stated earlier, insurance companies are heterogeneous in how they make reimbursement decisions. Medical necessity and consumer demand are phrases that tend to guide the process, but GH therapy (again, assuming it no longer was considered experimental) is exactly the type of treatment that could be decided in either direction. By analogy, for example, reconstructive surgery typically is paid for if it allows for greater mobility or is the result of an accident but not if it is solely cosmetic. Whether GH therapy would be judged as facilitating job performance or as cosmetic undoubtedly would be resolved differently by different companies. Also, companies are more likely to pay for procedures that have a measurable outcome, and self-esteem is not likely to fit into this category, although there certainly is a precedent for mental health services being covered. Again, isolated cases often have served to influence companies to alter their policies.

## The Arguments for Insurance Companies Reimbursing Human Growth Hormone Therapy for Non-GHD Children

The main argument for an insurance company's reimbursing for GH therapy for non-GHD children is founded in the principle of justice. If, indeed, GH therapy were shown to be as efficacious for the treatment of non-GHD short children as for GHD short children, then the argument can be made for treating like cases similarly. When a child is short to the point that he or she may be psychologically handicapped, and it has been shown that the psychologic handicap will be improved with the treatment, whether the child is GHD should be irrelevant. If the symptoms,

recommended course of treatment, cost of treatment, and outcome are similar for GHD and non-GHD children, then if treatment for one is reimbursed, so should treatment for the other.

A second argument used for reimbursement is that reimbursement means access, and lack of access means no hope of alleviating a psychologically handicapping condition. Numerous studies have demonstrated that tall people generally are more highly prized in this country than are short people. Similarly, there is ample evidence that tall people experience more career and social opportunities than do short people. However, this is quite different from whether children who were very short for several years have more opportunities and/or improved self-esteem after receiving GH therapy. First, they may not experience enough of a gain in height to be treated by others in a noticeably different manner. It should be remembered that the average change in height achieved in some studies was approximately 1 inch. Thus, it is not a comparison of short to tall people that we ought to be measuring but, perhaps, the difference in how a man who is 5 feet 1 inch is perceived compared with a man who is 5 feet 2 inches. Second, both children and their parents may be disappointed when this medical innovation still leaves the children considerably below average in height. Moreover, by the time treatment is initiated, the children may already have developed their identities, such that therapy could do little to alter self-perception. Again, although the argument legitimately is made that reimbursement, for most people, is the equivalent of access, access cannot be considered the equivalent of improvement until more information becomes available.

A final argument for reimbursement is more general and is founded in the principle of respect for autonomy. It says that individuals ought to have the right to decide which therapies they want in accordance with their beliefs about what will be beneficial to their health. Given the regimen through which GH therapy is given, it is unlikely that there will be a huge ground swell of people coming forth for the injections, and those who do surely are strongly committed in their belief in the potential benefit of the treatment.

## **The Arguments Against Insurance Companies Reimbursing for Human Growth Hormone Therapy for Non-GHD Children**

The main argument currently against reimbursement of GH therapy for non-GHD children is that there are far too many unknowns about the treatment. Studies concerning whether therapy affects the *rate* of growth or final height, whether there are harmful side effects to receiving supplemental GH when one is not deficient, and whether psychosocial well-being truly improves have been inconclusive and have not followed children into adulthood.

However, let us assume hypothetically that efficacy *were* established. There remain several arguments against companies

reimbursing this type of treatment. The first is the fear that parents will want to make superchildren out of perfectly normal offspring. The prototypical example is the parent who wants to make a basketball star out of a child whose height falls somewhere in the lower half of the growth curve. The situation may be thought of as analogous to perfectly adequate athletes using anabolic steroids to move their performance into the extraordinary range. This criticism points to the need to be very clear about who would qualify for the treatment, ie, children who, based on identifiable baseline characteristics, are likely to receive benefit from the therapy, not only in terms of increased growth but also in improved self-esteem or other psychosocial measures. Moreover, the therapy should be given only to that fraction of the population whom the medical community consensually determines to be of truly disabling stature. This is very different than having the hormone available for those who simply would like to be taller.

Another reason given for denying reimbursement is that this type of therapy is more "cosmetic" than medical, and insurance companies tend not to cover other elective, cosmetic procedures. Certainly, as stated above, prescribing GH therapy for children who fall within normal ranges but who just want to be taller clearly is cosmetic. Indeed, GH therapy for *all* children is cosmetic in the sense that it changes *appearance* rather than alleviating pain, reducing risk of disease, or increasing life expectancy. What might convince companies to reimburse for children whose height falls below the 1st percentile, however, is a belief that, like plastic surgery following an accident, the treatment places the person in the "normal" range of human appearance rather than taking them from the normal to the extraordinary. Nonetheless, it must be acknowledged that in approving this therapy, some endorsement is being made of values and pressures that say that variations from the normal will be scorned.

A final argument against reimbursement, exactly opposite of the autonomy-based rationale for reimbursement above, is that individuals do *not* have the right to decide to which therapies they are entitled. The purpose of insurance is to restore individuals to health or, in some cases, to prevent disease. Moreover, the process by which services are considered reimbursable is by careful attention to what most medical professionals would consider reasonable treatment for a condition; it is not in response to what a policy holder decides he or she wants.

## **Conclusion**

GH therapy is offered as a potential benefit. The only reason not to simply award it is its potential physical harms — the unknown risks of giving GH for a period of years to a child who is not GHD — and psychologic harms — exacerbation of a negative self-image by injections given to alleviate a perceived "inadequacy" as well as the disappointment that may ensue from realizing that, after years of treatment, relative SS remains. Finally, we must consider economic harms — the average cost



of treatment is \$20,000 a year, and treatments can last anywhere from 1 to 5 years — something that can be justified only if the benefits are proven to be quite significant.

The question, then, is which way to err. Certainly, reimbursement cannot be expected until more extensive studies examining the long-term efficacy of GH therapy have been conducted. Moreover, it is important for us to continue to scrutinize reimbursement decisions in order to see if they reflect our social priorities. However, if efficacy is demonstrated, and if we continue to categorize therapy for GHD children as a reimbursable service, then those non-GHD children who are equivalent in stature to GHD children and who are equally likely to respond to the therapy ought to be entitled to reimbursement by insurance companies for GH therapy.

## DISCUSSION IV: A

A: Growth Hormone Therapy for Children Who Are Not Growth Hormone Deficient: Should Insurance Companies Pay for the Treatment? - Nancy Kass, ScD

Moderated by Norman Fost, MD

WEISBARD: We can cite examples in American health care where disease is neither necessary nor sufficient for insurance coverage. However, where treatment is provided by physicians for a condition that we are prepared to call disease, coverage is generally presumed when the treatment passes beyond whatever we define, however problematically, as "experimental." If treatment does not fit that criterion, coverage is likely to be problematic *unless* there are other political forces operating. We could then examine what those forces might be, and how they might apply in the growth hormone context.

WIKLER: What is the role of competition for insurance companies? When I have asked insurance executives why a service is offered, they say, "We cannot compete if we do not offer it, because the competition makes it available." Otherwise the insurance companies will offer certain services as a marketing tactic to draw in the kinds of insureds they seek. For example, the insurance company will sponsor a sports fitness clinic to provide advice about what kind of running shoe to purchase based upon the logic that the clinic attracts runners and they (athletes) do not get as sick as often. Insurance companies will offer very elaborate fertility and obstetrical packages because this brings in young families. While these insureds will have childbirth expenses, they do not incur the usually greater expenses associated with more long-term care necessary in old age. So I wonder whether GH therapy may be caught up in the same kind of crossfire.

The other external factor is the desire of the employer. Insurance executives have told me, "We don't really determine what to offer. The employers determine it. We

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sell packages to companies where the company determines specifically what items and services they want covered, and we then agree to create a package to meet their specifications. The process is based upon what the employers believe they need to offer their employees to keep them happy, and so on."

KASS: You're certainly correct that multiple forces guide the insurance market. It must be remembered that companies are businesses and, as such, market to consumers whom they think will be dependable in terms of paying premiums but who are not likely to pose an unusually high risk of making large claims in the future.

In terms of negotiating with employees, insurance companies do not have an interest in offering particular types of coverage packages *per se*. Rather, they are willing to create whatever types of packages buyers want and are willing to pay for. Employers and insurers are constantly in the process of renegotiating benefits packages and costs. So, to some degree, insurance packages reflect consumer demand. Realistically, unless individuals are confronted with a situation in which growth hormone therapy is prescribed for a family member, they are unlikely to consider coverage for this treatment a priority when selecting or negotiating an insurance policy.

MACKLIN: It has been suggested that a practical approach to entitlement requires that we determine whether a particular condition or disease falls above or below the line of insurability. What does it mean to fall above or below the line when you have no criteria for where you draw that line? I am searching for a rational way to establish this line. You mentioned that Oregon devised an approach based upon preference rankings. I would argue vigorously that mere preferences do not provide a rational scheme nor do they establish a place to draw the line. A preferential ranking system is arbitrary, whimsical, capricious, and subjective. We do not have to start from scratch with everything, but we must establish baseline criteria to draw the line. One cannot arbitrarily decide that growth hormone therapy falls above or below the line. You must look at GH therapy relative to

MACKLIN: other therapies to rationally decide where the line falls. This decision must have specific criteria upon which it is based.

I recently heard that insurance companies recognize their profits in *life* insurance, and actually incur losses with health insurance. Now if this is the case, one might argue that the system is irrational and requires revamping. And, if we vote to discard the existing system, then we could proceed to argue for coverage of human growth hormone therapy and anything else deemed appropriate for coverage. Perhaps we should consider an approach that will let insurance companies reap profits with life insurance and galvanize the country under a more rational *health* insurance system with a single payor.

BAILY: Blue Cross does not do anything but health insurance, and I do not believe that Blue Cross would like to see themselves disappear. What private insurance would like is the best of both worlds: a system where they insure people who are able to afford premiums for the coverage they choose while the government takes care of everyone else; however, the government must not interfere with the privately-insured sector. This is the Blue Cross perspective.

CHARO: Is the definition of what is "experimental" and what is "therapeutic" a decision made separately among companies or made only by the most powerful and best positioned companies? I have never come across a good definition to distinguish these two terms.

KASS: The distinction between what is "experimental" and what is "therapeutic" seems very arbitrary and subject to consumer demand. If I were taking the role of a patient advocate, I would urge a patient or physician to present all the arguments relating to why a therapy is medically necessary, to look for examples of other companies that provide coverage for the therapy, and to document those insurers' rationale for providing coverage.

FOST: The only definition I have ever been able to come up with for "experimental" is that which insurance companies do not pay for. (laughter)

LANTOS: Why does the pediatric endocrinologist have to certify that a child is eligible for growth hormone therapy? The criteria that we are coming to agree on, (eg, extremely short stature and subnormal growth rate) are not the sort of criteria that you really need 3 years of fellowship training to assess. The necessity that a pediatric endocrinologist has to certify a patient as "growth hormone eligible" before insurance companies will pay for GH therapy seems to be simply a way of rationing growth hormone availability. It also corners the market for endocrinologists.

ALLEN: Most, if not all, of us look forward to the day when the criteria for the administration of growth hormone therapy are *appropriately* restrictive and within an affordable range so as to not unduly stress the healthcare budget. Nevertheless, another tier of restriction or gatekeeping is not necessarily a bad thing. For the last several years, pediatric endocrinologists have had one foot on the GH therapy accelerator and one foot on the brake. We (endocrinologists) keep discovering potentially new indications for GH but have not yet defined a clear end point for treatment. Insurance companies need to know the appropriate criteria for initiating therapy and goals and markers for terminating therapy also need to be designated. Unfortunately however, we have not identified criteria for ending therapy, so growth hormone is a "black box" for insurers. We have alluded to different end points such as height appropriate for genetic potential and within the normal range (or a "nonhandicapping") height. But there is a big difference between these criteria. Currently, the last two years of GH therapy for an adolescent, during which optimal final adult stature is achieved, now costs about \$30,000/year. Until we identify more specific and reasonable end points for treatment, the skepticism of insurance companies is understandable, in my opinion.

# ACCESS TO TREATMENT WITH HUMAN GROWTH HORMONE: ECONOMIC PERSPECTIVES



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## Introduction

Now that synthesis of human growth hormone (GH) has eased constraints on its availability, pressure is growing to expand access beyond the small group of patients currently treated. This paper is about the economic aspects of GH treatment: How much does it cost? How many would be treated? Who would pay for the treatment? And would the treatment be worth the expense?

## What Does Growth Hormone Treatment Cost?

Measuring the cost of treating a health condition is not easy. First, the condition must be specified. This paper considers only children who are unusually short, for whatever reason, and receiving GH to make them taller; it does not consider GH use by the elderly or by athletes. Second, the standard of care must be defined: the type and number of physician visits and diagnostic tests, the dosage of GH, the type and quantity of any other required medical services or supplies, and the duration of treatment. These may vary with the age and size of the child and the reason for short stature (SS). Third, unit costs must be estimated and applied to these quantities in order to get an estimate of the direct medical care cost of treatment per child treated. This is problematic in a financing system in which reimbursement levels vary significantly with source of payment, and there are no good data on real resource costs.

In addition to direct medical care costs, there may be mental health-care costs — counseling for family members to determine whether a child should enter treatment and/or to help them adjust if treatment is unsuccessful. Such costs should be adjusted by any savings in mental health-care costs if treatment prevents emotional problems related to SS.

There also will be nonmedical costs. These are usually ignored but may be substantial. A 4-year course of medical treatment that includes extensive testing, monitoring, and thrice-weekly or even daily injections requires at least expenditures of time, and may disrupt the child's and the family's life in other ways (eg, no summer camp or summer vacation because it would interrupt treatment).

Finally, the costs of treating side effects should be considered. This requires forecasting the number and type of such effects and measuring their medical and nonmedical costs.

These information requirements are formidable. They are difficult to meet even for simple conditions with well-established treatment regimens. In the case of GH, the effects of treatment are only partially understood and the standard of care is evolving.

Table 1 summarizes the numbers quoted in the literature on the cost of treatment.<sup>1-6</sup> Total direct medical care cost is usually estimated at \$20,000, with most of this amount allotted for the GH; there is, however, one low estimate of \$10,000.<sup>4</sup> The duration of treatment mentioned ranges from 6 months—a trial period to see if the child's growth rate responds and treatment cessation if it does not—to 10 years,<sup>5</sup> but the usual duration of treatment proposed is 4 to 5 years. The table assumes 4 years. The number of children who should be treated is particularly controversial, but the table arbitrarily chooses children in the lowest 1% of height distribution.

**Table 1: Direct Medical Care Costs of Human Growth Hormone Treatment**

|   |                       |
|---|-----------------------|
| Annual cost of treatment/child <sup>1</sup> | \$10,000 - \$20,000   |
| Number of years of treatment <sup>2</sup>   | 4 years               |
| Total cost/child treated                    | \$40,000 - \$80,000   |
| Treatment provided to:                      |                       |
| Lowest 1% of height distribution            |                       |
| Number of children <sup>3</sup>             | 37,170                |
| Total annual cost                           | \$1.5 - \$3.0 billion |

1. Estimated range for annual cost of treatment is derived from references 1 to 6.

2. References 1 to 6 indicate that treatment duration can vary from 6 months to as long as 10 years but is usually in the 4 to 5 year range. An average of 4 years is assumed for the table.

3. Based on total number of one-year-old children in 1989. US Bureau of the Census. *Statistical Abstract of the United States 1991* (111th edition). Washington, DC 1991.

Tallying up these numbers gives an annual cost of \$1.5 to \$3.0 billion to treat only the shortest of short children. To the extent that these numbers include only the direct medical care costs, they are underestimates.

## Are the Numbers Large or Small?

The answer depends on the frame of reference. Compared with the \$303.6 billion we spend on defense or the \$25.6 billion and \$23.0 billion we spend on alcoholic beverages and tobacco products, respectively, they seem small.<sup>7</sup> Compared with total federal and state Medicaid spending on children ages 6–20, \$6.2 billion,<sup>8</sup> or the federal allocation to the National Institutes of Health (NIH) for medical research, \$6.8 billion,<sup>9</sup> they seem larger. Looking at them a different way, if the total cost were spread over all the households in the population, it would cost each household \$16 to \$32 per year to treat 1% of children.

## Who Should Pay for Expanded Access?

The main sources of payment for health care are private insurance, Medicare, Medicaid, other public programs, patients and their families, and private charity. Table 2 gives the 1989 percentage distribution of expenditures on physician services and drugs and supplies by source of payment.

These numbers are not very useful, however, since the structure of coverage and source of payment vary with factors such as patient age and whether treatment is part of a catastrophic episode. Also, one cannot assume that the entire 1% of children would receive treatment. More than 15% of children under 15 years of age are uninsured<sup>10</sup> and many more are underinsured; their families would not be able to afford the costs of GH, the

careful diagnosis and monitoring by a pediatric endocrinologist, and other medical fees. It seems unrealistic to assume that donations of services by medical professionals would fill the gap.

Moreover, it is not reasonable to assume that third-party payers will passively accept the responsibility for the new costs. If they do, private insurance premiums will increase and government programs will cost more. Premiums and government program costs are already rising at an alarming rate. Third-party payers are more likely to react to this new situation by taking steps to avoid these costs.

What can they do? The obvious approach—explicitly exclude coverage of GH treatment, for all patients or for all except those who meet narrow medical criteria for GH deficiency—is actually not very likely. Once a treatment becomes part of the standard of care, third-party payers automatically cover it unless they take specific action to exclude it. Third-party payers sometimes do exclude treatment for certain conditions such as pregnancy and childbirth and procedures such as cosmetic surgery and orthodontia, but such exclusions are the exception rather than the rule. Public and private contracts are generally understood to cover “whatever care is medically necessary, and not experimental.” Although second opinions may be required for elective surgery and medical appropriateness reviews are growing more common, individual physicians still play the dominant role in defining what is “medically necessary” and thus third-party coverage.

Of course, there must be limits on coverage or the cost would be prohibitive. Limits do exist, and they can be very restrictive. Rather than exclusions of specific conditions, they usually take the form of exclusions of entire categories of services (medical devices, nursing home care, drugs), restrictions on the amounts of services covered, requirements for cost-sharing, exclusion of care for preexisting conditions, and constraints on who is eligible for coverage.

Table 2: Sources of Payment for Physician Services and Drugs, 1989 (Percent Distribution)

| Type of Expenditure         | Total | Out of Pocket | Third Party Payments |                   | Government |          |          |               |                       |
|-----------------------------|-------|---------------|----------------------|-------------------|------------|----------|----------|---------------|-----------------------|
|                             |       |               | Total                | Private Insurance | Total      | Medicare | Medicaid | Other—Federal | Other—State and Local |
| Physician Services          | 100.0 | 19.0          | 81.0                 | 47.7              | 33.3       | 23.4     | 3.6      | 1.4           | 4.9                   |
| Drugs and Medical Supplies* | 100.0 | 72.4          | 27.6                 | 15.7              | 11.9       | —        | 9.2      | 0.2           | 2.5                   |

Source: Health Care Financing Review 1990;12:24.

\*The total for drugs includes both prescription and over-the-counter (OTC) medications. Third parties pay only for prescription drugs. Assuming that all third-party payments are for prescription drugs, third parties paid for 42.3% of prescription drugs and consumers paid 57.7% out-of-pocket.



These limits often produce results that seem to serve neither fairness nor efficiency. It has been argued that drawing a distinction between children who are "growth hormone deficient" and children who are "genetically short" or short because of medical conditions such as renal failure is unfair, because the distinction is not medically meaningful.<sup>11</sup> Yet the third-party payment system is full of distinctions affecting access that are not medically meaningful. The distinction criticized here actually makes more sense than many others in the system.

If the standard of care plays such an important role in third-party payment, how does a particular treatment become part of it? There is no organized process. The treatment diffuses as a result of individual physician decisions based on the medical literature and personal assessment. As this occurs, there may be attempts to develop a consensus through conferences sponsored by various entities, but these are ad hoc and generally do not focus on issues of cost. New drugs must go through Food and Drug Administration (FDA) review for safety and efficacy (but not for cost-benefit analysis) before they can be sold. However, once a drug is approved for one use, other uses often are developed for it without any formal approval process.

If there is an expansion in the group of patients considered medically suitable for GH treatment, third-party payers will limit coverage temporarily by arguing that the treatment is "experimental." Once this is no longer a defensible argument, the treatment is likely to be covered. If the cost is significant (and it is), third-party payers will probably limit their exposure in ways that are not specific to GH.

In addition to increasing premiums (thus making private insurance even less affordable for the uninsured), private insurers may limit coverage of prescription drugs, drop dependent coverage from employment-related health plans, increase out-of-pocket payments for all care, and make SS a factor in screening applicants for family policies in the non-group policy market. Experience with other expensive-to-treat conditions suggests that small firms may experience prohibitive increases in the cost of their health coverage or outright cancellation if an employee's child receives GH treatment; parents of a child receiving treatment may find it difficult to remain employed or to change jobs.

The public insurance program that will be most affected is Medicaid, the federal-state program for certain categories of the poor, including poor children. In fact, recent federal legislation has greatly expanded the number of poor children who must be covered and has established a schedule for phasing in the new groups over time. Medicaid covers physician visits, laboratory tests, and prescription drugs; and there is a statutory requirement that the program not discriminate on the basis of medical condition. (The much discussed rationing process introduced in the Oregon Medicaid program required a special federal waiver of this rule.) This should mean that most poor children will automatically have access to GH treatment if it becomes part of the standard of care.

On the other hand, Medicaid already consumes an enormous share of state resources and a substantial share of federal resources. Medicaid enrollees already face significant limits on access because of low provider reimbursement rates, quantitative limits on covered care, and other restrictions imposed because of budget constraints. If GH treatment becomes an entitlement for Medicaid recipients and poor children are aggressively treated, the care is unlikely to be funded with new resources but rather with resources squeezed from somewhere else in the program.

To summarize, expansion of the medical indications for GH treatment would lead to increased pressure on a financing system in which rising costs are already a serious concern. It is unlikely that the resources to treat the entire 1% of children would be forthcoming. Moreover, the system's response might well have adverse effects on the availability of care to people with other health-care needs.

## Would Expanding Access to Growth Hormone Be Worth the Cost?

One must first ask, "How large are the benefits?" Somewhat to my surprise, the background material for this conference left me unsure that there are any net benefits, even judged by the standards of a confirmed "heightist." It seems self-evident to me, as an economist and payer of taxes and insurance premiums, that *medical treatments should not become part of the standard of care unless there is solid evidence that they produce net benefits for those treated*. This seems especially obvious for expensive treatments that do not save lives.

Of course, the principle is simpler to state than to apply. How does one measure benefits? What constitutes "solid evidence?" Should the standard of evidence be lower for treatments that may save lives or drastically improve the quality of life? Should promising but unproven treatments be available to patients who want to spend their own money on the chance of benefit? Should parents be allowed to choose unproven treatments for their children?

Even after acknowledging the complexity of these issues, it still seems absurd to consider spending millions of dollars on GH without strong evidence that the treatment has a significant probability of raising adult height by a significant amount—and one inch does not seem to be significant enough. It seems difficult to justify the personal cost to the child, let alone the resource costs to the family and society, for only a very small increase in adult height, and even more difficult to justify it for an acceleration in growth velocity in a child whose adult height will be normal but who is a "late developer."

This point made, suppose evidence accumulates demonstrating that GH has a significant effect on height and minimal side effects. Would it then be worth it to extend its use to 1% of children? The answer should depend on what we sacrifice to allocate resources to this end.

On the one hand, we could cut back on alcohol and tobacco or shave a few percentage points off the defense budget and easily pay for GH treatment. On the other hand, we are failing to address a host of medical and social needs many would consider more pressing than the needs of short children because we say we do not have the money. Unfortunately, health and welfare programs seem to be the "marginal expenditures," not cigarettes, alcohol, or defense.

For example, by spending the money it would cost to expand access to GH on the Medicaid program instead, 1.6 to 3.2 million children could be added to the rolls—highly significant given the fact that 8.6 million children under the age of 15 years are currently uninsured.<sup>12</sup> Or the money could be spent moving severely handicapped children closer to normal functioning through special training, rehabilitative care, and high-tech devices—items currently not well-covered by private or public payers. Moreover, since equality of opportunity is the issue, one should also consider expenditures outside health care that influence social opportunities—expenditures on education, nutrition, housing, family support, protection from child abuse, development of better employment opportunities, and so on.

## How Should We Decide—and Having Decided, How Should We Make It Happen?

The debate over access to GH highlights the fact that our society has no systematic process for assessing whether the value of a particular medical treatment justifies paying for it on a collective basis. Yet, as health care absorbs a larger and larger share of national resources, it becomes more and more important to set priorities wisely.

The difficulties are formidable, since neither the market nor the existing political process is equipped to handle the task. Yet allowing physicians to set priorities, in the guise of defining medical necessity, also is unsatisfactory. The example of GH illustrates this clearly, because the benefits, if any, are social. Doctors have no special expertise in deciding how valuable social benefits are and what priorities should be established among different kinds of social benefits.

### DISCUSSION IV: B

B. Access to Treatment With Human Growth Hormone: Economic Perspectives - Mary Ann Bailey, PhD

Moderated by Norman Fost, MD

**STABLER:** I am impressed that GH deficiency and extreme short stature are somewhat submerged, low-key disorders compared to other childhood disorders. Unfortunately, I have not heard our group actually clarify what it is that is wrong with these children and why we must do something for them. There is no Jerry Lewis Telethon for growth

We have, however, only begun to recognize the importance of this issue, and it will be a long time before it is resolved. In the meantime, physicians will continue to have great influence on defining the standard of care and, by extension, the allocation of resources to health-care needs. They will have to determine how to exercise this power responsibly. I hope they will recognize the economic implications of their decisions and take them into account.

For example, it is a well-established tradition in discussions of expensive new treatments for health professionals to argue "if we pay for treatment X (kidney transplants, coronary bypass surgery, GH for children with measured GH deficiency, etc), then we should also pay for treatment Y (heart transplants, liver transplants, GH treatment for all children below a specific percentile of height distribution). Is it too much to ask that the argument be reversed? Pediatricians should look at all the highly beneficial things we do *not* do for children and ask, "How can we think of paying for treatment Y until we pay for prenatal care, immunizations, prevention of lead poisoning, services to the severely handicapped, etc."

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hormone-deficient children. It is not a condition which generates a great amount of sympathy, and no one I know of has ever died of short stature (although people who have that condition may feel like that might happen in other ways). My question is this: How does the visibility of a disorder affect allocation of resources, and how successfully has short stature been promoted?

**BAILY:** In fact, children with growth hormone deficiency seem to be doing rather well in the system. As a nation, we have not guaranteed basic medical care to about 15% of our uninsured children who have conditions that, while they may drastically affect their quality of life, are relatively

BAILY: inexpensive to treat, but for which these children will not receive treatment.

WEISBARD: A typical methodology is to list 5 or 6 of the least advantageous, most expensive technologies that have been accepted into the system, to proclaim that we are already doing all of these, and then to claim that it would be unfair to deny this new one. In this system, new technologies are presumed innocent unless and until proven guilty. I don't think it's a very rational approach in a time of scarce resources and limited access to more cost-effective and socially compelling health care measures.

Suppose we are considering solely whether growth hormone therapy should be made available for people willing and able to pay out of private resources. Do such private expenditures have an adverse impact on the availability of resources that some would argue should be devoted to higher priority medical needs?

BAILY: If this were an adult treatment, I could support "consumer sovereignty" and allow people to pay directly for the therapy themselves at the full cost. There may be people that feel the expense is justified, similar to cosmetic surgery and certain types of sports medicine treatments. However, with regard to children, we have to determine whether the risk/benefit ratio is acceptable given that children themselves are not to be entrusted with this decision. If GH therapy is a safe and efficacious treatment, then we probably should leave the decision to the parents. And while it is true that rich parents will enjoy access and poor parents will not, there are many other types of considerations that are far more important. I would rather see the insurance system subsidize SAT preparation courses or remedial mathematics training for disadvantaged children than growth hormone therapy to increase stature.

GERTNER: This discussion about allocation of expensive health care resources has not addressed whether the high cost of GH therapy is necessary. U.S. orphan drug laws protect the manufacturers of growth hormone from competition and allow them basically to control supply and demand. Consequently, the price of growth hormone is tremendously high. Does society have the right, or perhaps even an *obligation* to do something about excessive costs for certain therapeutic agents especially where these costs are at least partially due to legislation?

BAILY: That is a good point. We have chosen to finance research and development in the pharmaceutical industry by charging the end user a very high price for the therapeutic agent. R&D is subsidized through the drug patent system and orphan drug laws are simply a refinement of that patent system. Control over the research pursuits is in the hands of the pharmaceutical companies; yet it is sometimes clear that marketing motives are not necessarily compatible with the greatest public welfare. In fact, there are some very good economic and theoretical reasons to argue that the public welfare is not being served once the R&D has been fully

realized, when the end user pays a very high price for the product. There is literature on marginal cost pricing available which suggests that pricing should be based on the production cost, because otherwise we unnecessarily limit the extent to which people are able to access the product.

UNDERWOOD: Your statement that the benefits of growth hormone are unclear is a bit global, and, in my opinion, inaccurate. In the treatment of growth hormone deficiency, the benefits are clear. They are becoming clear in the investigational treatment of patients with Turner syndrome, and also in the investigational treatment of children with chronic renal failure. The benefits are somewhat less clear in idiopathic short stature. I believe that the high cost of growth hormone therapy is a blessing, because it is a clear impediment to the widespread use and abuse of growth hormone. The cost factor is, in effect, extending the length of time we are allotted to answer some of the outstanding questions. Almost every day I thank the good Lord that growth hormone therapy is expensive, because I think the ultimate benefit of this therapy is potentially greater than the problem we are discussing today.

BAILY: I wish we could find a cheaper way to address and resolve this problem.

KASS: Saying that it is a blessing that growth hormone therapy is expensive once again points out the irrationality of our medical and insurance systems. If we think that limited access is appropriate, cost is not the best way to impose the controls.

LANTOS: The sicker you are, the harder it is to get health insurance especially if you are currently uninsured. The way insurance companies avoid paying is through the "pre-existing condition" exemption from insurance. I wonder whether short stature could be considered as a pre-existing condition.

KASS: The definition of a pre-existing condition is still somewhat gray. Generally, if the condition is something for which you have previously sought treatment, it will be regarded as a pre-existing condition.

HINTZ: You (Dr. Baily) said that we, as pediatric endocrinologists, have influence on healthcare decisions, but you feel that this should not be the case. You also suggested that we (physicians) all have good motives, but that the *system* is bad. So tell me who you feel should have the majority of influence in the system and describe a "good" system.

BAILY: I do not really mean to say that pediatricians and pediatric endocrinologists should not have considerable influence on how we allocate resources in medical care. What I do feel is that physicians currently have a disproportionately large influence. If we take the normal short stature group, where we are emphasizing psychosocial

**BAILY:** benefits and equal opportunity, these are not areas that physicians have any special expertise in evaluating. Therefore, it seems that the process should recognize and consider the input of others in addition to the medical profession. The medical profession, however, is the only group that can tell us what the efficacy is in physical terms. There should be societal preferences and values incorporated into whether insurers reimburse this therapy or not.

**HINTZ:** How do you propose we incorporate these opinions into a national system, and is this rational?

**BAILY:** We cannot have a perfect national system. All we can do is try to improve the present one. One avenue for improvement would be to institute a universal health insurance plan. The British, Canadian, and Western European systems do a better job of resource allocation. None of these systems could be adopted or incorporated into the United States' system because it is unique; however, I do think that there is room for improvement.

**MacGILLIVRAY:** Many pediatric endocrinologists are using GH treatment in children who are failing to grow and falling further behind each year. To say that GH therapy is experimental is really incorrect. This is a drug that has been used for 30 years. In spite of tremendous experience with GH, however, we continue discovering new things about it and we are continually reassessing and refining our knowledge and understanding of this powerful hormone.

**FOST:** "Experimental" refers to the newer, investigational applications of GH therapy. But the point about the feelings

is that bad feelings associated with short stature have to be weighed against the feelings associated with being premature, the feeling of being pregnant, the feeling of having a lump in your breast and not being able to go to a physician—in short, these feelings have to be weighed against all the other possible uses for available healthcare dollars.

**BAILY:** I remember vividly being in an organ transplantation conference and having presented the position which I genuinely believe, specifically, that there are other things that are more important than organ transplantation. A woman stood up and she said, "My daughter is alive because of a transplant and you want her to be dead." I thank God every day that I am not a physician practicing in our present healthcare system, particularly dealing with disadvantaged children, because it would tear at my heartstrings to be unable to treat a child. I do not want to trivialize the difficulties of a child who is miserable because of short stature. But you (endocrinologists) also do not have to see children suffering from some other, more serious disorders. If you recognized both and had to choose only one condition to treat, which would you choose? Recently, I cried watching a documentary about a family whose mother was a crack addict. Concerned neighbors had called the police because her children had nothing to eat. I cried as I watched those children being led away by the policemen. Our society should not let that happen. I have shared this because I feel it is important that you understand that I do not lack emotional contact with the problems of short children; it is simply that I think there are many other problems that we fail to address which are at least as important as the implications of short stature.

## ENTITLEMENT TO GROWTH HORMONE



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### Introduction

Curiously enough, for all the discussion of resource allocation in health care, there is one issue that has not received the kind of extended analysis it deserves. I am thinking here of the impact of constant medical progress on efforts to devise a just mode of allocation. Consider one simple, popular model of fairness—that of the pie which we try to cut in even pieces so that everyone gets a fair share. That is not a bad model for most

purposes. But what do you do when the pie itself keeps getting larger and changing shape from year to year, and when different parts of the pie turn out to have different nutrient value? We have no model for dividing that kind of pie. Yet it is precisely the one we have to deal with in contemporary medicine, which constantly generates new therapies—some good, some poor, and most of mixed value.

Growth hormone (GH) therapy is a pertinent example of the problem. Well before we have devised a political consensus on



the fairest way to allocate even immunization and other older, long-standing forms of preventive medicine to children, we are being asked to decide on a place for GH therapy. Here is a new, costly item that can help some children and that may be wanted by many more (or at least wanted by their parents), but the provision of which as an entitlement would be directly competitive with other needs of children. Should GH therapy immediately be granted equal standing or, to revert to my original metaphor, should the entitlement pie be reshaped to include this new item?

## When To Treat With Growth Hormone and Why

For my part, I have little trouble resisting the temptation—for the most part. The phrase “for the most part” is meant to allow an exception for medically classic GH deficiency (GHD). GH therapy should be provided as part of entitlement programs if, after appropriate tests, deficiency is established. What about borderline cases? There is a rule of thumb that could be used in that eventuality: when in doubt, do not treat. What about those cases when there is no GHD but the child is simply very short or has a growth trajectory that suggests he will be very short as an adult? No. Just say no.

Now, this is easy for me to say because I am persuaded by the argument put forward by Drs. Lantos, Siegler, and Cutler in a 1989 article appearing in the *Journal of the American Medical Association*.<sup>1</sup> They contend that GH therapy for non-GHD patients runs up against some serious objections: The risks and benefits of the treatment are unknown; there is no perfectly reliable way to determine which children might benefit; and the treatment itself is relatively burdensome. By contrast, the use of GH therapy for those with diagnosable GHD is efficacious and the burden of the treatment is outweighed by the benefits. To that judgment I would add two further considerations.

First, since “short” is a relative term, and since some percentage of children will always be the shortest children no matter how much GH therapy children as a group receive, it is an inherently losing venture to use this medical therapy to cope with the social disadvantages of being “too short.” Ironically, it could work only for some short children under the unfair condition that other short children not gain the same advantage and that those already tall be denied the chance to become even taller. My short child can be helped to become taller in comparison with his peers only by keeping them from becoming taller also; otherwise he is back where he started, at the bottom of the ladder.

Second, to expand the entitlement pie at a time when we hardly know how to divide the present pie, and at a time when many children do not get pieces of even that pie, would seem foolish. Unless we assume an increased budget to compensate for the increased demand on the system created by a new technology—an unsafe assumption these days—the net result of a new technology is likely to be a thinning of services for each child. The same amount of money must now be spread over more services.

## Reasons To Deny An Entitlement To Growth Hormone For Non-GHD Short Stature

There are, then, some good reasons to deny an entitlement to GH for nonclinical cases. Yet I suspect it will be hard to hold that line, and I want to examine what kind of response might be prepared for some likely eventualities. We should by now be familiar with two types of arguments that typically emerge to overcome initial obstacles to expanded entitlements. The first argument is that since the affluent will surely be able to gain the therapy even in the absence of diagnosable GHD, it would be unfair to deny a like benefit to the poor. The second argument is that even if in this case medicine is being used to treat what is at bottom a social problem—the deleterious impact of excessive shortness and the resulting social bias and stigmatization—it is done all the time and it would be unfair to draw a line here. Let me briefly examine both arguments.

*(1) The poor should be entitled to what the rich can buy.* Health care has long been seen as one of those basic human goods to which all should have access. Liberals and conservatives alike will usually agree that money alone should not be grounds to deny needed care; they differ only on the best way of bringing about, and paying for, that outcome. One outcome of this agreement might be called “escalatory egalitarianism”: if the rich can buy a medical good, it should in fairness also eventually become available to the poor as an entitlement. While this argument is likely to be far more vigorously deployed by liberals rather than conservatives, even the latter are uneasy about seeing obvious medical benefits denied to the poor and expect the market sooner or later to erase the discrepancy.

Whether in liberal or conservative garb, this argument should be resisted with GH therapy. The only pertinent standards for deciding whether the poor should be entitled to a medical service should be (a) actual individual benefit, and (b) the seriousness of the need to be met. Since there is great uncertainty about the long-term benefit of GH therapy for nonclinical cases, the fact that some rich people might want to buy it should be seen as irrelevant to the justice of the matter.

Most of us would prefer to be rich rather than poor because we could then, on whatever grounds we choose, buy all kinds of marginally useful or even totally useless but desirable goods. That is no reason, however, for those managing entitlement programs to believe they must do likewise. A higher standard is necessary and reasonable, not one fixed on the usually unlimited appetites, medical and otherwise, of the wealthy. Moreover, simply because some people with disposable income are able to define medical need with greater exquisiteness than others is also no reason for entitlement programs to follow them. The princess who suffered from a pea under her mattress is not the person to entrust with setting general standards of acceptable comfort. In the absence, then, of decisive evidence that shortness is a fundamental handicap to getting on with life—even if it might be harmful in gaining the presidency—the behavior of the rich should be irrelevant to the design of an entitlement program.

(2) *If medical treatment can alleviate the harm of social stigmatization, it should do so.* One of the great puzzles of our health-care system is how it should respond to those problems whose origins are social rather than directly biologic. On the one hand, it is thought deplorable that the health-care system is left to pick up the pieces of, and somehow put together again, lives and bodies ruined by diseases concomitant with poverty and social deterioration.

Why should it be medicine's job to counteract the stigma of excessive shortness? Is that not in the end much more a social than biologic failure? Yet in a less-than-ideal world, medicine may in fact be the only feasible remedy for some of those evils. It would, therefore, be wrong to wait for Utopia before applying an available medical treatment. Medicine has always picked up the pieces of broken societies. Why should it stop here? The latter argument usually wins the day. That is why, when faced with the potential stigmatization of their short children, some parents will turn to medicine rather than place their hopes in the social transformation of biases about size. They are not dumb in that decision, only realistic. But must medicine go along with them? No, not at all.

Medicine should have its own standards about what falls into its domain. It is right and proper, for example, that medicine should treat those gunshot wounds that result from the illegal drug trade. Even though the wounds do not have their origin in the ordinary biologic failings of the body, there is no other way to repair the injured bodies; and the injuries are undeniably real. But the treatment of shortness as a medical condition is an entirely different matter. The injuries imposed by that condition are, unlike gunshot wounds, of an uncertain kind. They will, for instance, be as dependent upon the response of the short people themselves to their condition as to the social attitudes toward them. Some short people seem to hate their condition while others respond with humor and flexibility, choosing for instance to be philosophers rather than corporate CEOs or the nation's President. If it is the case, moreover, that there will always of necessity be some portion of the population that falls into the lower percentile of height, then there is no ultimate solution to the problem other than a change in social attitudes about height. Nothing can be done biologically to eliminate those in the

lowest percentile of height, a logical—not a biologic—limit. By contrast, we know that there are societies, many indeed, that do not have a major gunshot wound problem. There is a problem that can be all but eliminated from a society.

What my comments in general imply should be evident. I do not see a serious moral issue in a two-tier health-care system in general or a two-tier system in allocating GH therapy in particular. Indeed, as medical progress yields one new remedy after another for biologically real or socially constructed maladies, there is all the more reason to have a way to distinguish between that which is imperative for good health and that which falls into a more optional category.

I hope that responsible physicians would not cater to anxious parents worried about the height of their child, but some probably will. It will thus be important to keep before our eyes not only the limitations of GH therapy for nonclinical cases, but also the hazards of medically capitulating to social pressures. As matters now stand, being too short falls in an ambiguous category of social ills—perhaps a real problem, perhaps not.

I believe we should just leave it there. This benign neglect means refusing it the culturally sacred legitimization of medicine as the remedy of choice. If anxieties about shortness become routinely medicalized, we can be sure that the pressures to make GH therapy an entitlement will be irresistible; such is the typical history of these matters. While I am short enough myself to understand some of the problems of that condition, so far as I can make out, it is possible to have a decent society even if some of its members suffer the burden of being too short; it is not a fatal or necessarily crippling condition, either physically or socially. To look to medicine to correct the entire human condition, down to feelings of inadequacy about shortness, is to take it even further down a road it may already have stretched too far. There must be some more serious problems out there to tackle.

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# ARE HEALTHY CHILDREN OF VERY SHORT STATURE ENTITLED TO GROWTH HORMONE TREATMENT?



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## Introduction

Entitlements originate either in prior agreements or in undeserved inequalities in opportunity for human welfare. Health care in particular is an example of how entitlements derive from one or the other of these grounds. Whether based in agreements or inequalities of opportunity, however, health-care entitlements have their limits. Some of our prospective care is of such great expense per unit of predicted benefit that we refuse to pay the necessary premiums or taxes to fund it.<sup>1</sup> Other care is similarly of too little benefit or too great expense to contribute as much to redressing inequalities in opportunity for human well-being as alternative investments of equivalent resources would.<sup>2</sup> The underlying logic of entitlements, then, also contains the reasons for detecting their limits.

## The Case Against Entitlement

I will argue that on neither a foundation of prior agreement nor the ground of unequal opportunity are healthy children of extreme short stature (SS) entitled to growth hormone (GH). Moreover, this holds even for children in the lowest 1% of height distribution. My argument is a moral one, and I will construct it without taking up the debate over the therapeutic merits or scientific facts about GH. I make at the outset a reasonably optimistic set of assumptions about those merits and facts: (1) Use of GH for 4 or 5 years, starting at the proper time, does not risk any harm to the child. (2) Seventy-five percent of the time such use will increase the height of a prospectively 62-inch male by 3 to 4 inches. (3) GH costs \$15,000 per year to administer. (4) Of the 75% of extreme SS patients for whom GH is thus physiologically successful, half would not have experienced serious psychosocial adjustment problems because of their SS, and half would have. (5) Of the half who would have experienced adjustment problems, half in turn will find those problems little diminished by their additional 3- to 4-inches of height, while half will be significantly helped, that is, 25% of the 75% who gain height from GH will experience significant psychosocial gain from the treatments. (6) The economic conclusion drawn from these data is that a 3- to 4-inch gain in height costs \$100,000, but that a major psychosocial gain costs \$400,000. (7) The new members of the subsequent bottom percentile in height will not suffer additional psychosocial problems because others have surpassed them.

Furthermore, I will grant completely, without question, the following conceptual and moral claims: (8) Medical needs are defined by "not only biologic norms but also by personal and cultural values."<sup>3</sup> (9) The lowest percentile of SS children can be considered to have a "handicapping height."<sup>4</sup> (10) It would be inconsistent to treat a non-GH-deficient (GHD) child any differently from a GHD child if both are predicted to grow to the same adult height without GH treatment, and both the same with it. The cause of SS should not matter.<sup>5</sup>

## Prior Agreement

One source of entitlements is the prior consent of insureds to contribute enough to a common fund to cover whatever set of services can then be financed. In turn, the proper conceptual framework for setting the limit of the resulting entitlements is not to ask what care people think is warranted despite its cost once they are insured and in need of the care. Once insured, people will of course demand all the care that stands any discernible chance of benefitting them virtually regardless of its cost — others, after all, are picking up all but a relatively tiny part of the tab. Since insured patients have thus lost most of their capacity to estimate opportunity costs (the value of alternative uses of resources), we should address the problem of keeping the use of care within costworthy limits at the *prior point* in the decision process — insuring — when the trouble of medicine's expansion without economic limits fundamentally begins. When subscribers to an insurance pool decide against paying the extra premium to provide coverage for a particular category of care, controlling costs by rationing that care is based ultimately in the will of the very people later denied coverage.

In many cases of congenital illnesses, however, this point gives us little if any ground for limiting the patient's care because of its expense. There is no prior point in time at which a severely ill newborn, for example, can be said to have consented to financial limits on its care. As an individual with even any imagined capacity to consent, the child has never been in a position in which we can in good faith conceive it to be likely to gain by withholding from it high expense-per-benefit care in order to reserve more to devote to it in other respects.<sup>6</sup> In the GH case, however, I propose that we can largely set this problem aside. The child does have a future, even usually a good one, without GH treatments. Furthermore, parents are

faithful representatives of the child's best interest in the decision over whether to insure for GH; the nasty potential conflicts of interest between child and parent are not present, at least not in remotely the same stark degree that they are in many neonatal life-support cases.

I will assume, then, that we can frame the question about agreement-based entitlement to GH treatment in the following way: Would loving, loyal parents choose to invest the extra family resources required by a policy that would cover GH for their healthy children in the lowest percentile for height? That is, given by assumptions stated earlier, would they agree that the significant amelioration of a very short child's psychosocial adjustment problems was worth \$400,000? Put it another way: in concert with 532 other couples parenting 1 child each, would they be willing to pay an additional \$150 per year for 5 years to significantly help (not just treat) *one* short child? They would thereby gain a 1:533 chance of significantly benefiting their own child.

The first thing we must ask ourselves in trying to answer such a question, of course, is what alternative investments we could make with the money. Those could be either investments in care for other sorts of potential health problems or investments in other things for the very same children who end up in the bottom percentile. For example, what about a \$75,000 trust fund (near-term value, which would be much higher by the time the child became an adult) for each of the 533 bottom percentile children in the pool? This kind of comparison brings us up in our seats on the GH entitlement issue. I suggest that it is indeed quite clear that almost all of us, as loving and faithful parents, would definitely prefer alternative investments, not GH treatments.

There is no argument for entitlement to GH treatment, then, in either the actual or presumed consent of subscriber parents. In fact, we have in this prior consent foundation of entitlements a convincing argument for precisely the opposite conclusion: *for a typical group of parents and their extreme SS children, GH treatment clearly and definitely ought not to be covered by insurance*. Note, of course, that it does not necessarily follow from this that GH treatment should never be prescribed. If particular parents wanted to purchase it out of pocket or if they had paid for an insurance policy at higher rates that made clear at the outset that the extra payments covered their children for the sort of ultimately psychosocial assistance occasionally accomplished in GH treatments, then their extremely short children might well receive GH treatments.

### *Unequal Opportunities for Welfare*

What does this other basis of entitlements tell us about GH? Should a "height-handicapped" child (bottom percentile) be helped to gain additional stature simply because that is what we owe a person who faces noticeably greater problems in life through no fault of his or her own? We owe roughly equal opportunities for future welfare to each and every individual child, and we would be reducing mildly handicapped children to impersonal items in an aggregate sea of socially efficient

benefits if we denied them coverage for GH treatment because its expense made alternative investments more attractive.

But there are 2 nasty questions here for any such argument. First, does extreme SS diminish opportunity for welfare enough to rank its GH treatment remotely close to the next-biggest rectification of less-than-equal opportunity achievable for children in our society? If we are going to use equalization of opportunity arguments, we need to use them consistently. That will get us quickly back to comparisons between what we can do for children of SS with GH and what we can do for other disadvantaged children not yet given a fair shake in our society. Put that way, I fail to see how any hard-thinking group of parents and citizens would ever decide to fund GH treatment for healthy SS in the current United States.

Second, even for height-handicapped children themselves, we must ask how we can best improve likely opportunities for well-being. There is simply no magic here in height or its remedies simply because height is one of the biologic bases from which we then develop personalities and go out and encounter the world. Health care often does get its importance from increasing or preserving a person's whole range of opportunities. But it is by no means unique in that respect (witness education and even certain financial investments), and major parts of health care do not represent future potential and opportunity any more than a variety of other things we desire. The added 3 to 4 inches likely brought about by GH treatments may look like a significant enhancement of some sorts of opportunity for a person whose opportunities are not otherwise hampered much at all. But if someone already faces other significant barriers, or if a person does not value as highly as some others might the sorts of opportunities that taller stature may provide, how can we justify spending *large* amounts of money on children to marginally raise their height?

The concept of a "handicapping height," too, does little work here in isolation from discriminating judgments about how best finally to use our resources to enhance a child's opportunities. Perhaps we have gotten bewitched by a label. Handicaps come in a huge variety of types and degrees, and a handicap *per se* hardly gives one an entitlement. How much of a handicap is extreme SS compared with other SS (62 inches for a male, say, compared with 65 inches)? Is it the *height* here that is really making the significant psychosocial difference? Even if it is, how might we frankly teach and help people to overcome this handicap without them actually growing taller? Handicaps *per se* do not create entitlements, nor do medical means of diminishing them.

Once this is noticed, we will not shrink from consistently following through our denial of entitlement to GH treatments for very short healthy children to also deny that GHD children are necessarily entitled to GH. In some cases, they probably are, for example, Turner syndrome patients and hypopituitary children, who carry larger disadvantages and will make especially significant gains from GH treatment. In any case, it is not the cause of short stature by itself that makes a difference



morally. If very short, non-GHD children are not entitled to GH treatments, then many GHD children with an equal height prognosis are also not entitled to GH treatments.

## Conclusions

Neither the prior agreement of faithful parents nor less than equal opportunities for overall welfare create an entitlement to GH treatments for healthy children of short stature. At current prices and likely benefits of GH, physicians who prescribe GH and insurance companies who pay for it are positively doing the wrong thing. Prescription of GH extracts resources from subscriber parents without their proper prior consent. It also represents a narrow focus on those dimensions of equal

opportunity connected only with stature and unquestioningly assumes that "handicaps" and the medical means for overcoming them automatically gain privileged position in the competition for scarce resources. This focus and this assumption are both to the detriment of the larger fight to equalize opportunities.

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## DISCUSSION IV: C & D

C. Entitlement to Growth Hormone - Daniel Callahan, PhD

D. Are Healthy Children of Very Short Stature Entitled to Growth Hormone Treatment? - Paul Menzel, PhD

Moderated by Norman Fost, MD

FOST: Suppose there were 2 people whose heights plotted within the first percentile and who, apart from stigma, were both unable to reach the gas pedal in an automobile. Would that change our perception of the problem or would it change our response?

CALLAHAN: Our response, I believe. This is an auto design problem. There is nothing wrong with the individuals.

LIPPE: It seems to me that we have a cultural bias against short people who have received a medical diagnosis. If our job is to make sure that children grow normally, we should be able to treat them.

CHARO: Specific types of functional impairments (eg, being unable to drive or to operate in a standard house, being unable to see over certain things, or to be ineligible for certain jobs) are not simply a matter of social stigma. These problems clearly fall within the definition of "disability," regardless of etiology or origin.

MENZEL: Let consumers indicate their preferences for what they want covered. I am convinced that if we take this approach to clarify entitlements, we will find very, very large numbers of American parents saying, "leave growth hormone treatment off the coverage listing if it costs \$30,000 to \$40,000 a year for a major psychosocial benefit."

WEISBARD: We need to distinguish more clearly among three concepts that are not fully synonymous. The first is "entitlement," the second, "allocation," and the third is "access." We need not adopt the same policy along each dimension. Another issue we have not talked very much

about is the potential use of GH therapy for enhancement, not for those at the very bottom of the height continuum, but for those of relatively normal stature who want (or whose parents want them) to be taller. I suspect few would advocate government subsidy or mandated insurance coverage, but what stance, if any, should society and the medical profession take regarding the use of growth hormone for height enhancement if it is paid for privately? Should this be permitted, or are there reasons to resist this use of GH "therapy?"

MENZEL: If purchasers of growth hormone therapy and physicians think it will work based on plausible medical grounds, then those parents ought to be allowed to purchase it if (— big "if" —) physicians themselves do not let the parent's purchasing ability of that care influence their prescribing habits for patients who, although insured, do not have coverage for GH therapy. If the insureds do not pay explicitly higher premiums to obtain this coverage, it is likely that a cut from the standard types of coverage has been made to offset the expense. As for entitlement: as long as physicians can work within a multi-tiered system and maintain these distinctions, then I do not worry about allowing a parent to purchase this therapy for their child. However, if physicians cannot keep this distinction straight, then we may end up, regrettably in my view, denying this therapy to the wealthy parent who was presumably not hurting anyone.

CALLAHAN: I am concerned about the phenomenon of escalating egalitarianism. Things start out as fulfilling a desire. Once they are established, they become needs, which then proceeds to perpetuate the perception that everyone deserves them. That is the logic of technological progress in our society. It might be very nice if there could be some agreement specifying certain standard deviations above which one ought not to treat.

MACKLIN: I want to understand whether "pathology" plays a role toward enabling us to make moral distinctions or whether pathology has the virtue of mere consistency. Let us go back to Johnny and Billy and assume they are both

MACKLIN: 4 feet 6 inches in height, thus we are not talking about "enhancement" of height. A biological abnormality (failure to produce a sufficient quantity of human growth hormone) enables you to consistently apply this criterion to all others. What would be wrong with using the criterion of predicted adult height? While it lacks the consistency of the biological criterion, it does set a limit and it has the virtue of justice. It seems to be morally superior and perhaps more compassionate to offer therapy that would help both of these children to achieve a height greater than 4 feet 6 inches even though this approach lacks consistency as a criterion to be used without exception.

CALLAHAN: I would prefer to establish moral standards for GH treatment and pose the fundamental question: What is the purpose of medicine and what should a healthcare system try to provide? My answer is that it should correct identifiable biological abnormalities that prevent people from having ordinary species functioning. A healthcare system should not try to satisfy certain human desires such as height preferences.

MACKLIN: Does that include psychiatric illness as well?

CALLAHAN: Yes. A person's mental status affects their quality of life as well as their physical well-being. I would like to be consistent, but if someone presents a convincing argument that certain short children without identifiable biological defects experience psychological distress, and GH therapy improves that condition, I would agree and approve of the therapy; however, the burden of proof remains with that person to prove their position.

ALLEN: Dr. Callahan, upon close scrutiny, many accepted medical interventions designed to correct or detect "identifiable biological defects" do not warrant the allocation of resources.

CALLAHAN: Appendectomy.

ALLEN: What about all the "elective" appendectomies? I am sympathetic to the resource allocation problem in this country, but it is unfair to imply that focusing on the restriction of growth hormone therapy will be a large step toward resolving that problem. The greater problem is the general disenfranchisement of children in our society and by our healthcare system. Funds required to provide adequate healthcare to children are not being made available. If the funds that are now going into terminal care in the last three months of life were redirected to child healthcare, the growth hormone issue would be minor. With regard to the issue of establishing prior agreements (which I do not find very convincing), insurance decisions revolve around the unpredictability of health. There is no way of predicting illnesses to which an individual will succumb. The fact that people may decide that the likelihood of their children having growth hormone deficiency is remote, and thus choose not to access coverage does not necessarily justify excluding coverage completely. If each of us were to analyze our

individual policies, we would probably gamble on excluding many of the conditions covered in order to reduce premiums.

MENZEL: I disagree. I think people are foresighted enough to perceive the risks that such an approach would expose them to. I think that this is precisely what insurance is based upon. Your assessment is correct, however, that these decisions should not be made by an uninformed subscriber.

LUSTIG: I think that is untenable, and I will give you an example. What is the deductible on your car for collision?

MENZEL: One thousand dollars.

LUSTIG: The chance that you are going to get a dent in your car is a lot larger than the chance that your child is going to need growth hormone therapy, but you are willing to risk a one thousand dollar deductible in the face of what is likely to happen out on the roads in Puget Sound. It is not that different.

MENZEL: Maybe so, but rational and informed subscribers will not choose to insure for everything, because the cost would be prohibitive. Informed subscribers seek coverage for the conditions/events that have a reasonable cost/benefit ratio. Facts can change my opinion; however, I think it is unlikely that even the most loyal and loving parents of a child with a predicted height (untreated) of 5 feet will determine that an expenditure of approximately \$400,000 for a major psychosocial benefit (and the subsequent improvement of final height to 5 feet 5 inches) is appropriate.

CALLAHAN: I agree with Dr. Allen that our priorities regarding the healthcare of children are misplaced; but if you seek to give priority to healthcare for children, you also have to decide which conditions warrant prioritization, with consideration to treatment with very expensive drug regimens versus increased provisions for preventive healthcare and conventional therapeutic options. How does the cost of GH therapy compare with effective prenatal care, assuming funds are available?

ALLEN: In many cases, GH therapy compares unfavorably; however we cannot base these decisions on such a single comparison.

CALLAHAN: Absolutely.

CHARO: I do not agree that healthcare is simply using medical technologies to address medical problems. Medical technologies are tools—tools developed in response to specific medical conditions—which often have applications beyond the specific condition, in that the technology can also relieve the emotional pain and suffering. Why should there be resistance to the transferring of medical technologies outside the medical context?

CALLAHAN: The first problem is to decide what will fall into the domain of the available allocation. One possible criterion would be a biological abnormality or insufficiency

CALLAHAN: that can be managed effectively. If this is the narrow view, then yours is the broad view. If available medical technology can change a situation, is the presence of a biological imperfection really key? I see a problem with bringing medicine in, time and time again, to correct for inadequate social systems. We have implemented medical technology to provide family planning for teenaged pregnancy, which is social pathology, not a medical problem. We compromise our allocation system by allowing so much into the system that even narrowly defined biological disorders do not compete very well. Secondly, we may divert attention from the deeper issues underlying teenage pregnancy. These are some consequences of having a broad standard.

ROOT: I define medicine as "a profession whose charge it is to maintain and restore health and function of the individual to the greatest extent possible." Medicine focuses on the individual. We take care of our patients 1 patient at a time. It is difficult for physicians to consider the broad population when we are interacting with an individual patient and their family.

MENZEL: I empathize considerably with your view of medicine as focusing on the individual, but focusing on the individual does not settle whether having a functional impairment justifies insurance reimbursement of any and every treatment to improve the situation of the person with the impairment. For the boy who is 5 feet tall, whom we hope with treatment to raise to 5 feet 5 inches, the benefit for that individual associated with spending \$75,000 may not be sufficient to claim that we are faithful to that individual by prescribing care. I do not see how physicians are really serving the interests of the individual or society by facilitating access to all potential treatments that an insurance company will pay for. Insurance companies ought to be serving the interests of their subscribers, including functionally impaired people, who participate in the determinations of resource allocation.

WIKLER: Here today, the medical scientists are talking about rescuing children who are functionally impaired. But tomorrow, the issue will shift. Because we have a medical means (GH therapy) to achieve a socially desirable goal (to have short children reach a normal height and presumably become achievers as well as social and professional successes), we must have the insurance companies reimburse for this therapy, because insurance companies pay for everything that is medical. Based upon such assumptions, we may have a major social policy problem on the horizon.

JOHANSON: I disagree with your assumption that we can make normal children tall. Since, from clinical studies, GH deficient children who are on average -3.5 SD in height, grow 10-11 cm/year with GH treatment, and non-GH deficient children, who are -2.5 SD in height, grow about 7-8 cm/year with treatment, it is quite logical to assume that normal statured children, ie, 0 SD height, will grow 5-6

cm/year with GH administration. That is a normal growth rate and will not add significantly, if at all, to the already normal height and growth rate. You are not going to change the height of an average size child with the doses of growth hormone that we are using, and much higher doses will dramatically increase the risk of side effects.

ALLEN: None of us are interested in using growth hormone to enhance height beyond the normal range during childhood or adolescence.

HINTZ: But, in 1994, when there are 6 growth hormone companies in the U.S. market—and it costs 10 cents a shot, just like it does for bovine growth hormone . . .

JOHANSON: Somebody will do it, but as I suggested, with out very high dosing, which imposes risk, I would expect no benefit.

DIEKEMA: First, endocrinologists choose a specific height percentile cut off because, in their minds, that is the most objective way of determining which children are functionally impaired. The goal of treating children whose heights plot in the lower percentiles is to bring them closer to the 50th percentile—or average—and to correct their functional impairment, not to enhance their stature in comparison to others. Second, the stigma associated with receiving growth hormone injections must be addressed as a potential risk. It is usually parents that are deciding for their child that he or she (the child) is too small, and it is the parents who decide that their child should undergo therapy, a therapy which involves injections. In a child's mind, the perception may be that she is being "treated" because she is, in some way, deficient. This may eliminate any gains in self esteem that might result from an extra inch or two.

TESCH: Whether GH therapy costs \$100,000 or \$400,000, there is a potential future benefit that is impossible to quantify at this point. No one has data on it, but it is there. We will not know for another 15 to 20 years to what extent these benefits offset some of these costs.

BAILY: In our discussion about functional impairment, I get suspicious when I hear there is a "difference" between men and women. As far as I am concerned, the only thing women do that men do not is have babies, and height does not really influence this ability. I do not think a male of 5 feet 5 inches is functionally impaired, because I am 5 feet 4.5 inches tall. I am also frustrated by the lack of answers from the medical sector to these questions: How tall is tall enough? How tall is the tallest child you want to treat? What medical indications are there to justify treatment beyond simple short stature? I also want to know how tall these children will be, or should be, when they reach their final adult stature.

JOHANSON: How short a daughter would you be happy with?

BAILY: But that's not the —

JOHANSON: Oh, it is, it is!

BAILY: We have already decided that because a parent wants a taller child this does not justify therapy.

JOHANSON: How large of an insurance premium would you pay to make your daughter, who is destined to be 4 feet 6 inches tall, a little bit taller?

STABLER: We keep talking about height and stature, but when you look at the research on how much height con-

tributes to what we call self-image and self-esteem, it is a very small percentage. However, an extraordinarily large number of these short kids have developmental impairments relating to academic and behavioral functioning.

LUSTIG: I would suggest that treating only the physical symptomatology of a child with these types of developmental impairments is not going to solve the problem.

STABLER: We do not know.

### *Session V:*

## **SUMMING UP: A DEBATE REGARDING POLICY PROPOSALS FOR ACCESS TO GROWTH HORMONE**

**Editor's comments:** It is clear that more complete data, obtained through prospective controlled trials, are needed to address issues of GH toxicity and efficacy in the treatment of non-GHD children. However, while current expectations for GH may be exaggerated, the prospect that GH will prove only to have toxicity without efficacy seems unlikely. For several reasons, discussions of the ethical use of GH should proceed while such studies are in progress.

First, determining what *can* be done with GH is not the same as determining what *should* be done. Successful clinical trials with GH will create demand, and objective thinking about *responsible* GH allocation inevitably will be compromised when pressures of parental demands, profit, and other self-interests arise. Second, GH use should be directed toward goals deemed important enough to justify the necessary allocation of resources. There is currently no consensus about precisely what these goals are. Third, due in part to heterogeneity in responsiveness among diagnostically related groups (eg, Turner syndrome and chronic renal failure), clinical trials alone are unlikely to provide complete answers to the questions of who is entitled to GH and for how long.

In this session, two divergent views of entitlement to GH are discussed. This conference was not a consensus conference, and these presentations are not policy statements. They are, rather, preliminary explorations of medical, ethical, and social issues that need to be addressed to deal rationally and cost-effectively with the likely prospect of expanded GH use.

David B. Allen, MD



# WHY GROWTH HORMONE SHOULD NOT BE USED FOR NON-GROWTH HORMONE DEFICIENT CHILDREN



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## Introduction and Assumptions

Much of the discussion about the indications for growth hormone (GH) has focused on criteria for the initiation of therapy. While this is a difficult problem, it may be more difficult to determine the endpoint of therapy. Indications for the initiation of therapy may be arbitrary, but at least they can be consistently applied: we can say, for example, that everybody with a predicted adult height below the 1st percentile ought to be treated. Once treatment is begun, however, variations in response to GH therapy and different therapeutic goals will make decisions about when to stop treatment even more contentious than decisions to initiate treatment. I will argue that certain features of GH therapy will make it impossible to limit the use of GH to the shortest children. Instead, we are moving towards a world in which GH will be allocated based primarily on parental preferences. Furthermore, I will argue that pediatricians should resist this trend.

For the purposes of this discussion, I will make 4 assumptions. First, I will assume that there are no long-term side effects of GH therapy. If any major side effect of GH is discovered, it will make treatment of even GH-deficient (GHD) children (and certainly non-GHD children) morally questionable. At present, the practical question is whether, or at what point, we are willing to say that GH is safe. I will assume that evidence of GH safety will continue to accumulate.

My second assumption is that GH will increase final adult height of many non-GHD children. Again, if the data show that this is not the case, there will be no real argument in favor of treatment (except, perhaps, the more limited argument that certain children with growth delay would benefit psychologically by reaching their predicted adult height faster, but I will ignore that issue). The moral argument will center on the use of treatment that is effective.

I will assume that money is not a factor. The money issue too easily cuts both ways. If GH is good for children, it should be provided regardless of cost. If it is not good for children, it should not be provided even if it is free. An intermediate position would be to view GH as a consumer good, rather than a medical treatment, and its allocation based on ability to pay. To

do this is essentially to assume that there is no compelling argument for the use of GH in short children and so no serious concerns about injustice. Furthermore, questions of economics and justice must fix a price for GH in order to compare its value with other goods. But the price is both relative and variable. Therefore, it is justifiable, for the sake of argument, to set it very low, so that it drops out of the moral equation. By ignoring economics, I will better focus on the primary question of whether GH is good or bad for children. Only after that question is answered can we discuss its relative worth.

Finally, I will assume that GH therapy will require daily injections for years. In the final part of the paper, I will discuss the implications of any change that would allow oral or transdermal administration of GH.

## Short Children Are Healthy

Arguments about whether short stature (SS) is or is not a disease generally focus on the difference between social and biologic conceptions of disease. Such arguments turn on whether something must have a biologic substrate or explanation before it can be classified as a disease. Generally, arguments about the relative contributions of biology and sociology to the classification of an entity as a disease ignore or abjure the idea that there is a thing called health. I believe that health is a nonarbitrary quality that may be present or absent in all living things. Although I cannot define health in a way that is precise and inclusive, I believe, along with Kass, that health is not relative but that it is "a state or condition unrelated to, and prior to, both illness and physicians."<sup>1</sup> Health is not social or cultural, and is not defined in relation to others. It is a property of biologic entities.

In spite of the fact that people who are short may suffer as a result of their stature, just as amputees may suffer as a result of their disability, they are generally healthy. Most of the bad outcomes associated with SS, such as poor self-esteem, poor school performance, lower earning potential, etc, may lead to poor health but are not themselves inconsistent with health. Healthy people may not do well in school or may be poor, but this does not indicate that they are diseased. We generally do

not give otherwise healthy children shots to improve their school performance or improve their earning potential; we give them better teachers and a better education.

The lack of association between stature and health has important implications for the role of pediatricians in dealing with SS. Because stature is not associated with health, there is no height below which we can call someone intrinsically unhealthy, and no height above which we can define someone as being healthy enough. Whatever goods come from height are relative. Generally speaking, the more height one gains, the more such goods will come. Thus, anybody who would want treatment to achieve some gain in height would likely want as much such treatment as possible.

## Treatment of Short Stature: The End Point Dilemma

Suppose we see 2 sisters in a clinic — one has a predicted adult height of 152 cm (5 feet 0 inches; just below the 5th percentile) and a growth rate of <4 cm/yr. The other has a predicted adult height of 155 cm (5 feet 2 inches). We treat the first but not the second. At the end of a year of therapy, the first has responded with a growth spurt and now has a predicted adult height of 156 cm. The second still has a predicted height of 155 cm. Do we continue to treat the first, in order to make her 165 cm? Do we stop treatment, since her predicted adult height is now in the normal range? If we continue to treat the first child, do we offer treatment to the second child, since her predicted height is now less than the child whom we are treating? Suppose GH works even better, and after 3 years, our treated child now has a predicted height of 168 cm. Do we continue treatment, or do we say that it has been too successful and so is no longer justifiable?

Such decisions will be manageable if GH hardly works at all, so that the first child moves only from a predicted adult height of 152 cm to 155 cm. If it works well, so that we can titrate doses to allow almost anyone to reach almost any height, we will create unavoidable inconsistency in our treatment. We will inevitably care for children who are too tall to meet eligibility criteria for the initiation of GH treatment but whose predicted adult height is shorter than that of children who are being treated and who are responding. If these children are not candidates for GH therapy, their SS will be relatively more significant, and any psychosocial sequelae of SS will be worsened. If they are treated, it will create a continuously sliding scale of eligibility that will eventually include children of any height.

This dilemma leads to an allocation paradox governing GH therapy for non-GHD children.

Let  $p$  = the final adult height that we consider to define disability, and thus to justify treatment.

Let  $n$  = the number of inches above one's predicted adult height that GH will allow one to grow.

Then, either  $p + n = p$ , the new height cutoff of children who will have to be considered candidates for GH therapy if GH is to be used consistently (and we would have to revise the formula to be  $p + n = p$ , etc. . . .). Or, we will be treating people with a higher predicted adult height than people whom we refuse to treat.

If we follow this allocation rule, we will eventually be treating anybody who wants treatment, or else arbitrarily stopping treatment once a minimally acceptable height has been reached. It seems unlikely that anyone who was willing to undergo GH therapy in order to be taller would want to stop GH therapy before he or she attained the maximal height that could be safely achieved.

This allocation paradox shows that, to the extent that GH works and confers benefits on children who are treated, the benefits cannot be nonarbitrarily limited to children below a certain height. The response to GH inevitably creates a sliding scale of eligibility by which children of any height will soon be candidates for treatment. Allocation of GH will then reflect either very individualized assessments of the psychosocial consequences of SS, or very imperfect associations between particular heights and particular problems. Since there are very little data on such associations, and no predictive data, parental assessments of the psychosocial sequelae of SS for their child should be considered as reliable (or as unreliable) as physicians' assessments.

The question, then, is whether pediatricians should prescribe GH for any child whose parents want the child to have GH. The stakes for pediatricians in this debate are high. We ask society to recognize us as having the moral authority to speak about what is in the best interests of children. As our part of the bargain, we agree to be so careful and conservative in our assessments of the interests of children that our views and our opinions will be allowed to override the decisions that parents make for their children.

We are granted such power primarily because we have earned a reputation as the guardians of and spokespersons for the well-being of children. The moral regard in which we are held, and society's willingness to respect our views, is conditional. We cannot say whatever we want. We need to base our views on knowledgeable statements about the health of children. I don't think that we can now make knowledgeable and unambiguous statements that the treatment of non-GHD children with GH is in the best interest of any particular child. Furthermore, I think we can say that widespread use of GH will be detrimental to the interests of children as a whole.

The benefits of GH are necessarily relative. Whether SS is conceived of as disease, disability, or normal variant, GH can alleviate the sequelae of SS only by changing the relative height of some children in relation to others. This highlights the difference between SS and ill health. Stature is relative in a way that health is not, and interventions that preserve or protect health are generally beneficial in a way that GH is not. If all children are immunized against polio or pertussis, they are all

better off. If everybody is screened for and treated for anemia or lead poisoning, then everybody will be better off. The general health of the population will improve. But if everybody was treated with GH and if they all responded, then nobody would be better off. The shortest people would still be relatively short. In fact, everybody would be worse off, since in order to maintain their relative state of well-being, everyone would require a daily injection. If only some people are treated or only some people respond, they will be better off in relation to others, who will be relatively worse off.

Seen in this way, pediatricians who administer GH will be either increasing the net burden of medical treatment for children without any compensatory benefit or selectively conferring benefits on some children by creating detriments for others. GH therapy could be unique among pediatric therapies in that it can confer benefits to some children only at the expense of other children.

## Conclusions

What, then, is to be done? This is the point in a talk when it is customary to say that we need further research. However, I'm not sure further research, *per se*, would help. It depends upon what type of research. Most current research, which tries to answer the narrow question of whether GH actually increases

final adult height is irrelevant. I have argued against GH therapy for non-GHD children assuming research results that would be most favorable to children — that it is safe, effective, and affordable. Even under those circumstances, I argue that it should not be used for non-GHD children. Any data indicating that it is ineffective and/or has side effects, and certainly any consideration of social justice, would only strengthen these arguments.

Two lines of research might change my conclusions. One would be the discovery of a method of administering GH by mouth. This would minimize the burden of therapy. If, as I've assumed and as research shows, GH remains safe and effective, an orally administered version should probably be sold over the counter, like vitamins. Parents could then decide for themselves whether they wanted to alter their children's height. Another line of research that might change my conclusions would be research delineating a clear-cut association between SS and psychiatric conditions, and a convincing demonstration that GH not only alleviates those problems but also alleviates them more effectively than alternative psychiatric interventions, such as counseling. Such research is not currently being done.

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## GROWTH HORMONE THERAPY FOR THE DISABILITY OF SHORT STATURE

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## Introduction and Conceptual Guidelines

Limited availability of human growth hormone (GH) once provided a barrier to expanding its use beyond children who were unequivocally GH deficient (GHD). By necessity, strict arbitrary criteria were established to identify classic GHD children entitled to GH. Today, increased availability of recombinant DNA-derived GH has allowed investigation of its growth-promoting effect in short children who do not fit traditional definitions of GHD. Increased supply has created increased demand; more than twice as many children received GH therapy in 1989 and 1990 than in 1985 and 1986 at an average annual cost per child of \$10,000.

Advantages conferred by increased height in social, economic, professional, and political realms of Western society are well-

documented. Stigmatization and discrimination are shared by *all* extremely short children, whether GHD or not. *If* GH is shown to have growth-promoting effects in non-GHD children and *if* treatment of such children can be accomplished without toxicity, then what ethical criteria should determine entitlement to long-term, invasive, and (currently) expensive therapy? Would it be justified to restrict access to GH based on the diagnosis of GHD? And whatever the indication for GH therapy, to what attained height should GH therapy be considered an entitlement?

Answering these questions requires rethinking of the medical indications for GH therapy. Toward the goal of achieving both controlled but fair access to GH, the following conceptual guidelines are proposed: (1) GH be viewed as a treatment for the disability of short stature (SS) and not for the diagnosis of GHD;

(2) GH-responsiveness, not GHD, be the central criterion for GH treatment; and (3) entitlement to (and reimbursement for) GH therapy be guided by the degree of disability and the degree of GH-responsiveness rather than by a child's diagnosis.<sup>1</sup>

## The Continuum of Growth Hormone Secretion: Disease, Potential, and Handicap

The once clear boundary between GHD and GH sufficiency has become blurred. Traditional criteria for the diagnosis of GHD do not identify all children who are GH-responsive. A continuum of "inadequate" GH secretion likely spans classic and partially GHD children,<sup>2</sup> children with delayed growth and puberty, and other poorly growing short children who pass provocative tests but still secrete less GH than their peers.<sup>3</sup> Furthermore, GH augmentation therapy in short children with no detectable abnormalities of GH secretion increases growth velocity and, if given for sufficient time prior to puberty, may increase eventual adult height.<sup>4,5</sup>

Arguments emphasizing proven GHD as the primary criterion for GH therapy are often rooted in notions of disease, handicap, or potential. The treatment of disease, "an abnormal condition of an organism that impairs normal physiologic functioning" (*American Heritage Dictionary*, 1985), is one function of medicine. One might argue that GH therapy be confined to those with the "disease" of GHD. Restoration of hormonal equilibrium by supplementing deficient or suppressing excessive levels of hormones is a justifiable, time-honored principle in endocrinology. The GHD child is viewed as more entitled to therapy because something has been taken away that needs to be restored. The American Academy of Pediatrics statement recommending GH therapy only for GHD children concludes with the old adage, "If it ain't broke, don't fix it."<sup>6</sup> But what exactly is "broke" when it comes to SS and GH therapy? This view ignores both the likely, though yet unrecognized, physiologic "defects" that lead to genetic SS and its accompanying psychosocial impairment. Both GHD and non-GHD short children, if they have a disease at all, have the disease of SS.

If the legitimate function of medicine includes the alleviation of handicap, "a disadvantage or deficiency, especially a physical or mental disability that prevents or restricts normal achievement," then the short child's well-being is viewed in the context of his or her interaction with the environment. GH therapy is justified by recognition that extreme SS interferes with normal activities such as driving a car and reaching shelves, as well as competition for jobs, schools, incomes, and mates. After all, preventing handicapping SS is the primary impetus for treating GHD children. Other beneficial physiologic effects occur with GH therapy, but these are of secondary importance. Growth rate and final adult height are the measures by which we judge therapeutic success. Whether burdens associated with SS of a given degree qualify for designation as a handicap is not the central question. The point is that short children of equal height have the same handicap regardless of the cause.

The concept of potential is also invoked to distinguish treatment of GHD and non-GHD children. For some, a GHD child with parents of normal height is "meant," by virtue of genetic endowment, to be taller than the child with familial SS. He or she is entitled to treatment with GH until a height appropriate for the genetic endowment is attained. GH supplementation of the familial short child who appears to be GH-sufficient is "tampering with nature" and outside the proper province of medicine. But this analysis fails, since both children (given an equal height prognosis) are equally unlucky, one by virtue of having GHD and the other by virtue of having short parents. For both, attaining maximum adult height requires "tampering with nature" by providing exogenous GH.

## Equitable Restriction of Growth Hormone Therapy

While concepts of disease, handicap, and potential do not distinguish GHD from GH-responsive children with regard to entitlement to GH therapy, it does not follow that *all* GH-responsive short children are entitled to therapy. Resolving that question requires consideration of balancing benefits and risks and asking further questions about allocation of health-care resources.

Response to GH is not an "all or none" phenomenon. GHD children are likely to be *more* responsive than non-GHD children, justifying their preferential treatment as a class. Possible GH toxicity in non-GHD children, while apparently rare, still requires further study. Risks of psychosocial stigmatization also require careful consideration; short, otherwise normal children exposed to injections to promote growth may conclude (with some accuracy) that their bodies are unacceptable in the eyes of their parents and physicians.<sup>7</sup> Statistically significant increments in final adult height may not actually improve psychosocial adaptation, failing a primary objective of GH therapy. Finally, unrestricted access to GH would shift the bell-shaped curve of height upward without changing the handicap for those at the lower percentiles in competing for social, professional, and athletic status.

Assuming that clinical trials of GH in non-GHD children show efficacy with acceptable risk, how might access to GH therapy be equitably restricted? First, the goals in treating SS must be clarified. If the goal is to achieve each child's maximum height potential, GH therapy would (ethically) need to be offered to any potentially responsive short child. Providing GH therapy only to those with documented GHD and treating them until maximal adult stature is reached would be unfair to equally short, non-GHD children who could grow with GH supplementation. On the other hand, if the goal is to alleviate the disability of extreme SS (from any cause), GH-responsive short children should have equal access to treatment until they reach a height no longer considered a handicap.

This latter goal, bringing short children into the normal opportunity range for height, coincides with society's duty to



provide basic needs to its citizens. There is no duty to provide the *very best* opportunity for all, and an insistence on equal access to GH by those who have already achieved a normal final height compromises this goal. To improve opportunities for those truly disabled by height, GH must be selectively available to them. The challenge is to define this group, and to apply criteria of disability consistently in deciding when to commence and when to *discontinue therapy*. The diagnosis of GHD should not be rewarded with unlimited access to GH while access is denied to equally handicapped non-GHD but potentially GH-responsive children.

## Toward Responsible Use of Growth Hormone

Any definition of "handicapping height" would be arbitrary, but the difficulty in defining boundaries precisely should not be an obstacle to making distinctions. Decisions about treatment are always based on probability, not certainty. While current methods for height prediction remain suboptimal, *some* determination of a height considered a handicap needs to be made if GH allocation in the future is to be both controlled and fair.

Emphasizing degree of disability and GH-responsiveness as selection criteria for therapy equitably fulfills reasonable goals of growth-promoting therapy. (See Figure 1.) Children disadvantaged by stature, regardless of pathogenesis, would be brought closer to or within the normal opportunity range for height. The attainment of maximum height potential would not

be a valid treatment goal, and the use of GH to make normal-statured children taller would be opposed. The normal range of height would not be altered, but rather the disparity between percentiles—for example, between the 0.1th and 1st percentiles—would be lessened. By restricting GH therapy to those seeking only to achieve the normal opportunity range for height, we would not exploit the perception that taller is better.

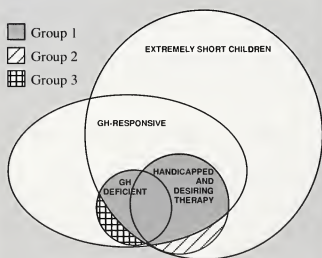
Widespread distribution of GH has been deterred in part by high drug prices<sup>8</sup> and concern about toxicity. Assuming efficacy of GH in increasing final adult height, the relevant question is not how much should be spent on GHD versus non-GHD children but rather how should health-care resources be responsibly and fairly expended on the treatment of SS in general.<sup>9</sup> Resources for this endeavor may in fact be limited, but treatment of severely SS individuals can still be approached with *consistency*. If our goal is to help (all) children attain a height closer to the normal opportunity range, the cause of the SS really should not matter. The central question about allocation of GH is this: To what maximum height should any GH-treated child be entitled to receive private or public support?

Moreover, the crisis in GH allocation will expand not with its failures but with its successes, and not as the cost of therapy rises but as it falls. These impediments, which may be resolved soon, have distracted attention from the issue of responsible use of GH. What we can do with GH therapy is not necessarily what *we should* do. We who prescribe GH should now ask how we would respond if families who do not require insurance reimbursement strongly request GH therapy. Without guidelines for restriction based arbitrarily on likely final adult height, access to treatment would increasingly reflect ability to pay, providing yet another societal advantage to those already well-off. Rather, a consistent goal of growth-promoting therapy should be to lessen the burden for those who are so short as to be handicapped; that is, to provide GH therapy to those disabled by height only until a height within the normal opportunity range is attained. Consideration of degree of disability, rather than diagnosis, both when commencing and when discontinuing GH therapy, will most responsibly contain an expanding cohort of candidates for GH treatment.

The physician's duty to respond to the needs of each child does not necessarily extend to parental aspirations or hopes for the child. In an era of plentiful GH, child advocacy requires consideration of the needs of all children, bringing as many as possible into the normal opportunity range of height without deliberately trying to make some taller than others.<sup>10</sup> The paradox of GH therapy is that no policy regarding its use will ever eliminate the 1st percentile. GH cannot replace parental love and nurturing of a child, regardless of the child's height. Prudent use of GH will recognize these limitations, encouraging physicians to respond to concerns about SS more often with counseling than with injections.

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Figure 1: Allocation of Growth Hormone to Children



(Group 1) Equitable, but restricted entitlement to GH therapy based upon preferential allocation to children *disabled* by height who demonstrate GH-responsiveness. (Group 2) Children unresponsive to GH or (Group 3) GH-responsive but not sufficiently disabled by small stature, including GH-deficient children who have achieved *non-handicapping adult stature* would not receive public or privately subsidized therapy.

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## DISCUSSION V: A & B

- A. Why Growth Hormone Should Not Be Used for Non-Growth Hormone Deficient Children - John Lantos, MD
- B. Growth Hormone Therapy for the Disability of Short Stature - David Allen, MD

Moderated by Louis Underwood, MD

**FRASIER:** I am disturbed about the prospect of discriminating against children who have the disease of growth hormone deficiency. We do not only treat short stature, we correct the deficiency as completely as possible. Stopping the treatment of a growth hormone-deficient child before he or she reaches their final height based upon a debate regarding fairness is, I think, ludicrous in the extreme. At the same time, to argue that insurance coverage should not be an entitlement to short children when there is a disease that we are treating is also ludicrous. It is a mistake to lump a disease entity with short stature. I do not think the same rules apply. If a growth hormone-deficient child, when fully treated and fully grown, is 5 feet 10 inches tall, I do not see any reason why the growth hormone therapy should have been stopped when that individual was 5 feet 2 inches tall. If this seems unfair, well, life is unfair.

**LANTOS:** I agree that this sort of arbitrary selection does not make sense. It creates the worst of all possible scenarios. To set an arbitrary cut-off height seems silly; however, the implications of treating someone who is going to be 4 feet 6 inches tall to reach a height of 5 feet 10 inches, while, on the other hand, denying treatment to another individual who untreated, will reach a final height of 5 feet 2 inches and with treatment could reach a height of 5 feet 10 inches, also does not make sense.

**FOST:** Doug, there are people who have disorders of heart muscle function, which by anyone's criteria would be considered a disease. If one desired, we could spend half the gross national product and give many quality years of life to these individuals. It does not follow that because we can do it we *should* do it, or that such an approach represents a responsible use of limited healthcare resources.

**FRASIER:** I do not believe in "Mount Everest" medicine either. We do not treat just because the technology is

available, but true growth hormone deficiency is a medical condition that exists affecting 5,000 to 7,000, and by some estimates as many as 15,000, children in the United States alone.

**FOST:** It is illogical to say that because somebody has a disease which results in a disability, they are *entitled* to whatever treatment will manage or cure the disability.

**FRASIER:** When a patient comes into my office, it is my job, as a physician, to provide information and access to whatever treatment is available. If I cannot do that, I would choose not to be a physician!

**ALLEN:** I want to respond to that. I feel that I have provided a valuable service to GH-deficient patients by treating them with growth hormone throughout their life to a height of 5 feet 6 inches. This is a fulfilling response for growth hormone treatment. I do not believe that this person then has a right to demand of their insurance company or of our government the continued payment of \$20,000 to \$40,000 a year to allow them to acquire additional inches of height just because growth potential remains. That is where we disagree. My goal in GH therapy is to achieve a final adult height which is no longer considered a handicap or a disability. This does not imply compromising the quality of treatment provided to growth hormone deficient patients.

**LUSTIG:** Dr. Frasier, when you give growth hormone to correct a deficiency, why do you stop when a patient reaches final height? Why don't you continue to treat? After all, you're continuing to replace a deficiency.

**FRASIER:** At this time, we do not know conclusively that continuing therapy replaces a significant physiological deficit. If there were physiological consequences of that deficiency, then I might continue treatment.

**ROOT:** I think eventually the answer to that question will be, "yes." I also believe that the aging process will ultimately be impacted or managed to an extent by growth hormone therapy. It makes a lot of physiological sense.

**MENZEL:** Beside cost efficiency, which justifies prior agreement of payment into the insurance pool, another basis for potential entitlement is that short-statured individuals are truly disadvantaged. You must show that the benefit exceeds

STABLER: the expense of rectifying the inequality and handicap. Given those qualifications, I do not oppose continuing growth hormone treatment for some growth hormone-deficient patients.

BRASEL: It behooves physicians who will prescribe these agents for patients, to track, record, and develop data that will be meaningful, so that in 10 or 20 years we will not be asking the same questions nor be in the position of having put alot of money down a rat hole.

LANTOS: The idea of calling this treatment research rather than therapy is superficially appealing, but unless you define the research question, it is meaningless. No one has presented data on the psychological effects of growth hormone treatment.

STABLER: There is preliminary data from a study of normal short-statured children in which we measured self-concept before therapy. Not surprisingly, these children felt good about their appearance, their school work, their family, and their friendships. Following 12 to 24 months of GH therapy, there did not appear to be any statistical difference in self-concept and, presumably, no clinical difference. This interesting—although unpublished—data supports the notion that these children, who are normal in many ways, do not experience psychological harm because we treat them with growth hormone.

LANTOS: It seems a weak justification for growth hormone treatment that treated children are psychologically no worse off than untreated children. I'd like to see some benefit before I began prescribing it.

CLOPPER: I do not think the relevancy will be limited to a *single* outcome. Potentially, there will be several relevant outcomes.

UNDERWOOD: We have done poorly by identifying the relevant outcome as final adult height, rather than the quality of life during childhood, which contributes to the quality of life during adulthood.

STABLER: One of the best treatments for what we might call body image disorder is counselling. Psychotherapy has a proven track record for modifying and improving body-image disorder.

HINTZ: Much of what we have been talking about is drawing lines. Dr. Allen was drawing a line on the basis of responsiveness. We have talked about drawing a line at  $-2.5$  standard deviations for growth rate or upon the basis of whether the individual is disturbed or not disturbed. All of these lines are fuzzy lines. I think growth hormone responsiveness is a more fuzzy line than peak growth response,  $-2.5$  standard deviations, or annual growth rate. We are merely replacing one fuzzy line—the diagnosis of growth hormone-deficiency—with another fuzzy line.

ALLEN: I agree. Trading one arbitrary criterion for another is a poor choice. However, growth hormone responsiveness is actually the effect that we desire to achieve.

HINTZ: I could argue that the effect we are after is an improvement in final adult height. That is a criterion that we might agree on, but it will take 5 to 10 years to verify!

ALLEN: Agreed. I do not know where to draw the line that determines adequate response, but I do believe that we, the community of pediatric endocrinologists, will need to do this in the future. GH responsiveness is closer to our therapeutic objective than merely raising growth hormone levels in the blood.

HINTZ: That is correct. What I am saying is that your therapeutic view may be philosophically attractive, but it has certain practical problems.

MACKLIN: I would like to identify several propositions on which we seem to sharply disagree. First, pediatricians have an obligation to treat children for measurable growth hormone-deficiency. It is a disease and it is treatable. There is no medical obligation however, to treat extremely short-statured non-GHD children because this is merely a *psychosocial* condition, notwithstanding the fact that it can be a very serious one. Second, one goal of treatment is to achieve a certain height, designated by a cut-off below which short stature is defined as a handicap. In that case we would not distinguish between those who are growth hormone-deficient and those who are not. This contrasts with treating a growth hormone-deficient child long enough to achieve their genetic potential. Do you stop the treatment when you have achieved a "nonhandicapped" height regardless of the fact that the child could grow taller based on his or her genetic potential? I would also like to ask why this is relevant to the ethics issue.

HINTZ: We are doctors. We are not ethicists.

MACKLIN: I want to know why treating to achieve one's genetic potential is more ethically relevant than treating to achieve what is in the best interest of the child.

HINTZ: Why is treating to achieve the individual's genetic potential different from what is in the best interest of the child?

MACKLIN: Let us leave that for a later discussion. We need to consider whether a consistent approach to treating extremely short children is ethically important or not. I have heard several arguments or propositions regarding this issue. Dr. Allen argues that consistency is critically important from an ethical point of view, and he maintains that either equal treatment or nontreatment of Billy and Johnny is acceptable. Others suggest that a consistent approach is not ethically important, but feel that it would not be an injustice to treat

MACKLIN: children (with short stature of similar etiologies) in an inconsistent manner, regardless of the etiology. The fourth point of disagreement appears to involve this group's assessment of the risk/benefit ratio. Dr. Lantos argues against treating both the growth hormone-deficient child and the non-GHD child on the basis of uncertainty regarding the risk/benefit ratio, due to a lack of sufficient data showing benefit and/or the stigmatizing effects associated with daily injections. Others contend that presently there is a favorable risk/benefit ratio that justifies GH treatment for growth hormone deficiency and possibly other types of extreme short stature. Now the arguments will continue and these propositions might get lost, but I thought it would be useful to identify them.

UNDERWOOD: I think your points are well taken, but I do not know what to do with them.

MACKLIN: I do not think that you have to do anything.

UNDERWOOD: I am relieved.

FOST: Whether or not a consensus develops in the conference depends in part on how the conference is set up. You have identified several issues for which there may not be a consensus, but there may be consensus on others that are important to identify. For example, there appears to be consensus against treatment of a non-GHD child growing within the normal distribution. Since there are people out there with entrepreneurial instincts, it is important just to point this out, even if nothing else. I would urge that we consider a follow-up consensus conference.

GERTNER: Clearly, there is a tremendous divergence of views among endocrinologists that, to some extent, is being swept under the carpet. I would like to address three endocrinologists. To Dr. Root, I agree that things are still to be considered experimental and that treatment of disabling short stature should proceed and has justification, but only if we add an obligation to do this in academic and research centers where the information on safety and efficacy will be collected and provided to the world as a whole.

GERTNER: To Dr. MacGillivray, I sense the feeling that if a patient was not severely handicapped by size, then we should not be inflicting growth hormone on them. That is an extreme view.

And to Dr. Frasier, I hear an extreme opposite view. In my opinion, I am an average-sized person. When I treat a male child with growth hormone deficiency and he reaches my height, I usually stop treatment.

ALLEN: My argument concerning the treatment of *disabling* short stature is based on the premise that these kinds of policy recommendations be considered only if growth hormone is shown to be safe and effective. I am undecided and unconvinced whether treatment of these youngsters is presently justified.

MacGILLIVRAY: Dr. Allen are you saying there is no distinction between these two groups?

ALLEN: No. One group is growth hormone-deficient, the other group is not, or only partially so. However, when you ask what disability they share, this is not really distinguishable, and alleviating the *disability* of shortness is the main reason for treatment with growth hormone.

CLOPPER: We have focused almost entirely on 1 therapy for extreme short stature. Future conferences could profit by considering the range of therapeutic approaches for extreme short stature, including psychosocial and psychoeducational interventions as well.

BAILY: I knew something before attending this conference: prescribers of GH are not going to be able to level down; they will only be able to level up. Therefore, I was hoping to be convinced that there is real *medical* distinction between nongrowth hormone-deficient children and growth hormone-deficient children. Practically speaking, the history of medicine shows us a therapy already established as standard of practice will not be removed, even if there are good non-medical reasons for doing so.



## Session VI:



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## CONCLUDING COMMENTS

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Clarence Thomas recently testified that he was, apparently, the only lawyer in the United States who had not discussed *Roe -v- Wade* and had no views on it over the course of the last 20 years. This was found to qualify him for sitting on the Supreme Court of the United States. I, unfortunately, have already blown it in terms of *Roe -v- Wade*, but until about 2 months ago I had very little to say about human growth hormone (GH). This was felt to qualify me to sit in on these discussions and share some reflections.

Let me begin with the bottom line. All parties were staggered on several occasions, although I did not count any knockdowns. There were several occasions on which I was tempted to call a technical knockout, but as I went over to confer in each corner, I discovered that the managers of the respective teams could not understand my vocabulary.

We have been through this together and it would be presumptuous of me to announce any conclusions that we have reached as a group—except for one, which is that, in contrast to most conferences and events that I attend these days, at this one I actually learned something. From conversations I have had with my colleagues on the ethics side of the divide, that is a shared perspective. However, some questions have not been entirely answered.

The most fascinating aspect of the conference has been the challenge of communicating across disciplinary divides. There has been some very real frustration over the fact that we have different mental pictures of who it is we are talking about, what the nature of the interventions are, what kind of impact these interventions will have, and what this bodes for the future. One of the benefits of having this kind of conference is to be fairly self-conscious about when we are failing to communicate, about trying to ask our questions more precisely, and about finetuning the issues we need to work on in the future.

There was a statement in one of the articles that “in the absence of clearly defined criteria, there is a need to turn to the ethical norm of pediatric practice.” Those of us based here at the University of Wisconsin wondered if that was a covert allusion to Norm Fost, the “ethical Norm” we deal with in our daily lives. One of Norm’s credos is that good ethics start with good facts. I was struck by the fact that the dominant paradigm and

image of our conversations together involved Johnny and Billy and what we are to make of that rather arresting comparison that was put forward in David [Allen] and Norman’s [Fost] paper and taken up in our discussions. The hypothetical cases of Johnny and Billy focus attention on the ethical discomfort we have in the lack of seemingly morally relevant differences between these 2 parties and the issue of whether we can justify differences in our treatment approach and the underlying financial approach of entitlement to treatment in these cases. Even given the extremely problematic character of coming to a shared sense of what is meant by disease in this context and how it is evaluated, I will express my own concern about whether the categories of GH-deficient (GHD) and non-GHD children quite capture what it is we want to look to here in a way that is useful.

We seemed to have enumerated four possible responses. There was, in fact, support for each of these positions in the way we think about Johnny and Billy. First is that they are really different and they should indeed be treated differently. This response is rooted in a disease model and a notion that that disease model should drive our moral analysis and our policy. This response seems to be articulated primarily by pediatric endocrinologists understandably committed to existing practice. A second view, expressed in several variations, is that from some important perspectives they may not really be different, that drawing a really compelling ethical distinction between Johnny and Billy is indeed very difficult; nonetheless, we should treat them as *if* they are different even if they are not. That reasoning came from those of us who are concerned about how to fit this case into a broader ethical framework for health-care access and financing in order to develop lines of thinking and rules that could be applied consistently in this and other areas of potentially very expensive health care. Some form of “disease” driven model seemed the only plausible candidate to apply here, even though many people who made that argument (particularly Norman Daniels and Dan Callahan) expressed considerable discomfort in the basis for that. There were also 2 views that these 2 parties cannot be distinguished in any meaningful terms and we therefore have to treat them the same. Some taking that position said, “Since we are comfortable in the established practice of treating one of them as an entitlement, then we should treat the other the same way using a definition of extreme short stature as a handicapping condition affecting

participation in the normal range of life opportunities." Others suggested: "These are the same. They should be treated the same. We should not treat any of them." That summary is a bit frustrating in terms of drawing us toward a consensus regarding a particularly critical issue.

As I understood him, whatever criteria one uses to distinguish "growth hormone deficient" from "non-growth hormone deficient," height was not the only issue. Between those people identified as classically GHD and those individuals of extreme short stature not meeting those criteria, the range of social and psychologic considerations differed significantly. GH therapy might be relevant to those issues, but other interventions might be as effective, or more effective, and more or less cost-effective. If that turns out to be correct, then there may be some interesting reasons to think about GHD and non-GHD individuals as distinct cases calling for distinct responses. That is a bit frustrating for the ethicists among us who want to draw this line in a neater way. That information confounds some initial assumptions and requires additional work to figure out how best to proceed.

We now face the issue of feeding through the mill of social policy these four very different perspectives. Most of the discussion dealt with the term "entitlement," connoting some notion that private insurance and/or governmental programs funding health care should be required to finance these interventions. A near-consensus was heard that, given the burdens on the existing health-care system, it is difficult to provide a morally satisfying justification for giving priority to these treatments, given their expense, the nature of the benefits, the uncertainty of the benefits, and the risks associated with them. That raises the continuing difficulty of doing good for each individual patient while thinking about the larger picture of health care and how GH therapy fits into it. We heard some suggestions of a Bolshevik approach to collapse the entire system as the necessary ground clearing before systemic reform. I do not know that we are likely to resolve this issue.

Let me conclude with a reflection on what I think the bioethicists can bring to the discussion with the pediatric endocrinologists. We know a whole lot less than you folks do about the technical issues of GH. No question about that. I, on the other hand, bring some conceptual equipment that may be useful, a fund of experience with problems that have arisen elsewhere in health-care decision making and from which we may learn some useful things in thinking about this set of problems. A classic case in bioethics was the evolution of the social response to end-stage renal disease and the developing governmental policy on paying for dialysis. That struck me as one of the most compelling metaphors for where we are at in this discussion about GH. At the time, dialysis was an extremely

expensive and extremely limited resource. The relevant health-care community, largely consisting of subspecialists at academic centers interested in research objectives as well as the needs of these patients, defined rigid criteria concerning when dialysis would be highly efficacious and when it should be used. We then entered into public discourse and lobbying efforts to persuade Congress to mandate public entitlement and payment for dialysis treatment.

Poignant discussions about empathy and identification with people suffering from a particular condition were very much a part of that experience. As some of you know, the culminating moment involved dialyzing a patient in a congressional hearing room and suggesting to the assembled congressmen that if they did not act, they would be responsible for the death of this individual and many others. It was a very compelling demonstration, but, as we have learned, it is one that can be made on behalf of numerous different causes. The Congress did act. Public funding was provided and we can now look and learn from what transpired. What happened is that the nature of the provision of dialysis changed remarkably. The people offering dialysis services ceased to be largely academic researchers and became commercial enterprises making considerable money in the context of assured government payment. The indications for dialysis, again, expanded beyond anyone's imaginings: people who clearly were believed to be inappropriate candidates in the early days now have to go into the courts to get permission to discontinue treatment when they think it doesn't serve their purposes anymore.

That is the kind of case that gives pause to some of us when we consider where we may be going with GH. There has to be some discussion of motives. I have been extremely impressed with the integrity and thoughtfulness of all the people participating in this discussion. I want to suggest, however, that you may not be the folks making the decisions and putting care into effect if GH therapy is safe and efficacious, and can be of use not just in the fraction of a percent but along fairly broad lines as enhancement therapy rather than treatment for a disease or extreme short stature. Drawing lines to control that will be very, very difficult and articulating rationales for control will be tremendously important. For myself, I am doubtful that anything measured by standard deviations or by percentages, not to say anything expressed in terms of centimeters, will be promising in that regard. And, as Norm suggested, the virtue of holding this meeting early in the development of the technology is that this relatively small group has an opportunity, one that will not present down the road, to initiate the development of professional consensus and standards of professional practice as to what is appropriate and, maybe more important, what is not appropriate use of GH and to articulate the rationale for that policy.

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# GROWTH

## Genetics & Hormones

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## Growth Hormone Deficient-Like Syndromes and Their Etiologies

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Children with a growth hormone deficient (GHD)-like phenotype but with normal or increased levels of immunoreactive growth hormone (GH) have been reported, beginning with the descriptions of a group of Jewish children by Laron et al.<sup>1</sup> Most of these children with a GHD-like phenotype have low insulin-like growth factor 1 (IGF-1) levels. Some fail to respond to human GH (hGH) injections with increased IGF-1 concentrations,<sup>1</sup> while others respond to hGH injections with increased IGF-1 concentrations and significant increases in growth velocity.<sup>2,3</sup> A few patients with a GHD-like phenotype have normal or increased IGF-1.<sup>4,5</sup>

Since GH acts mainly through its induction of somatomedins or growth factors, mainly IGF-1 and to a lesser extent IGF-2, a derangement of any event in the entire sequence of GH → GH receptor → IGF-1 generation → IGF-1 receptor → post-receptor activation of biochemical processes leads to growth retardation. The purpose of this review is to consider those conditions producing a GHD-like phenotype in the presence of normal or increased GH concentrations.

### **Possible Defects In GH-IGF-1 Axis When GH Immunoreactivity Is Normal Or High**

In Table 1 (page 2), the possible defects in GHD-like syndromes are listed for those patients with (A) low IGF-1 concentrations and (B) normal or high IGF-1 concentrations. In the first group, an abnormal GH structure, a defect in the binding of GH to the GH receptor, or a defect in activation of a post-binding biochemical event at the GH receptor site could produce a low IGF-1 level. In the second group, an abnormal IGF-1 structure, a defect in IGF-binding protein (IGFBP), or a defect in the IGF receptor either in respect to binding or a defective post-binding event could produce a normal or high IGF-1 concentration but a GHD-like syndrome.

Investigation of GHD-like children who have significant immunoreactive GH can advantageously include in vitro radioimmunoassays (RIAs) and radioreceptor assays (RRAs) for GH, determination of the RRA:RIA ratio of GH, evaluation of serum GH with high radioimmunoreactive GH with different monoclonal antibodies, measurement of IGF-1 and IGFBPs

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**Table 1**  
**Possible Defects Producing Growth Hormone Deficiency-Like Syndromes**

|   | <b>SCREENING TESTS (ABNORMAL FINDING)</b>   |
|---|---|
| <b>A. Low IGF-1 Concentrations</b>                                    |   |
| 1. Abnormal GH Structure  | 1a. Monoclonal Antibody Testing of GH (Variability of GH by Different Tests)<br>1b. RRA:RIA for GH Using hGH Receptors From Human Liver (↓) |
| 2. GH-Receptor Defect<br>a. Binding Defect<br>b. Post-Binding Defect  | 2. Serum IGF-1 After hGH (No Change)<br>2a. GHBP (↓)<br>2b. GHBP (Normal)   |
| 3. GH Antibodies Present  | 3. GH Antibodies of High Binding Capacity in Serum  |
| <b>B. Normal or High IGF-1 Concentrations</b>                         |   |
| 1. Abnormal IGF-1   | 1. RRA:RIA for IGF-1 (↓)  |
| 2. IGFBP Inhibition of IGF-1 Action<br>a. In Serum<br>b. In Tissue    | 2a. IGFBP Ligand Binding (↑)<br>2b. IGF-1-Stimulated AIB Uptake (↓)   |
| 3. IGF-Receptor Defect<br>a. Binding Defect<br>b. Post-Binding Defect | 3. IGF-1 Variants (See Text)<br>3a. Binding in Fibroblasts (↓)<br>3b. AIB Uptake in Fibroblasts (↓)   |

**LEGEND:**

AIB aminoisobutyric

hGH human growth hormone

IGFBP insulin-like growth factor-binding protein

RIA radioimmunoassay

GH growth hormone

IGF insulin-like growth factor

RRA radioreceptor assay

in serum, determination of the RRA:RIA concentration of IGF-1, evaluation of the IGF-1 receptor binding capability for IGF-1, measurement of aminoisobutyric (AIB) uptake to evaluate post-receptor activity of the IGF-1 system, and testing of fibroblasts to evaluate their ability to generate IGFBPs in vitro. Measurement of IGF-1 in serum following GH administration also is useful. Unfortunately, several of these techniques are available only in research laboratories. Nonetheless, the endocrinologist needs to comprehend the possibilities for diagnostic evaluation in GHD-like children. Therefore the types, advantages, limitations, and possible interpretation of the results of each of these laboratory tests are discussed briefly.

### **Abnormal Growth Hormone Structure**

Several suspect cases of abnormal GH structure<sup>2,3</sup> have been reported, including the first reported cases by Kowarski and colleagues.<sup>2</sup> These patients had a very distinctive GHD-like phenotype but elevated serum GH levels. It was thought that they

might have GH with normal immunologic activity but deficient biologic activity.<sup>2,3</sup> They had low levels of serum IGF-1 and responded to GH treatment with generation of IGF-1 and increased growth. This suggested a structural defect in the GH molecule or, alternatively, the unexplained need for pharmacologic doses of GH to be present in order to generate IGF-1.<sup>2,3</sup>

Abnormal circulating GH species were reported<sup>2</sup> when researchers utilized RRAs from liver membranes prepared from pregnant rabbits<sup>5</sup> and <sup>125</sup>I-labeled hGH. This assay is now regarded as questionable because the rabbit GH receptor differs greatly in its specificity from the human GH receptor. For this reason, GH receptors from a human lymphoid cell line (IM-9 cells) have been used subsequently in the GH RRA.<sup>6</sup> This method has a disadvantage in that the IM-9 cells must be maintained in culture. Also, different batches of IM-9 cells differ greatly in their expression of GH receptors. For these reasons, membranes obtained from the liver of human donors were prepared in our laboratory. These bind well to <sup>125</sup>I-labeled hGH. These specific membrane GH receptors

are stable for over 3 years when properly stored and provide reliable assays. Standards are prepared in normal sera with GH concentrations of less than 1  $\mu\text{g/L}$ , which minimizes interference from GHBP. Reliable results can be obtained with sera in which the GH by RIA is greater than 10  $\mu\text{g/L}$ .

Interpretation of the assay requires comparison of RRA with RIA. Because of the combined error in each assay, only ratios of RRA:RIA less than 50% on repeated testing should be considered indicative of a possible GH abnormality. Since development of this assay and with this criterion, no cases of abnormal GH have been recognized. Furthermore, Dr. P. Rotwein, also of Washington University School of Medicine, has not found abnormal GH mRNA in the very short children he has studied. Unfortunately, the patient reported by Kowarski et al<sup>2</sup> was not studied by this method. The one patient reported by Bright et al<sup>3</sup> was tested utilizing a panel of monoclonal antibodies and the liver hGH RRA method, and no abnormality was found.

The patient reported by Valente et al<sup>7</sup> needs to be mentioned. The investigators reported an abnormal GH in a patient with growth failure. After neutral gel filtration of the serum, much of the GH eluted with molecular weights of 45 kd and 85 kd, rather than 22 kd, which suggests the presence of dimers and tetramers. These oligomers were dissociable with 8 molar urea. The molecular defect in this patient was never further characterized. The fact that this patient had a normal serum somatomedin (IGF-1) concentration makes it unlikely that the abnormal circulating GH complexes were related to the growth failure.

In summary, regarding patients with a GHD-like phenotype and low IGF-1 concentration, no conclusive evidence of a defect in GH structure has been demonstrated, although such defects may exist. Theoretically, patients with severe GHD-like syndromes who respond to GH treatment with increased IGF-1 concentrations would best fit into this category.

### **Abnormal Growth Hormone Receptors**

The growth hormone receptor (GHR) is the next critical step in the cascade. The receptor abnormality could be either in its ability to bind GH or in its post-binding signaling. Patients with abnormal GHRs have elevated serum GH and low serum IGF-1 concentrations. Both growth and IGF-1 generation are unresponsive to exogenous GH. Recognition of cases of abnormal GHRs has been greatly

facilitated by study of serum GHBP,<sup>8</sup> as this binding protein is derived from the extracellular domain of the GHR. The circulating GHBP is the presumed product of an enzymatic cleavage of the receptor near the cell membrane and usually has been measured by incubating an aliquot of serum with <sup>125</sup>I-labeled hGH and separating the <sup>125</sup>I-labeled hGH bound to GHBP from the free <sup>125</sup>I-labeled hGH by size-exclusion gel chromatography. For this purpose, we have used a 0.9 x 15 cm Sephacryl<sup>®</sup> 200 column. Specific binding is determined by repeating the procedure with excess unlabeled GH. A correction is made for GH that is present in sera, and the results are compared with those obtained with a normal reference serum. Because there is a progressive rise in serum GHBP during childhood, results must be compared with age-appropriate controls.

A number of simpler methods of measuring GHBP have been proposed. We have used a method in which the <sup>125</sup>I-labeled hGHBP complex is precipitated with a monoclonal antibody directed against the extracellular domain of the GHR. Specific RIAs for GHBP are under development in several other laboratories. Such assays would permit recognition of immunoreactive GHBP that might be deficient in its ability to bind GH.

### **In Future Issues**

#### **The Effects of Irradiation on Endocrine Function in Children: Past, Present, and Future**

by Stephen M. Shalet, MD

#### **Sleep, GH Secretion, and Short Stature**

by Richard Wu, MD, and Paul Saenger, MD

#### **Teratogens and Growth**

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#### **Contiguous Gene-Deletion Syndromes**

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#### **The Relevance of Developmental Genetics to Human Malformations**

by Golder Wilson, MD, PhD

#### **The Spectrum of Undernutrition and Poor Growth**

by Fima Lifshitz, MD



GHBP is virtually undetectable in Laron-type dwarfism, a condition characterized by elevated serum GH, very low serum IGF-1, and the phenotype of severe GHD.<sup>1</sup> The condition is heterogeneous, with some patients homozygous for GHR gene deletions<sup>9</sup> or nonsense mutations in whom no GHR protein is produced.<sup>10</sup> In other similar cases, detectable immunoreactive GHR occurs. These patients may have simple amino acid substitutions that prevent specific binding of GH. In affected members of one family, a substitution of thymidine for cytosine resulted in serine replacing phenylalanine at position 96 of the GHR.<sup>11</sup> This mutation, however, did not result in decreased GH binding to GHBP synthesized by recombinant methods.<sup>12</sup>

In African Pygmies an abnormality in GHR appears to be present.<sup>13</sup> GHBP in the sera of young Pygmy children is only slightly reduced but fails to rise normally as childhood progresses, so that by puberty, mean values are only 30% of those of normal-statured African controls. This pattern parallels the growth pattern of these individuals, which becomes abnormal only in later childhood and puberty. Although the precise defect responsible for the decrease in GHBP in the Pygmy cannot be identified at the molecular level, a defect in the regulation of the GH gene seems likely.

Many children with apparently normal GH secretion and serum GHBP still have serum IGF-1 concentrations that are 1 or 2 standard deviations (SD) below the mean for chronologic age. In these patients, GH insensitivity seems to be only relative, as serum IGF-1 rises after GH administration and some acceleration of growth velocity occurs. These patients appear to have a sluggish response at the GHR level or beyond. Theoretically, this explanation could be a contributing factor for constitutional short stature. Unfortunately, after GH is bound to the GHR, the mechanism of signal induction across the cell membrane and the mechanisms responsible for the initiation of intracellular responses are poorly understood. Since none of these response sequences are well known, their contribution to impaired growth remains conjectural.

A functional defect in the ability of GH to stimulate IGF-1 synthesis occurs in a dramatic form in kwashiorkor and less markedly in many forms of clinical malnutrition, including uncontrolled diabetes mellitus. Serum GH is elevated but IGF-1 levels are low. Defects in both GH binding and in post-binding events probably contribute.

Acquired resistance to GH in patients who have received therapeutic GH with initial satisfactory responses suggests the development of immunologic resistance. This is readily determined by testing for GH antibodies in serum. The spontaneous development of antibodies to GH without prior administration of exogenous GH remains a potential cause of GH resistance, similar to the mechanism recognized in the development of insulin resistance.

### **Abnormal Insulin-Like Growth Factors**

Abnormalities of the IGF-1 gene or its expression might result in reduced or absent secretion of a functionally impaired peptide with retained immunologic determinants recognizable by RIA. We have screened children with severe unexplained growth failure for this latter possibility by comparing serum IGF-1 concentrations, as determined by a human placental membrane RRA, with concentrations determined by RIA, and Dr. P. Rotwein has looked for IGF-1 gene mutations by endonuclease protection assays. Thus far, no abnormalities have been recognized in this limited survey. It is possible that a severe homozygous defect would be lethal.

### **Abnormal Insulin-Like Growth Factor—Binding Proteins**

Another possible abnormality to account for a GHD-like syndrome with increased IGF-1 is increased concentrations of plasma IGFBPs. Increased IGFBPs are known to be capable of acutely inhibiting IGF action. Most of the plasma IGFs are complexed to IGFBP-3, and normally the concentrations of this binding protein are closely coordinated with the total concentration of IGF-1 and IGF-2. It is not known whether increased concentrations of IGFBPs could sustain inhibition of IGF-1 action, but we know that genetic conditions associated with increased concentrations of transcortin (corticosteroid-binding globulin) or thyroxine-binding protein are not associated with recognized clinical abnormalities of hormone action.

IGFBPs are produced locally by many tissues, and the type and amount of binding protein produced by various tissues differ greatly. Most is known about the secretion of IGFBPs by human fibroblasts. These cells secrete binding proteins into conditioned media, and also retain binding proteins associated with the cell surface that greatly decrease the response to added IGF-1. We

found that fibroblasts of a short girl with elevated concentrations of serum IGF-1 required almost threefold higher concentrations of IGF-1 to stimulate uptake of a model amino acid ( $\alpha$ -AIB) than did normal fibroblasts.<sup>14</sup> However, the response of these fibroblasts to IGF-1 variants lacking in binding protein determinants was normal. The conditioned media from these fibroblasts contained greatly increased concentrations of IGFBPs, and there also was increased concentration of cell-associated binding proteins, particularly a 32 kD species that may be IGFBP-5.<sup>15</sup> These observations suggest that local production of IGFBPs might blunt IGF-1 action and contribute to short stature.

### Abnormal Insulin-Like Growth Factor 1 Receptors

The last rung in the cascade of GH  $\rightarrow$  somatomedin  $\rightarrow$  IGF-1 is the IGF-1 receptor. Two patients have been reported with what could be an abnormality of the IGF-1 receptor.<sup>4,5</sup>

Bierich et al<sup>4</sup> reported a patient with elevated GH and IGF-1 concentrations and a GHD-like phenotype with delayed dentition and skeletal age as well as hypoglycemia. Both immunoreactive and bioactive IGF-1 levels were present. Fibroblasts from a skin biopsy specimen taken at 21 months of age were incubated with <sup>125</sup>I-labeled IGF-1. Binding was diminished by 50% as compared with controls. The laboratory data regarding this case are not convincing because the method used would not distinguish <sup>125</sup>I-labeled IGF-1 bound to cell-associated IGFBPs from that bound to IGF-1 receptors. Earlier, Lanes et al<sup>5</sup> reported a similar patient with a GHD-like phenotype with normal integrated concentrations of GH but elevated IGF-1 levels by RIA, RRA, and bioassay (SO<sub>4</sub> uptake).

To date, no molecular abnormalities have been recognized in the IGF-1 receptor, although several have been observed in the structurally related insulin receptor. IGF-1 variants such as the (des 1-3) IGF-1<sup>16</sup> and the [Q,<sup>3</sup>A,<sup>4</sup>Y,<sup>15</sup> and L<sup>16</sup>] IGF-1<sup>17</sup> permit characterization of the IGF-1 receptor of isolated cells without interference from secreted binding proteins.

In summary, GHD-like syndromes are still difficult to study, but increasingly refined molecular techniques should provide the capability to clarify the pathophysiology or normal physiologic variations that account for these syndromes.

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*This new column, which will highlight specific clinical conundrums, is added at the instigation of the editors. How do you explain the findings? Is there a problem? If so, is it 1 problem (adrenal) or 2 problems (adrenal and gonadal - possibly, hypogonadotropism)? You are invited to contribute your thoughts and/or speculate what these unusual findings signify. Please send your thoughts, ideas, and explanations to Dr. R. M. Blizzard, PO Box 386, Department of Pediatrics, University of Virginia, Charlottesville, VA 22908. We will provide a consensus from our respondents in the next issue.*

J.H., a 7 6/12-year-old male referred for pubic hair of 1 year's duration, tall stature, HA 10 3/12 without a history of a growth spurt, and obesity and promiscuity, had a negative prepubertal physical examination except for stage III pubic hair, and testes noted to be "very small." The midparental height (183 cm) fell on the 80th percentile for adult males. The BA was 11 years; serum testosterone, <25 ng/mL; 17-OH.progesterone, 265 ng/dL; 17-OH.pregnenolone, 19.7 ng/mL; cortisol, 24 µg/dL; 11.desoxycortisol,

28 µg/dL; and cortisol, 27 µg/dL. All levels except that for testosterone were modestly elevated for age.

At CA 7 3/12 years samples for serum LH, FSH, and testosterone were drawn every 20 minutes from 2200 to 0200 hours. The range of LH values was 4.3 to 5.2 mIU/mL; FSH, 6.9 to 8.3 mIU/mL; and all testosterone concentrations were <25 ng/mL. Corticotropin (ACTH), 250 mg intravenously, was given at 0801 hours. The results were:

|                              | <u>Normal</u> | <u>0800 Hours</u> | <u>0900 Hours</u> |
|------------------------------|---------------|-------------------|-------------------|
| Cortisol (µg/dL)             | 10 - 20       | 23                | 43                |
| 17-OH.progesterone (ng/dL)   | 50 ± 50 SD    | 242               | 426               |
| 17-OH.pregnenolone (ng/dL)   | 0.41 - 1.83   | 19.7              | 25.5              |
| 11-Desoxycortisol (µg/dL)    | <120          | 318               | 395               |
| DHEA-SO <sub>4</sub> (µg/mL) | 24 - 122      | 148               | 130               |
| Androstenedione (ng/dL)      | 31 - 71       | 70                | 90                |

At CA 8 7/12 years, J.H. returned, after being lost to follow-up, with essentially the same physical findings, a BA of 12 3/12 years, and comparable steroid values. Following 10 days of dexamethasone at 500, 500, and 750 µg during each day, all steroid values were suppressed.

At CA 11 7/12 years he returned because of bilateral cryptorchidism. The height was 165.6 cm (HA, 14 6/12 years) and the BA, 13 6/12 years. The testes (2 mL bilaterally) could be brought into the scrotum. Physical examination findings were those of a prepubertal boy with stage III pubic hair. Laboratory values were: 17-OH.progesterone, 88 ng/dL; 11 desoxycortisol, 104 µg/dL; testosterone, 23 ng/dL; androstenedione,

52 ng/dL; DHEA-SO<sub>4</sub>, 257 µg/mL; LH, <1.1 mIU/mL; FSH, 2.8 mIU/mL. His lack of sexual development and "effeminacy" reported by the mother prompted a recommendation for depot testosterone administration (50 mg every month).

At CA 12 9/12 years his height was 171.2 cm (HA, 15 1/2 years). He had received only 2 testosterone injections. Twelfth-year molars had erupted. The serum LH was 1.6 mIU/mL; FSH, 4.4 mIU/mL; testosterone, 93 ng/dL; the testes were 2 mL each in volume.

**Please assist us in solving this clinical conundrum.**

**R.M. Blizzard, MD**

## Growth Hormone Insensitivity: Clinical Spectrum, Regulation of Growth Hormone, and Related Factors

Several cooperative studies were recently presented at a symposium<sup>1-4</sup> in which the clinical and hormonal aspects of growth hormone insensitivity (GHI) were extensively reviewed.

The first of the 4 reports<sup>1</sup> involved a multicentric clinical analysis of 29 patients (14 male, 15 female) with suspected GHI from 11 countries (9 European countries, Saudi Arabia, and Australia), ages 3.2 to 22.6 years. Their heights ranged from +2.6 to -8.9 standard deviations (SD) (mean, -6.0 SD); their weight was +1.2 to -5.2 SD for height (mean, -2.9 SD). Basal levels of GH were extremely variable ranging from 0.8 to 158.2 mU/L, and were detectable in all. In contrast, insulin-like growth factor 1 (IGF-1) was subnormal in all, 20 to 97 ng/mL (mean, 33.6 ng/mL), in comparison with a normal range for age of 120 to 180 ng/mL, and IGF-1 did not increase in response to short-term administration of GH. In this series, some features varied, showing a spectrum of clinical signs of GHI. Micropenis was found in 9 of the 13 males whose penile size was documented.

The second paper<sup>2</sup> details the regulation of GH secretion in 2 of these patients. Their nighttime pattern of GH secretion was pulsatile, with major peaks exceeding 200 mU/L. Their response to 2 sequential bolus injections of GH-releasing hormone (GHRH) in a 3-hour study was strongly positive, with peaks of 512 and 216 mU/L after the first injection, and of 486 and 150 mU/L after the second bolus. In both subjects, serum GH fell to very low levels during an infusion of somatostatin, and there was a rebound following the end of this infusion. This study showed that the normal dual hypothalamic control mechanism by GHRH and somatostatin is preserved in GHI, and that the lack of negative feedback control of GH secretion is at the pituitary level, probably as a failure of the direct inhibition by GH of its own release.

The third report<sup>3</sup> details the clinical and genetic characteristics of 2 large groups of very short patients with GHI living in 2 separate areas of Ecuador (Central America). The morphology of these individuals was similar to that of GHI patients initially described by Laron in Israel and then by others in different parts of the world. However, some differences were observed: the upper/lower segment ratio remained infantile in adult patients, the hands and feet measurements were above the 10th percentile in all, and three quarters of the patients had a limitation of elbow extension. A history of probable hypoglycemia was reported in half of the cases. The genetic studies showed that 1 of the 2 geographic groups (the area of El Oro; 26 cases: 12 males and 14 females) proceeded from a single extended consanguineous pedigree, while the other (the area of Loja; 21 patients: 19 females and 2 males) was probably inbred but not clearly from a common consanguineous origin. The female predominance in the Loja group of GHI patients suggested the possibility of an association between the GHI trait and a trait lethal for male fetuses.

The last paper in this series<sup>4</sup> presents the results of biochemical studies in the Ecuadorian Loja patients and their heterozygous parents, compared with normal-sized, sex- and age-matched controls from the same area. Patients with GHI had markedly reduced serum levels of both IGF-1 and IGF-2 as well as reduced levels of GH-dependent binding proteins, that is GH-binding protein (GHBP) and IGF-binding protein 3 (IGFBP-3). This was in contrast with increased levels of the non-GH-dependent protein IGFBP-2. IGFBP-2 and IGFBP-3 showed an inverse correlation. Moreover, IGFBP-3 correlated positively and IGFBP-2 correlated inversely with the age of patients. The study of heterozygotes did not show such abnormalities except for a slight reduction of IGF-2 that overlapped with normal control values. Thus, no reliable biochemical marker for heterozygosity was found.

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4. Fielder PJ, et al. *Acta Paediatr Scand* 1991;377(suppl):104.

**Editor's comment:** Laron-type dwarfism, one type of GHI, is a very rare familial disease, which has been a model allowing important discoveries in the physiology of the GH receptor and in its genetic mapping. The orientation of the reports summarized here is first and foremost clinical, since no such large-scale studies had been possible previously. The investigators show that the main features of GHI are common to all cases, but that individual variations, and the presence of peculiar features in certain cases or families produce a clinical spectrum. The orientation is also biochemical, with one study suggesting that the excess of GH secretion in GHI results from the lack of receptivity directly at the pituitary level and another study demonstrating that all GH-dependent growth factors and binding proteins are dramatically reduced in GHI, with an inversely correlated increase of one non-GH-dependent binding protein. The lack of a marker for heterozygosity detectable by serum biochemistry also is reported. But heterozygosity can be recognized by appropriate DNA analysis when an index case of GHI has been found and studied within a pedigree, as demonstrated by several authors in recent years.

Jean-Claude Job, MD

## Special Announcement

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## Vitamins in the Prevention of Neural Tube Defects

Recently, a randomized, double-blind prevention trial was completed by the Medical Research Council (MRC) Vitamin Research Group to determine whether periconceptual supplementation with folic acid (one of the vitamins in the B group) or a mixture of 7 other vitamins (A, D, B1, B2, B6, C, and nicotinamide) could help to prevent neural tube defects (NTDs) such as anencephaly, spina bifida, and encephalocele. A 72% protective effect was found for folic acid supplementation; the other vitamins showed no significant effect.

It has long been suspected that diet plays a role in the causation of NTDs, which are among the most common severe congenital malformations. The possibility that folic acid might be important was raised as early as 1964.<sup>1</sup> In 1980 and 1981, 2 intervention studies<sup>2,3</sup> were published in which periconceptual vitamin supplementation was given to women who had experienced a previous NTD pregnancy and were thus at increased risk for another such pregnancy.<sup>2</sup> These 2 studies yielded somewhat equivocal results, but suggested that folic acid or other vitamin supplementation might indeed reduce the risk of recurrence. The MRC group's randomized, double-blind trial with a factorial design utilizing data from 1,195 at-risk

pregnancies from 33 centers in 7 countries removes concerns regarding a lack of randomized controls or the introduction of bias. It has thus been concluded that folic acid supplementation can now be firmly recommended for all women who have had an affected pregnancy and that public health measures should be taken to ensure that the diet of all women of childbearing age contains an adequate amount of folic acid.

Wald N, Sneddon J, Donson J, et al. *Lancet* 1991;338:131.

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2. Smithells RW, et al. *Lancet* 1980;1:339.
3. Laurence KM, et al. *Br Med J* 1981;282:1509.

**Editor's comment:** Definitive results identifying folic acid prophylaxis as an effective preventive measure for NTDs are indeed welcome. Routine periconceptual folic acid supplementation for all women planning to conceive a child should now become standard practice, and it represents a powerful addition to the arsenal of preventive health care.

Judith G. Hall, MD

## Cystic Fibrosis and Congenital Absence of the Vas Deferens

It is well known that most, if not all, male children born with cystic fibrosis (CF) also have bilateral congenital absence of the vas deferens (CAVD). In most studies, the converse has not been observed, as males referred for CAVD have not generally been tested for CF in the past. Because it is now possible to test directly for common mutation of the CF gene, Rigot et al<sup>1</sup> have recently analyzed a group of men with CAVD for carrier status for the  $\Delta F_{508}$  mutation of the CF gene. They found that 8 of 19 CAVD patients were heterozygous for this deletion. This frequency of  $\Delta F_{508}$  carriers is much higher than the expected carrier rate for the general population, which is 1 in 25. In addition, all but 1 of the 8 carriers had chronic sinusitis, and 2 patients had abnormal sweat chloride tests.

The results of Rigot et al have been confirmed by Anguiano et al,<sup>2</sup> who found that 12 of 20 patients with CAVD carried confirmed mutations in the CF gene. These patients all had normal sweat chloride tests and no other signs of CF. It must be that the  $\Delta F_{508}$  mutation is contributing to the CAVD in some way. Perhaps there is still another allele at the CF gene locus that does not cause CF but does lead to CAVD, and the patients in the study by Rigot et al who showed signs of CF represent compound heterozygotes.

In such a population, the risk of having a child with CF would be between 1 in 100 and 1 in 200. In the past, no one with CAVD has been able to father a child, but Silber et al<sup>3</sup> have achieved successful in vitro fertilization with epididymal sperm from patients with CAVD. Thus, as Rigot et al emphasize, their data on the frequency of CF carriers in CAVD males suggest that testing for the CF  $\Delta F_{508}$  allele should be performed in these men and their partners whenever in vitro fertilization is planned. In addition, the Silber et al<sup>3</sup> plan a detailed study of the offspring of the CAVD patients. The condition does seem to have a genetic basis—many affected siblings have been observed as well as concordant monozygous twins. If a

substantial number of the male children of these patients have unilateral CAVD, it would imply that the condition is due not to a sex-linked recessive transfer from mother to son, but possibly to an autosomal dominant gene.

### References

1. Rigot JM, et al. *N Engl J Med* 1991;325:64.
2. Anguiano A, et al. *Proc Intl Congr Hum Genet* 1991; 49(suppl):22.
3. Silber SJ, et al. *N Engl J Med* 1990;323:1788.

**Editor's comment:** The concurrent development of in vitro fertilization technology and the ability to identify carriers of CF mutations will have, in this case, a twofold benefit. Now that CAVD patients are able to conceive, the CF analysis will allow counseling regarding their high risk for transmitting a CF gene to their children. The mapping of the CF locus, which appears to be linked or identical to CAVD, combined with inheritance studies of CAVD, will hopefully help to identify the mutation responsible for CAVD.

Judith G. Hall, MD

### Erratum:

In GGH Vol. 8, No. 1 (March 1992), an error on page 14 incorrectly references Dr. Yarasheski, et al's abstract entitled "Effect of Growth Hormone and Resistance Exercise on Muscle Growth in Young Men." The correct reference is: Yarasheski KE, Campbell JA, Smith K, et al. *Am J Physiol* 1992;262:E261-E267.

## Systemic Delivery of Human Growth Hormone by Injection of Genetically Engineered Myoblasts

The ability to deliver recombinant proteins into the systemic circulation could facilitate the treatment of a variety of acquired and inherited diseases. The ideal recombinant protein delivery system requires a cell that is easily isolated from the recipient, reproduced in vitro, transduced with recombinant genes, and conveniently reimplanted into the host. The secreted recombinant protein from these cells needs to gain ready access to the circulation. Such cells need to survive for long periods while secreting the transduced protein product without adversely interfering with body function. Until the possible use of myoblasts was considered and tried in the experiments reported here, the production of stable and physiologic levels of circulating recombinant proteins in normal animals has been relatively unsuccessful. The use of myoblasts may open new therapeutic horizons, as discussed in the 3 presentations from a recent December 1991 issue of *Science*. These articles are abstracted here.

Myoblasts can easily be obtained from muscle tissue, and genetically engineered myoblast cells can easily be returned to muscle without causing damage. This technique was used by Dhawan and colleagues<sup>1</sup> at Stanford University, who introduced a recombinant gene that encoded human growth hormone (hGH) into cultured myoblasts from mice. A modified gelatin (MFG) retrovirus vector was utilized. These cells secreted hGH at levels ranging between 1,400 to 4,600 ng/10<sup>6</sup> cells per day in vitro. These cells were injected into muscle and hGH was demonstrated to be secreted into the serum at increasing levels over an 85-day period. It was demonstrated that hGH can be continuously produced and secreted by myoblasts that are implanted into muscle tissue. The authors state that "this type of delivery system may be useful in the treatment of children with GH deficiency." They also state that "these findings suggest that somatic cell therapy using myoblasts may have application in delivering to the circulation a number of recombinant proteins."

Barr and Leiden,<sup>2</sup> in the same issue of *Science*, published an article entitled "Delivery of Recombinant Proteins by Genetically Modified Myoblasts." They used a plasmid carrying the hGH gene as the vector to insert these genes into the murine C2C12 myoblast cell line. The cultured transfected myoblasts were then placed into the muscles of mice and circulating hGH was measured over a 3-week period. Thus, the results were corroborated in the 2 experiments. Histologic examination of muscle tissue injected with myoblasts demonstrated that many of the injected cells had fused to form multinucleate myotubes.

A concern regarding such injections with a continuous cell line, such as the C2C12 line, is the possibility that these cells have malignant potential. Long-term studies will be needed to evaluate this possibility. In addition, it remains to be determined if this system can be used to produce physiologic levels of circulating proteins in large animals.

In the same issue of *Science*, Michelle Hoffman discussed in an editorial entitled "Putting New Muscle Into Gene Therapy"<sup>3</sup> the potential applications of these techniques, including the possibility of treating the genetic defects that cause muscular dystrophy and other diseases. Myoblasts do better than the cells used in previous systems because they eventually differentiate and fuse into existing muscle tissue. Hoffman cautions against excessive and premature enthusiasm, however. The results achieved could be different using primary cell lines obtained from the individual receiving therapy, in contrast to the cells used in the mouse experiments, which were from cell lines perpetuated in culture over many years. A remaining unanswered question is: Does one get sustained expression in primary cells? Also, cell lines are probably unacceptable for use in humans because they are too frequently tumorigenic.

In spite of these difficulties, some investigators such as Barr and Leiden are optimistic about the future of myoblasts for gene therapy. Some researchers are projecting their ideas even further into the future, such as the potential to inject DNA directly into muscle cells, a technique pioneered recently by Wolff et al at the University of Wisconsin and by investigators in San Diego.

## References

1. Dhawan J, et al. *Science* 1991;254:1509.
2. Barr E, Leiden JM. *Science* 1991;254:1507.
3. Hoffman M. *Science* 1991;254:1455.

**Editor's comment:** My editorial comment is simply this: Wondrous innovations in science never cease. The use of myoblasts as protein carriers is only one of many recent scientific innovations, but one that will undoubtedly receive much attention in the future from the readers of *GROWTH, Genetics, & Hormones*. Congratulations to Dhawan, Barr and colleagues for these stimulating ideas and studies.

Robert M. Blizzard, MD

## Uniparental Disomy in Beckwith-Wiedemann Syndrome

Henry et al have demonstrated that 3 of 8 cases of Beckwith-Wiedemann syndrome (BWS), a fetal overgrowth syndrome, are associated with demonstrable paternal uniparental disomy of chromosome 11. Uniparental disomy is a phenomenon in which both copies of a chromosome have been inherited from a single parent, in this case the father, with a concomitant deficiency of the maternal copy. This result was in contrast with 0 of 18 unrelated controls carrying uniparental disomy for chromosome 11. Furthermore, these authors found an overall increase in the frequency of homozygosity for several 11p15.5 markers in 21 cases of sporadic BWS, suggesting that uniparental disomy probably accounts for an even higher proportion of sporadic BWS cases than the 3 of 8 cases of unequivocal paternal uniparental disomy.

Henry I, et al. *Nature* 1991;351:665.

**Editor's comment:** Both BWS and uniparental disomy are of particular interest in the field of genomic imprinting. Many regions of the human genome, including the region of chromosome 11 implicated in BWS, appear to be imprinted, ie, they are differentially expressed depending upon whether they were inherited from the mother or the father. Because of genomic imprinting, 2 copies of an imprinted gene from a single parent, ie, uniparental disomy, might result in a strikingly different phenotype than 1 copy from each parent. The results of Henry et al support this theory and demonstrate that uniparental disomy can be associated with BWS, a cancer-predisposing genetic syndrome.

Judith G. Hall, MD

## Growth Prognosis and Growth After Menarche in Primary Hypothyroidism

Pantsiotou et al examined growth data of 20 girls and 9 boys with primary hypothyroidism from the beginning of thyroxine treatment to final height. Bone age (BA) was determined by the method of Tanner. At diagnosis, girls had a mean age of 8.8 years (range, 3.0 to 13.0 years) and a mean BA of 5.4 years. The mean age of diagnosis in the boys was 9.5 years (range, 3.7 to 14.2 years) with a mean BA of 6.3 years. All patients were treated with thyroxine ( $100 \mu\text{g}/\text{m}^2/\text{d}$ ). In girls, the mean height standard deviation score (SDS) for BA before treatment was  $-0.59$ . At final height (17.5 years) the mean height SDS for BA was  $-0.55$  ( $P < 0.01$ ). In boys, the mean initial height SDS for BA was  $-1.6$ , at final height (16.5 years) this was decreased to  $-0.87$  ( $P < 0.02$ ). All patients, except 1 girl, were below the 50th percentile at final height. The onset of puberty in boys was at age  $13.3 \pm 1.4$  years, or 1.7 years later than in the normal population. The onset of puberty in girls was at 12.4 years, or 1.2 years later than in the normal population. The mean age of menarche was 13.8 years compared with 13.5 years in normal girls. Therefore, the time from the onset of puberty to menarche (1.4 years) in girls with primary hypothyroidism was reduced as compared with that of normal girls (2.3 years). Unlike normal girls, whose growth velocity decelerated markedly with the onset of menarche, the girls with treated hypothyroidism had a mean growth velocity of  $5.1 \text{ cm}/\text{yr}$  during the year after menarche and  $4.1 \text{ cm}/\text{yr}$  during the second year following menarche. Thus, there was a permanent height deficit in treated primary hypothyroid children and the growth characteristics were markedly different from normal.

S. Pantsiotou, et al. *Arch Dis Child* 1991;66:838.

**Editor's comment:** The results of this study confirm earlier work by Rivkees et al (*N Engl J Med* 1988;318:599-602) who showed that at maturity girls and boys treated for acquired hypothyroidism were approximately 2 SD below normal adult stature. Both girls and boys in that study were somewhat older at diagnosis than those in the present study, and disease duration was longer. No description of growth characteristics was given in the Rivkees et al report, although both studies report that BA advanced at a greater rate than height age in treated hypothyroid children.

In an editorial accompanying the study by Rivkees et al (*N Engl J Med* 1988;318:632-634), Fisher suggests that the average age at diagnosis of the children in the study may have limited the period of catch-up growth available to them to about 3 years. It is significant that the children in Pantsiotou's study were younger than those in the Rivkees et al report, yet they showed a similar pattern of growth deficit at final height. The significance of growth following menarche is unclear, as it did not contribute (significantly) to these patients achieving a normal final adult height. It is important that pediatric endocrinologists use caution when predicting final height in children being treated for primary hypothyroidism.

William L. Clarke, MD

## Translocation Chromosome Associated With Both Angelman and Prader-Willi Syndromes in a Single Family

The Angelman and Prader-Willi syndromes have been associated with a deletion in the same region of chromosome 15. Almost all cases of Angelman and Prader-Willi are sporadic; thus, it had not been possible to prove unequivocally that a 15q deletion was responsible for the different phenotypes seen in these 2 syndromes. Hulten et al<sup>1</sup> have now reported a translocation chromosome transmitted within a family in which both Angelman and Prader-Willi children are seen.

Other studies have shown that the only apparent cytogenetic difference between patients with the 2 syndromes is that Angelman is associated with a deletion in the maternal chromosome 15q, while Prader-Willi is associated with a deletion in the paternal chromosome.<sup>2</sup> Thus, it has been postulated that these 2 syndromes represent an example of genomic imprinting, the process by which a gene or chromosomal region produces a different phenotype depending upon whether it is inherited from the mother or from the father. Genetic contributions from both parents usually play complementary but sometimes opposing roles, and both are necessary for normal phenotype. In regions that are imprinted, the phenotype produced by a mutation is determined by the sex of the parent transmitting the mutant allele. In the Hulten et al study, the index child with classic Angelman syndrome had a maternally derived unbalanced 15;22 translocation leading to a deletion 15pter→q13. Another branch of the same family had 2 children with Prader-Willi syndrome who had the same unbalanced translocation but of paternal derivation.

The authors note that the unbalanced translocation in the index children was overlooked at first and classified as the

typical 15q11-q13 deletion. The detection of this translocation was achieved only through the application of more specialized in situ cytogenetic techniques. The authors stress the importance of obtaining detailed pedigree information and for the cytogenetic reinvestigation of apparently sporadic cases of both syndromes to look for familial chromosomal translocations.

## References

1. Hulten M, et al. *Lancet* 1991;338:638.
2. Magenis ER, et al. *Am J Med Genet* 1990;35:333.

**Editor's comment:** This report further supports the theory of genomic imprinting. The fact that both Angelman and Prader-Willi syndromes occur in a single family and are associated with the same chromosomal translocation provides striking evidence for parent-of-origin differences in phenotypic expression for certain areas of the genome. It seems likely that there are actually 2 different closely linked genes on the 15q11-q13 region, one maternally imprinted and one paternally imprinted, with both deleted by the translocation in this family. However, the mechanism of imprinting is unknown at this time. It suggests other chromosomal translocations may produce 2 phenotypes, depending upon parent of origin. Particular care may need to be given to submicroscopic deletions with translocations.

Judith G. Hall, MD

## Chorionic Villus Sampling Versus First- and Second-Trimester Amniocentesis: An Update

Two large randomized controlled trials comparing conventional second-trimester amniocentesis with first-trimester chorionic villus sampling (CVS) have shown that CVS carries a small but significant additional risk of fetal loss, a higher rate of false-positive diagnoses, and a higher laboratory failure rate.

First-trimester CVS which can be performed as early as 8 weeks of gestation has an advantage over second-trimester amniocentesis, which usually has been performed between 15 to 18 weeks of gestation, in that it allows for earlier prenatal diagnosis of genetic and cytogenetic fetal disorders and, therefore, earlier termination of affected pregnancies. However, the relative safety and diagnostic accuracy of the two methods have been unclear. A 4-year Medical Research Council (MRC)<sup>1,2</sup> trial has just been completed in Europe, following the Canadian Randomized Trial<sup>3</sup> published in 1989, to address questions of comparative risks, diagnostic accuracy, and subsequent malformation rates in the 2 methods.

The spontaneous pregnancy loss associated with second-trimester amniocentesis is 0.5% to 1.0%. The European results indicate that a woman who received CVS had a 4.6% less chance of a successful pregnancy outcome than a woman allocated to second-trimester amniocentesis. In Canada, there was a difference of 1.7% in total loss rates between the 2 groups.

In both trials, these differences related to all losses, spontaneous and induced, among women allocated to the 2 groups. As Garattini's editorial<sup>4</sup> accompanying the MRC report stresses, a complication in making such comparisons is introduced by the different periods of gestation during which the 2 tests are conducted. CVS is performed during a time when higher natural loss rates are expected, while amniocentesis is done after natural loss rates have peaked. Consequently, because both spontaneous and induced losses were regarded as risks of procedure in both trials, a woman choosing CVS during the first trimester had a priori a lower chance of achieving a successful pregnancy than a woman waiting until the second trimester and choosing amniocentesis. In addition, it probably is psychologically easier to decide to terminate in the first trimester, thus adding to the likelihood of the reported outcome for CVS vs amniocentesis.

Congenital abnormalities in infants following CVS also have been reported. Based on a number of studies, it appears that the possibility of some risk of post-CVS vascular disruption at a very early stage of gestation, which might lead to these abnormalities, cannot be dismissed. It is prudent not to undertake the procedure prior to the 10th week of gestation, pending further analysis.<sup>5</sup>

As Garattini also points out, the ultimate accuracy of the cytogenetic results is as important, and in some instances more so, than concerns of safety. In both the Canadian and European trials, accuracy was somewhat higher for amniocentesis than for CVS; in addition, the accuracy of CVS results is more dependent upon the skill of the laboratory conducting the analysis.<sup>1</sup> The primary reason for reduced accuracy of CVS is confined mosaicism, ie, chromosome abnormalities confined to the placental villi that are not found in the fetus. There also seems to be an increased risk of maternal cell contamination in CVS specimens.

Clearly, a prenatal diagnostic approach is needed that is as safe as second-trimester amniocentesis but that can be conducted earlier in gestation, thus decreasing the risk of physical and psychologic complications for women who choose to terminate an abnormal pregnancy. This new approach could be early amniocentesis (11 to 14 weeks). At a recent conference at St. Mary's Hospital in London,<sup>6</sup> the relative merits

and shortcomings of this approach were reviewed. Evidence from a number of centers indicates that early amniocentesis produced a fetal loss rate similar to that obtained with conventional second-trimester amniocentesis. However, one series that included 55 cases done before 12 weeks of gestation showed a loss rate of 14.8% for these cases. Also on the negative side, the loss rate after early amniocentesis in twin pregnancies was 8 of 35. Among 1,000 procedures done at 11 to 14 weeks of gestation at Pennsylvania Hospital (Philadelphia, Pennsylvania), 13 cases of minor orthopedic deformities were identified. The impression was that leakage of amniotic fluid might have occurred in a considerable number of women after early amniocentesis. However, on the positive side, the Belfast, Ireland group obtained a lower spontaneous abortion rate and found no congenital anomalies among 880 live-born babies following early amniocentesis.

Lung hypoplasia, possibly secondary to oligohydramnios, also may be a greater risk with early amniocentesis, as the proportion of amniotic fluid withdrawn is greater for early amniocentesis than for second-trimester amniocentesis.<sup>4</sup> A new technique, amniocentesis, whereby amniotic cells are filtered off as up to 40 mL fluid is withdrawn and the filtrate then returned to the amniotic cavity, might reduce the risk of oligohydramnios while increasing the harvest of cells. It remains to be seen whether the increased concentration of chorionic cells in the amniotic fluid in early amniocentesis increases the number of cases of mosaicism detected and the false-negative rates.

CVS does appear to carry a slightly increased risk of both loss of pregnancy and inaccurate results. Thus, these risks must be weighed against the advantage of earlier diagnosis and termination of an abnormal pregnancy when counseling women contemplating CVS. With the advent of high-resolution ultrasound techniques earlier amniocentesis has become a possibility, and may present a viable solution to this dilemma. We all will watch these developments with interest and hope for a safer and more accurate diagnostic procedure that can be performed at an earlier time than previously has been possible.

Judith G. Hall, MD

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## A Difference in Hypothalamic Structure Between Heterosexual and Homosexual Men

The question has long been asked: "Are differences in sexual preference dictated solely by psychosocial factors, or does biology play a determining role?" LeVay<sup>1</sup> has examined the brains of heterosexual and homosexual males to determine whether there might be neuroanatomic differences that are related to sexual preference. Such a difference was found in the interstitial nuclei of the anterior hypothalamus (INAH-3) cell group.

Allen et al<sup>2</sup> have shown that the volume of the cell group called INAH-3, which participates in the regulation of male-typical sexual behavior in nonhuman primates, is more than twice as large in males as in females. LeVay postulated that the brains of members of either sex who are attracted to females might differ from the brains of those who are attracted to males. Examining the anterior hypothalamus in 41 middle-aged individuals (16 presumed heterosexual males, 19 homosexual males, and 6 heterosexual females), LeVay found that the INAH-3 cell group differed in size between the homosexual and heterosexual males and that the INAH-3 cell group of the homosexual males resembled the structure found in females. This finding suggests that the INAH region is dimorphic with regard to sexual orientation, and may represent a biologic basis for sexual preference and orientation. In this study, LeVay examined the brains of homosexual men but not homosexual women. Previous studies in rats by Rhee et al<sup>3</sup> have shown that size differences in this part of the hypothalamus are influenced by levels of circulating androgens during a sensitive perinatal period; thus, exposure to altered androgen levels during this period may, at least in rats, affect sexual behavior in adult life.

### References

1. LeVay S. *Science* 1991;253:1034.
2. Allen LS, et al. *J Neurosci* 1989;9:497.
3. Rhee RW, et al. *Dev Brain Res* 1990;42:17.

**Editor's comment:** LeVay's study indicates that structural differences may indeed exist between the brains of heterosexual and homosexual males, a possibility that would move homosexuality further from the outdated definition of a psychiatric "disease" to one of a normal biologic variation. These findings, together with the hormone studies in rats, raise some interesting ethical questions regarding whether attempts might be made to prevent homosexuality in the future by prenatal hormone treatment or other biologic manipulation. Since there have been animal studies indicating that increased prenatal exposure to maternal stress hormones results in a higher frequency of homosexual offspring, it is also interesting to speculate that homosexuality might actually serve as an ecologic contraceptive — overcrowding results in increased maternal stress hormone production, which results in more homosexual offspring, which results in fewer productive unions, etc.

Finally, these studies are complicated in that some of the subjects (both homosexual and heterosexual) died of AIDS, but certainly the results will trigger additional and much-needed work on this question.

For further speculation, the reader is referred to an article entitled "Are Gay Men Born That Way?" (Time magazine, September 9, 1991).

Judith G. Hall, MD

## Decreased Stature Associated With Moderate Blood Lead Concentrations in Mexican-American Children

The association between blood lead concentration and growth has been difficult to ascertain because the high concentrations of lead are usually found in populations living under poor socioeconomic conditions. This study evaluated the relationship of blood lead concentration to stature and socioeconomic status in a large, representative sample of Mexican-American children.

The Hispanic Health and Nutrition Examination Study (HHANES) was conducted between 1982 and 1984 and characterized the health and nutritional status of 3 geographically distinct Hispanic groups in the United States. Poverty index, stature, hemoglobin, transferrin saturation, and blood lead data were available for 1,454 Mexican-American children. Their ages ranged from 5 to 12 years and their blood lead levels ranged from 0.14 to 1.92  $\mu\text{mol/L}$ . The poverty index ratio (PIR) developed by the National Center for Health Statistics (NCHS) was used to assess socioeconomic status. A PIR >1.0 implies that the family should be able to fulfill its basic necessities. The relationship of blood lead concentration and growth was analyzed through multiple regression analyses. Blood lead concentrations were classified into 2 groups: low lead and high lead, depending on whether blood lead concentration was above or below the age- and gender-specific medians for lead concentration.

The mean blood lead concentrations were 0.51  $\mu\text{mol/L}$  for males and 0.45  $\mu\text{mol/L}$  for females. Age, hemoglobin, and blood lead concentrations were the best statistically significant predictors of stature in males and together they accounted for 82% of the variance. For females, age, PIR, and blood lead concentrations were the best predictors and also accounted for 82% of the variance in height. The mean heights of children whose blood lead concentrations were low or high, adjusted for the effects of age and

hematocrit in males or age and PIR for females, revealed that children whose blood lead levels were above the median for their age and sex were approximately 1.2 cm shorter than children with blood lead concentrations below the median. The correlation of serum lead with stature was not due to an intercorrelation of serum lead with any of the other variables.

According to the Centers for Disease Control (CDC), lead values <1.20  $\mu\text{mol/L}$  are considered normal. However, this study demonstrated an inverse relationship between stature and blood lead concentrations, although mean blood lead levels were well below 1.20  $\mu\text{mol/L}$ . In addition, this inverse relationship was not due to collinearity of lead and socioeconomic factors as measured by the PIR. The study's finding of an inverse association between stature and blood lead concentration is in agreement with the analysis of the second National Health and Nutrition Examination Survey (NHANES II).

Frisancho AR, Ryan AS. *Am J Clin Nutr* 1991;54:516.

Wapnir RA, et al. *Pediatr Res* 1977;11:153.

Wapnir RA, et al. *Am J Clin Nutr* 1980;33:1071.

Wapnir RA, et al. *Am J Clin Nutr* 1980;33:2303.

**Editor's comment:** This well-designed study provides the strongest evidence to date that moderate lead levels, previously considered safe, are associated with reduced stature. The large, representative sample allowed examination of the confounding factors, such as poor socioeconomic status, that plagued other studies. The finding that children of all ages whose lead levels were above the median for age and gender were shorter than children with blood lead concentrations below the median leads to speculation of other effects of chronic lead exposure among this

population of Mexican-Americans. While the exposure was of sufficient duration to interfere with growth, the more toxic effects of lead and/or other sequelae of lead exposure were not evaluated. Further, the mechanisms by which lead retards growth were not addressed in this study; however, there are indications that nutritional factors may play an important role. In studies of oral lead ingestion in young rats, the intestinal transport capacity for glucose, amino acids, and sodium is altered even before the deleterious effects of lead on the kidney are evident. In addition, dietary inadequacies have been implicated. Epidemiologic studies report an inverse relationship between dietary calcium and serum lead concentrations. Interestingly, because lactase deficiency is more prevalent in Hispanic populations, the correlation of increased blood lead levels and shortened stature may reflect deficiencies in the intake and absorption of dietary calcium in these Mexican-American children. Additionally, elevated blood lead levels are

associated with anorexia, either as a primary determinant or secondary to iron deficiency anemia. Progression of weight gain, while not evaluated in this study, would be an important consideration for future studies. Even though the stature reduction associated with these blood lead levels is mild, if it is also associated with compromised nutritional status, then the adverse effects would be of greater concern since the consequences of lead exposure are exacerbated by energy and/or protein restriction.

Although additional research is required to elucidate the nutritional aspects of lead-induced short stature, this paper provides strong support for the reevaluation of the current CDC standards for acceptable blood lead concentration and renewed emphasis should be placed on minimizing the exposure of growing children to lead.

Fima Lifshitz, MD

## Standards For Selected Anthropometric Measurements in Prader-Willi Syndrome

Butler and Meaney present anthropometric measurements for children between the ages of 0 to 24 years with the Prader-Willi syndrome (PWS). Seventy-one white subjects who met the clinical criteria for the diagnosis of PWS (infantile hypotonia, hypogonadism, delayed psychomotor development and/or mental deficiency, early-childhood obesity, small hands and feet, and short stature) were included. High-resolution chromosomal analysis demonstrated that 52% of these individuals had an apparent deletion of the proximal long arm of chromosome 15. Anthropometric measurements included weight, length, sitting height, head circumference, head breadth, head length, total hand length, middle finger length, palm length, hand breadth, total foot length, foot breadth, triceps skin-fold thickness, and subscapular skin-fold thickness. Children below 2 years of age had length measured in the supine position; measurements after this age were made with a balance beam scale and anthropometer. Longitudinal data on several individuals were collected for up to 6 years. Subjects were grouped at either 3- or 4-year age intervals (eg, 0 to 4 years, 4 to 8 years, 8 to 12 years, 12 to 16 years, 16 to 20 years, and 20 to 24 years) and criteria of a sample size of 5 or more subjects per age group were utilized.

The results for height of males and females are presented in the figure. Sitting height was decreased proportionate to total height.

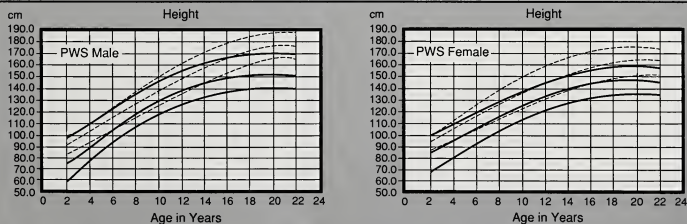
50th percentile head circumference and head length for PWS males and females during childhood were on approximately the 25th percentile for normal male children until approximately 20-22 years of age when the final measurements fell on the 5th percentile for normal males. Of all measurements taken, only those of the skin folds (triceps and subscapular) were greater than the data reported for normals. The 50th percentile for skin folds in PWS approximates the 95th percentile of normal females and is above the 95th percentile of normal males and reflects the obesity characteristic of these patients.

Butler MG, Meaney FJ. *Pediatr* 1991;88:853.

**Editor's comment:** This is a very detailed anthropometric study of 14 variables in a group of children who are seen frequently in pediatric endocrinology and/or genetics clinics. The incidence of PWS is estimated to be 1 in 16,000 live births, and it is a common form of dysmorphic obesity. The growth curves for height, weight, and other parameters that have been produced for all these variables (see original text) should be useful to clinicians interested in evaluating growth in individuals with this syndrome.

William L. Clarke, MD

Figure 1  
Standardized Curves for Height of Prader-Willi Syndrome



Standardized curves for height of Prader-Willi syndrome (PWS) male and female patients (solid line) and healthy individuals (broken line).

Reproduced by permission of *Pediatr* 1991;88:853.

## Effects of Therapy in X-Linked Hypophosphatemic Rickets

Verge et al prospectively studied 24 children (ages 1 to 16 years) with X-linked hypophosphatemic rickets who were treated from 0.3 to 11.8 years with daily calcitriol ( $25.6 \pm 16.9$  ng/kg/d) and oral phosphate ( $100 \pm 34$  mg/kg/d) administered in divided doses every 4 hours. Patients were evaluated every 3 months for serum electrolytes, blood urea nitrogen, creatinine, calcium, phosphate, and alkaline phosphatase and for height. Calcium, phosphorus, and creatinine were measured in 24-hour urine samples and in random urine samples collected to determine calcium to creatinine ratios. Glomerular filtration rates were measured in 20 patients. Annual height measurements were converted into height standard deviation scores (SDSs) according to data from the US National Center for Health Statistics. To exclude the effects of variation of the onset of puberty and the difficulty of measuring small infants, the first measurements reported were after the age of 2 years and the last were taken before the age of 10 years in girls and 12 years in boys. The mean interval of study of these 13 patients was  $5.0 \pm 2.3$  years.

Growth data were compared to those of 16 untreated prepubertal Australian patients with X-linked hypophosphatemia whose height SDSs were previously reported. Height SDSs also were computed according to the standards of Tanner. Renal ultrasonography was performed every 6 to 12 months. Duration of therapy, age at which therapy was begun, mean and total doses of vitamin D and phosphate, mean serum calcium, number of episodes of hypercalcemia, mean and maximum levels of urinary calcium and phosphorus excretion, number of episodes of hypercalciuria and the mean and maximal products of urinary calcium and phosphorus concentrations were examined as potential risk factors for nephrocalcinosis.

Patients treated with combined calcitriol and oral phosphate for at least 2 years had a mean height SDS (method of Tanner) of  $-1.08$  as compared with  $-2.05$  for the untreated control group ( $P=0.01$ ). However, the mean change in height SDS of the 13 patients treated for at least 2 years changed only from  $-1.42$  to  $-1.25$  ( $P=0.05$ ). When the change in the height SDSs were analyzed only with regard to the period of calcitriol and phosphate therapy, mean height SDSs increased from  $-1.58$  to  $-1.25$  ( $P=0.05$ ). No significant correlation was found between the change in the height SDSs and the duration of treatment or the age at which it began. Nineteen of 24 patients (79%) demonstrated nephrocalcinosis on renal ultrasonography. Regression analysis demonstrated a significant association between nephrocalcinosis and mean daily phosphate dose ( $r=0.60$ ,  $P=0.002$ ). Mean serum calcium concentrations in the 24 patients ranged between  $2.15$  to  $2.53$  mmol/L ( $8.6$  to  $10.1$  mg/dL). Fifteen patients had serum calcium concentrations of more than  $2.5$  mmol/L ( $10.0$  mg/dL) on 1 or more occasions. Eight of the 19 for whom urinary measurements were available had 1 or more episodes of hypercalciuria. These received significantly more calcitriol than those who never had hypercalciuria ( $29.9$  vs  $17.3$  ng/kg/d,  $P=0.007$ ); 4 of 20 had a decrease in glomerular filtration rate.

The authors conclude that since X-linked hypophosphatemic rickets is a benign disease compatible with a normal life span, the potentially serious side effect of nephrocalcinosis requires that the treatment regimen be reevaluated. Since the advent of combination therapy with calcitriol and phosphate, few patients now require surgical osteotomy; however, their data demonstrate only a modest effect on final height. They further suggest that since the growth pattern of untreated patients has not been well

documented, a prospective controlled trial of combination therapy needs to be undertaken to evaluate its effect on linear growth. The authors recommend the conservative use of phosphate and calcitriol during therapy and regular monitoring for both nephrocalcinosis and periodic determination of glomerular filtration rates.

Verge CF, et al. *N Engl J Med* 1991;325:1843.

**Editor's comment:** This very important and interesting article contributes significantly to the information concerning the effects of calcitriol and phosphate in X-linked hypophosphatemic rickets. The authors are correctly concerned with the high frequency of nephrocalcinosis in their patients.

An accompanying editorial (*N Engl J Med* 1991;325:1875) by Glorieux reviews the classification and therapy of all forms of rickets. Glorieux notes that data developed by Verge et al confirm earlier reports suggesting that phosphate and calcitriol improve the growth rate of children with X-linked hypophosphatemic rickets. However, at least one retrospective study (Stickler GB et al. *Lancet* 1989;2:902) concluded that failure of treatment to promote growth and the risks of renal failure suggested that it might be better not to treat these patients at all. Glorieux does not agree with Verge et al that a randomized, placebo-controlled trial should be undertaken. He points to the central role of hypophosphatemia in retarding growth as demonstrated by Harrison et al in 1966 (*Am J Dis Child* 1966;112:290-297). In that report, a girl with dwarfism and X-linked hypophosphatemic rickets had severe vitamin D intoxication that permanently reduced her glomerular filtration rate; her serum phosphate had increased to a normal level. Surprisingly, her final adult height reached the 50th percentile. Glorieux concludes that the frequent assessment of renal function is important in caring for these individuals.

Rickets and growth was recently reviewed in GGH (7:4:1-3). In that review it was suggested that if treatment starts before the age of 5 years, catch-up growth can be achieved. Readers need to realize that not all investigators agree on the extent of treatment benefit, but all agree that close observation is needed.

William L. Clarke, MD

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# Growth Hormone Resistance and Inhibition of Somatomedin Activity by Excess of Insulin-Like Growth Factor-Binding Protein (IGFBP) in Uraemia

Blum et al measured somatomedin bioactivity (SmBA), insulin-like growth factor 1 (IGF-1) and IGF-2 by radioimmunoassay (RIA); IGF-binding protein 1 (IGFBP-1) and IGFBP-3 by RIA; and free somatomedin-binding capacity (SmBC) in 2 groups of children. Group 1 consisted of 31 children with a mean age of  $10.5 \pm 4.8$  years who had end-stage renal failure (ESRF) and were on dialysis. Group 2, 11 children with a mean age of  $7.3 \pm 3.1$  years, had chronic renal failure (CRF) but with some residual glomerular filtration. All blood samples were taken in the morning.

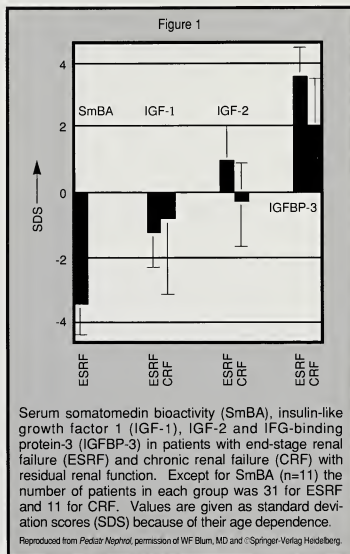
SmBA, as determined by a porcine cartilage assay, was subnormal in all subjects, while IGF-1 was in the low normal ranges, and IGF-2 was slightly elevated or normal (Figure 1). IGFBP-3 was markedly elevated in those with ESRF and was at the upper range of normal in group 2. In normals, a linear correlation has been found between the sum of IGF-1 and IGF-2 vs IGFBP-3 ( $r = 0.91$ ) and an exponential correlation has been reported between IGF-1 and IGFBP-3. In renal failure there is a marked deviation from these correlations, with higher IGFBP-3 levels for both IGF-1 plus IGF-2 vs IGFBP-3 and IGF-1 vs IGFBP-3. SmBC was increased in both groups, suggesting that the IGFBP was biologically active. IGFBP-3 of a molecular weight ranging from 12 kd to 150 kd is present in normal serum. However, IGFBP-3-like material in sera from ESRF subjects eluted in the range of 20 kd to 60 kd.

The authors demonstrated that SmBA is significantly decreased in patients with ESRF while serum IGF-1 and IGF-2 levels are in the normal range, and that IGFBP-1 and IGFBP-3 are elevated. They concluded that the excess of IGFBP-3 related peptide rather than IGFBP-1 plays a role in the inhibition of IGF activity. Indeed, the molar ratio of total IGF and IGFBP-3 is approximately 1.0 in normals but a large excess of IGFBP-1-like and IGFBP-3-like materials are present in uremia, suggesting a relative deficiency of IGF in these individuals. Since free SmBC was increased in the patients with CRF, the authors suggest that the excess of IGFBP is, in fact, biologically active. The authors further suggest that when renal function is impaired, low-molecular-weight IGFBPs accumulate, leading to an excess of IGFBP. This excess IGFBP results in a "sequestration" of IGF and an inhibition of IGF activity. Thus, IGFBP in uremia acts as a somatomedin inhibitor. The authors also conclude that IGF production itself is decreased in uremia, possibly as a result of an impaired growth hormone post-receptor event.

The following hypothetical schematic model is proposed. IGF-1, IGF-2, and IGFBP are produced in the liver and free IGF-1 and IGF-2 bind to IGFBP-3, which binds to a nonbinding subunit to form a high-molecular-weight complex, which remains in the circulation. While in equilibrium in normals, IGFs are released to act on their target cells, and low-molecular-weight IGFBP forms are cleared by the kidney. In renal failure, this clearance is impaired, and the IGFBPs accumulate in the circulation, leading to an excess of IGFBP over IGF. Thus, free biologically available IGF is lowered. In addition, IGF secretion is low.

Blum WF, et al. *Pediatr Nephrol* 1991;5:539.

**Editor's comment:** This is an elegant paper that demonstrates important findings concerning circulating growth factors and their binding proteins in uremic children. The findings suggest a possible mechanism for growth failure in uremic children whose growth hormone secretion may be within normal limits. It also suggests reasons why these children may respond favorably to the



administration of exogenous growth hormone with an improvement in height velocity. The paper would have been more interesting had the authors included more information concerning the children who were studied. Of particular interest would have been information concerning growth velocity and growth hormone secretion at the time of the study. Combined with previously reported data on GH failure in CRF (Schaefer et al, *GGH* 1991;7:2), and GH pulsatility and linear growth response to exogenous GH (Rees et al, *GGH* 1991;7:2), the data concerning the pathophysiology of growth in CRF is becoming more understandable.

William L. Clarke, MD

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**July 12-15, 1992** 24th Ann March of Dimes Clinical Genetics Conf, Stanford, CA. Info: Prof Svs Dept, March of Dimes Birth Defects Found. Tel: 914-428-7100.

**July 20-31, 1992** Bar Harbor Mammalian Genetics Course, Bar Harbor, ME. Info: J Musetti. Tel: 207-288-3371 ext. 1253.

**August 5-9, 1992** The David W Smith Workshop (Malformation and Morphogenesis), Wake Forest Univ, Winston-Salem, NC. Info: J Dean. Tel: 803-223-9411, Fax: 803-227-1614.

**August 30 - September 5, 1992** 9th Int'l Congress of Endo, Nice, France. Info: NICE 92, c/o SOCF1, 14 Rue Mandar, 75002 Paris, France.

**September 7-10, 1992** 31st Ann Mtg of the ESPE, Zaragoza, Spain. Info: Dr A Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hosp, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-76-355700.

**September 10-12, 1992** Int'l Congress on Growth Hormone and Somatomedins During Lifespan, Milan, Italy. Info: Drs D Cocchi/V Locatelli, Dept of Pharm, Univ of Milan Sch of Med, Via Vanvitelli, 32, 20129, Italy.

**October 8-9, 1992** Int'l Symp on Growth '92 - 2 Decades of Experience in Growth, Santiago de

Compostela, Spain. Info: Dr S Rossetti, Ares-Serono Symposia, Via Ravenna 8-00161 Rome, Italy. Fax: 39-6-44291324.

**October 10-14, 1992** 44th Postgrad Assembly of the Endo Soc, Boston, MA. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

**November 4-7, 1992** The Role of Insulin-like Growth Factors in the Nervous System, Arlington, VA. Info: The New York Academy of Sciences. Tel: 212-838-0230; Fax: 212-888-2894.

**November 9-13, 1992** Ann Mtg of Am Soc of Human Genetics, San Francisco, CA. Info: M Ryan, ASHG. Tel: 301-571-1825; Fax: 301-530-7079.

**December 3-6, 1992** Growth Hormone II: Basic and Clinical Aspects, Tarpon Springs, FL. Chairpersons: Drs B Bercu/R Walker. Info: Sero Symposia, Dr B Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

**June 3-7, 1993** 4th Joint Mtg of the LWPES/ESPE, San Francisco, CA. Info: Prof M Grumbach, Univ of CA Sch Med. Tel: 415-476-2244; Fax: 415-476-4009.

**June 9-12, 1993** 75th Ann Mtg of the Endo Soc, Las Vegas, NV. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

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# GROWTH

## Genetics & Hormones

Vol. 8 No. 3

September 1992

### Current Status of Somatic Gene Therapy

**Fred D. Ledley, MD**

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*Departments of Cell Biology and Pediatrics*

*Baylor College of Medicine*

*Houston, Texas*

Genetic therapy for human disease has been variously heralded as a panacea or as a threat to fundamental human and ethical values.<sup>1-3</sup> Since the initiation of clinical trials involving gene transfer<sup>4</sup> and gene therapy<sup>5</sup> at the National Institutes of Health (NIH) in 1989, the hyperbole that has often surrounded this debate has ceded to a more critical assessment of the clinical indications for gene therapy for specific disease processes. While the therapeutic efficacy of somatic gene therapy has not yet been established for any specific disease in either animal models or clinical trials, experimental data demonstrate that the safety of somatic gene transfer is commensurate with that of conventional experimental therapeutic agents. Various regulatory bodies, including the Recombinant DNA Advisory Committee and the Food and Drug Administration, have demonstrated their willingness to approve judicious clinical trials aimed at establishing the feasibility and safety of somatic gene transfer procedures. The number of clinical trials involving gene transfer and gene therapy is increasing at a rapid rate, and it is likely that the first commercial products will be in Phase II or Phase III trials and licensed within several years. This review describes current methods that may be employed for somatic gene therapy with particular reference to those that may be applicable to endocrine disorders and disorders of growth.

#### PRINCIPLES AND APPROACHES TO SOMATIC GENE TRANSFER

The basic principle of somatic gene therapy is that recombinant genes can be introduced into somatic cells to alter the course of a disease process. Most research has focused on single-gene disorders based on the paradigm that inherited defects in essential genes might be treated by introducing a normal copy of that gene into somatic cells. While single gene disorders represent models for basic research, these disorders are rare. If somatic gene therapy is to have a significant impact on medical practice, it will be for polygenic, multifactorial, and acquired diseases, which are more common.

The initial clinical trials of somatic gene therapy at the NIH provide examples for gene therapy of both monogenic and polygenic disorders. The clinical trial of gene therapy for adenosine deaminase deficiency<sup>5</sup> involves introducing a normal copy of this gene into genetically deficient T cells to correct severe combined immunodeficiency disease (SCID).

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Another clinical trial involves treating solid tumors by introducing a gene for tumor necrosis factor into a select population of T cells (tumor-infiltrating lymphocytes) that are capable of migrating to the tumor and delivering a high level of this natural antitumor agent specifically to these sites.<sup>6</sup>

Somatic gene therapy as currently conceived does not involve the repair or removal of genes bearing pathogenic mutations; most importantly, it does not involve any manipulations of the inherited germ line. While technologies for site-specific modification (homologous recombination) of genes in embryonic cells are well developed in mice, these techniques have not yet been applied successfully in other species. These technologies also raise difficult ethical and social issues, and it is unclear when such interventions would be clinically indicated.

## METHODS FOR SOMATIC GENE TRANSFER

The enabling technology for somatic gene therapy is the ability to transfer recombinant genes into somatic cells. Two major methods can be distinguished: *DNA-mediated gene transfer* involves introducing pure DNA or complexes of DNA bound to various carriers into cells and is referred to as *transfection*. *Viral-mediated gene transfer* involves the use of viral particles as vehicles for delivering genes to cells by the process of infection and is commonly referred to as *transduction*.

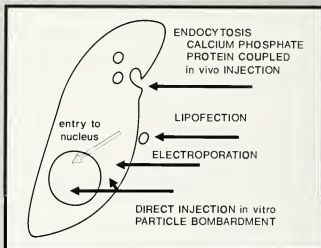
### DNA-Mediated Gene Transfer

DNA-mediated gene transfer is based on the observation that cells in culture that are exposed to DNA in various forms can take up these molecules and express the gene products that they encode. The classic methods for transduction is *microinjection*, in which DNA is physically injected into the nucleus of cells in culture.<sup>7</sup> Other methods include administration of calcium phosphate precipitates,<sup>8</sup> *electroporation*,<sup>9</sup> *lipofection*/*liposome fusion*,<sup>10</sup> particle bombardment with DNA bound to magnetic particles,<sup>11</sup> and receptor-mediated uptake by DNA protein complexes.<sup>12</sup> The mechanism by which cells take up and express DNA remains unclear, but for several of these methods it is thought to involve endocytosis of DNA into cells and release from endosomes before degradation is complete (Figure 1). Recent data suggest that the admixture of DNA with adenoviral particles, which enhance the release of endosomal contents into the cytoplasm, can increase the

efficiency of some methods of transfection more than a thousandfold.<sup>13</sup>

When DNA is taken up by cultured cells, recombinant gene expression is detected for a short period; this is referred to as a period of *transient expression*. *Stable* integration of DNA into the chromosomes of transfected cells is rare.

Figure 1  
Mechanisms of DNA-Mediated Gene Transfer



Schematic of DNA uptake into cells by DNA-mediated gene transfer. Exposure of cells to DNA, DNA precipitated with  $\text{CaPO}_4$ , or DNA coupled to trophic peptides results in uptake by cells by endocytosis. Exposure of cells to DNA packaged in liposomes leads to uptake by membrane fusion. Exposure of cells to DNA in the presence of a strong electric pulse results in the creation of pores in the membrane and electrophoresis of DNA into the cell. DNA also can be introduced directly into the cell or nucleus by injection or bombardment with DNA-bound magnetic particles. The mechanism by which DNA enters the nucleus remains unknown.

The possibility of DNA-mediated gene transfer in vivo was suggested by early experiments in which the injection of viral DNA into animals resulted in the production of infectious virus particles. Recent research demonstrates that injection of DNA into muscle<sup>14</sup> and thyroid<sup>15</sup> leads to uptake and expression of recombinant sequences into these cells in vivo. DNA-mediated gene transfer into hepatocytes has been achieved by injecting DNA/asialoglycoprotein complexes, which are specifically taken up into the liver via the asialoglycoprotein receptor.<sup>12</sup> In the liver and thyroid, transient expression of injected genes is observed for several days. In muscle, more prolonged, although not permanent, expression has been observed.<sup>14</sup>

## Viral-Mediated Gene Transfer

The principle of viral-mediated gene transfer, or transduction, is that viral vectors could be used as a "Trojan horse" for introducing recombinant gene sequences into cells. Since viral infection is highly efficient, and since some viruses stably integrate their genome into the chromosomes of the infected cells, viral-mediated gene transfer has been viewed as the preferred method for attaining efficient long-term expression of recombinant genes.

Viral vectors based on the Moloney murine leukemia virus<sup>16</sup> have been employed in each of the clinical trials performed to date and are presently the vectors of choice for somatic gene therapy. The use of retroviral vectors for gene therapy is based on the fact that it is possible to separate the process of producing a viral particle from the process of packaging a recombinant gene into these particles (Figure 2).

A cell that is genetically modified to express the viral proteins *gag-pol* and *env* in the absence of an intact viral genome will assemble viral particles and is referred to as a *packaging cell line*. If another gene is introduced into these cells, which contains a human cDNA along with the  $\psi$  (packaging) sequence and dual *long terminal repeat* (LTR) sequences, the

transcript bearing the  $\psi$  sequence will be packaged into the empty viral particles. This produces a *defective retrovirus*, which is capable of infecting target cells and permanently introducing the recombinant gene into the chromosome but which does not contain genes encoding any viral proteins and is incapable of expressing any viral functions.

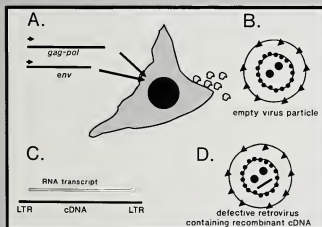
Extensive safety testing in nonhuman primates,<sup>17</sup> the results of initial clinical trials,<sup>4</sup> and the fact that there is no known pathology caused by similar viruses that are ubiquitous in our environment suggest that these agents are relatively safe. There continues to be concern about the theoretical risk of malignancies due to insertional mutagenesis and the potential for homologous recombination with wild-type viruses, but these risks are calculably small.<sup>18</sup>

Retroviral vectors have been shown to be capable of transducing a variety of cell types that could be targets for gene therapy, including hematopoietic progenitors, lymphocytes, hepatocytes, fibroblasts, endothelial cells, keratinocytes, thyroid follicular cells, and others. One of the limitations of retroviral vectors is that stable gene transfer requires cell division, thus restricting the potential targets for these vectors. Recently, adenovirus<sup>19</sup> and adeno-associated virus<sup>20</sup> have been proposed as vehicles for somatic gene therapy. These vectors may have advantages for certain clinical applications, although there are presently less data on feasibility and safety than there are for retroviral vectors.

## SOMATIC TARGETS FOR GENE THERAPY

Many somatic targets may be considered candidates for somatic gene therapy (Figure 3, page 4). The major focus of gene therapy research has been to develop methods for introducing recombinant genes into different somatic sites and achieving stable and appropriately regulated expression of recombinant genes. Each target tissue presents different problems of molecular biology, gene regulation, cell biology, and surgery, which will not be reviewed in detail here. Three somatic targets are currently the object of clinical trials. Bone marrow was initially considered an ideal candidate for somatic gene therapy since transduction of a discrete population of stem cells would theoretically replenish the entire mass of marrow-derived elements. Progress has been inhibited by the complexity of recognizing the pluripotent stem cell and attaining appropriate expression of recombinant genes through the process of differentiation. The first clinical trials of somatic gene transfer involved introducing genes

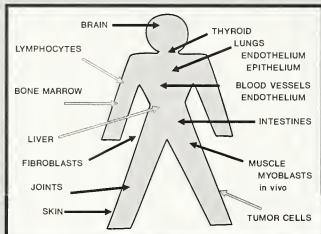
Figure 2  
Mechanisms for Synthesizing Defective Retroviral Vector



Schematic of strategy for producing defective retroviral vectors for viral mediated gene transfer. If a cell is transfected with genes encoding the 3 major retroviral proteins *gag-pol*, and *env* (A), these gene products will self assemble and bud off empty virus particles (B). If an expression vector containing the packaging signal ( $\psi$ ) and LTR sequences is introduced into this cell, the RNA transcript from this vector will be packaged into the empty viral particles, producing a defective retrovirus capable of being used for gene transfer (D).



**Figure 3**  
**Targets for Somatic Gene Therapy**



Potential targets for somatic gene therapy. Many cells and organs have been considered targets for gene therapy in *in vitro* or animal experiments. Those targets indicated by shaded lines are currently under clinical investigation.

into T cells collected from tumors (tumor-infiltrating lymphocytes) or peripheral blood (peripheral blood lymphocytes).<sup>4,6</sup> The limited life span of these cells after transplantation limits the scope of this approach to gene therapy. Hepatocytes represent another target for clinical trials of somatic gene therapy.<sup>21,23</sup> The demonstration that hypercholesterolemia in the low-density lipoprotein (LDL) receptor—deficient Watanabe rabbit could be ameliorated by introducing a recombinant LDL-receptor gene into hepatocytes represents the first successful application of somatic gene transfer to alter a systemic phenotype in animals.<sup>24</sup> This approach to somatic gene therapy is limited by the difficulty of large-scale hepatocyte cultivation and the lack of clinical precedent for hepatocellular transplantation.

DNA-mediated gene transfer into the liver,<sup>11</sup> muscle,<sup>13</sup> and thyroid<sup>14</sup> has been demonstrated in animal models. This method is generally simpler than viral-mediated gene transfer, although it generally results in only transient expression of the gene product. There is considerable interest in using gene transfer into muscle to treat muscular dystrophy, produce hormones or enzymes, or provide vaccines.

#### **APPLICATIONS OF SOMATIC GENE THERAPY IN GROWTH AND DEVELOPMENT**

In considering somatic gene therapy for a specific disease, it is necessary to have a clear understanding of the molecular nature of the

disease process, how a discrete molecular intervention would alter the course of the disease,<sup>25</sup> and how the potential benefits balance the risks of this highly experimental therapy.<sup>26</sup> Inborn errors of metabolism are frequently associated with poor growth and impaired development and are considered important models for somatic gene therapy.<sup>25</sup> Inherited disorders of endocrine factors such as growth hormone, parathyroid hormone, or thyroid hormones also are attractive candidates for somatic gene therapy, and progress has been made in developing a gene therapy for each of these disorders. Somatic gene therapy not only is applicable to rare inherited defects in single genes but also can be used to alter the course of polygenic or multifactorial processes too numerous to list. Gene transfer could be used to provide hormones that are deficient because of acquired diseases such as diabetes mellitus (type I) as well as increasing expression of hormones such as growth hormone to alter complex disease states or even aging. Recent reports demonstrate that significant systemic levels of growth hormone can be achieved *in vivo* by gene transfer into muscle cells using either viral or DNA vectors.<sup>27-28</sup>

The critical element in using gene transfer methods to supply essential endocrine factors is to achieve proper regulation of the recombinant gene product. This can be achieved by constructing vectors with regulated promoter elements, by the choice of target tissue that responds to different endocrine or paracrine factors, and by considering different modes of gene delivery (stable versus transient).

In considering somatic gene therapy for genetic diseases affecting growth and development, one rapidly confronts the specter of using the same technologies to enhance normal growth. While somatic gene transfer as a means for treating human disease has come to be almost universally accepted by ethicists, theologians, and legal bodies,<sup>22</sup> the notion of enhancement engineering continues to be viewed with concern. There is no way to ensure that genetic technologies will not be abused by individuals seeking personal enhancement. What is critical is to ensure that gene therapy is seen only as a means for allopathic correction of recognized diseases and that any application of gene therapy meets the highest possible standards of safety, fairness, and voluntary informed consent. With these cautions, it is likely that somatic gene therapy will be widely studied in the coming decade as a means for treating a wide variety of genetic and acquired diseases, and that this technology may have a significant impact on the practice of medicine.

## ACKNOWLEDGMENTS

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## Letter From the Editor:

Dr. Herbert Miller, a highly respected senior pediatrician with a longtime interest in neonatology, submitted the following paper as a follow-up to that published by Dr. Joseph Warshaw in *GGH* Vol. 8, No. 1. It will be of interest to those who work with small or intrauterine growth restricted infants.

Robert M. Blizzard, MD

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# Intrauterine Growth Retardation: Past, Present, and Future

**Herbert C. Miller, MD**  
*Professor Emeritus of Pediatrics*  
*University of Kansas Medical Center*

The summary of a recent review<sup>1</sup> of fetal growth retardation makes the strong statement that epidemiologic research of intrauterine growth retardation (IUGR) continues to be "hampered by the lack of a broadly used standard for defining fetal growth." The need for such a standard is long overdue, but the problems associated with all aspects of IUGR in the United States go beyond just the need for a "broadly used standard." Investigators do not agree on whether there are different types of IUGR or if IUGR is an entity to be diagnosed solely by a low birth weight for gestational age; the causes and prevalence of IUGR differ widely among investigators.

Many of the faults associated with IUGR in the United States are illustrated in a recent meta-analysis<sup>2</sup> of 6 published studies that suggested the occurrence of IUGR among infants born to mothers with pregnancy-induced hypertension was reduced by 44% among mothers taking daily doses of aspirin in amounts of 50 to 150 mg during the second and third trimesters. The diagnosis of IUGR in the meta-analysis was made on the basis of a birth weight below the 10th percentile for the infant's gestational age. No mention was made of which of the several standards was used in diagnosing IUGR, which of the several types of IUGR was reduced in frequency by prophylactic aspirin, whether the mothers had additional risk factors in their pregnancies, whether the women were white or black, or whether the infants were preterm or full term.

In his review of IUGR, Warshaw<sup>1</sup> recognized 2 types of IUGR: the symmetric and the asymmetric. In the symmetric type, he indicated that body weight, crown-heel length, and head circumference showed the same degree of growth restriction at birth, and stated that most infants with this pattern born after 36 weeks gestation continued to exhibit sluggish postnatal growth. It is not clear whether the reduction in body weight was solely dependent on the reductions in crown-heel length and head circumference or also involved a reduction in soft tissue mass. Some newborn infants have small skeletal dimensions for their gestational ages, including short crown-heel lengths and small head circumferences, that occur in combination with significant reductions in soft tissue mass. These infants with the combined pattern of reductions in skeletal size and soft tissue mass have the most severe type of IUGR, a condition that occurs less frequently than infants with the asymmetric type.<sup>3</sup> Warshaw does not describe which parameters of growth are retarded in the asymmetric type.

As described by Gruenwald<sup>4</sup> nearly 30 years ago, asymmetric infants at birth can have significant reductions in soft tissue mass with normal lengths for their gestational ages, or they can have significant reductions in crown-heel lengths for their gestational ages without reductions in soft tissue mass.<sup>4</sup> Warshaw does describe distinct differences in postnatal growth in infants with the asymmetric type of IUGR resulting from nutritional compromise; one group showed rapid catch-up growth after birth, but approximately one third of the asymmetric infants were still below the 5th percentile at 2 years of age. The differences in postnatal growth suggest that the infants who had rapid catch-up growth also had reductions in soft tissue mass and normal crown-heel length, and infants with sluggish growth in the first 2 postnatal years had short crown-heel lengths for their gestational ages and normal amounts of soft tissue, especially subcutaneous fat. These conclusions are based on follow-up studies of infants born at the University of Kansas Medical Center with these 2 different types of asymmetric fetal growth.<sup>5</sup>

Diagnosing IUGR by types increases the reported frequency of IUGR substantially, compared with diagnosing IUGR solely by low birth weight for gestational age.<sup>6</sup>

Warshaw's recent review of IUGR suggested that the standards developed by Brenner and colleagues<sup>7</sup> might serve as an example for evaluating fetal growth in newborn infants. Their standard was based on infants born during the period from 1962 to 1969 and was limited to birth

#### In Future Issues:

##### **Fragile X Syndrome: Review and Current Status**

by David L. Nelson, PhD

##### **Effects of Drugs and Other Chemicals on Fetal Growth**

by J.M. Friedman, MD, PhD

##### **Relevance of Developmental Genetics to Human Malformations**

by Golder N. Wilson, MD, PhD

##### **Sleep, Growth Hormone Secretion, and Short Stature**

by Richard H. Wu, MD, and  
Paul Saenger, MD

weights of infants with gestational ages of 21 to 44 weeks.<sup>7</sup> Corrections were made for parity, race, and sex, with gestational ages ranging from 36 to 42 weeks inclusively. No standard was provided for evaluating whether infants had abnormally short crown-heel lengths or had normal body lengths at birth. No standards were provided for evaluating head circumferences at birth.

It is important to recognize that the types of IUGR that occur at birth also occur in older infants and young children and are diagnosed by careful measurements of height and weight. There are no valid reasons for not making careful measurements of crown-heel lengths, head circumferences, and weights at birth. The types of IUGR that occur in newborn infants can be suspected by simple inspection of the infants; however, for the sake of the infant, parents, and other physicians caring for newborn infants, it is important to carefully make and document measurements.

The problems associated with the diagnosis of IUGR in the United States are so manifold and so important that a national committee of obstetricians and pediatricians should be given the task of trying to establish guidelines for diagnosing IUGR in newborn infants, including standards of fetal growth.

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# The Effects of Irradiation on Endocrine Function in Children

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Radiation may directly impair hypothalamic, pituitary, thyroid, and gonadal function or, alternatively, induce the development of hyperparathyroidism, thyroid adenomas, or carcinomas. Clinical presentations include short stature, failure to undergo normal pubertal development, precocious puberty, hypothyroidism, thyroid tumors, gynecomastia, infertility, and varying degrees of hypopituitarism.

## GROWTH IMPAIRMENT

A number of factors may adversely affect growth in children with tumors, including radiation damage to the hypothalamic-pituitary axis (HPA), a direct radiation effect on growing bones, hypothyroidism and precocious puberty secondary to irradiation, cytotoxic chemotherapy, malnutrition, residual tumor, corticosteroid therapy, and graft-versus-host disease in those receiving bone marrow transplantation.

### Hypothalamic-Pituitary-Adrenal Axis

Deficiency of one or more anterior pituitary hormones is a recognized sequel of external radiotherapy to the HPA in childhood. The radiotherapy may be part of the treatment for a brain tumor distant from the HPA or for acute lymphoblastic leukemia (ALL), retinoblastomas, or nasopharyngeal tumors. With radiation of the HPA, growth hormone (GH) is always the first hormone to be affected. Gonadotropin (Gn), corticotropin (ACTH), and thyrotropin (TSH) deficiencies usually follow in that order. Littley et al<sup>1</sup> reported that 5 years following 3.75 to 42.5 Gy, all patients treated were growth hormone deficient (GHD), 91% Gn, 77% ACTH, and 42% TSH deficient. The degree of hormonal deficit is related to the radiation dose. Following lower radiation doses, isolated GHD occurs, while higher doses may result in panhypopituitarism. The speed of onset of GHD also is dose-dependent; the higher the radiation dose, the sooner GHD ensues.

Moell et al<sup>2,3</sup> described reduced spontaneous GH secretion using 24-hour profiles in prepubertal and pubertal girls following cranial irradiation for ALL (20 to 24 Gy). The expected

increase in GH secretion at puberty did not occur, and there was attenuated pubertal growth.

The dose of irradiation employed in prophylactic cranial irradiation for ALL recently was reduced to 18 Gy. Results of recently completed studies by Crowne et al<sup>4</sup> of GH secretion after 18 Gy of cranial irradiation in children differed from those obtained by Moell et al.<sup>3</sup> Spontaneous GH secretion was normal in prepubertal children, but pubertal children showed abnormalities of spontaneous GH secretion despite the fact that each underwent puberty spontaneously and showed normal sexual progression. There was both reduced secretion of GH and significant disturbance in the periodicity of GH secretion in these children.<sup>4</sup>

### Spinal Irradiation

Brain tumors with potential to disseminate within the central nervous system usually are treated with craniospinal irradiation, and the whole spine is included within the radiation field. The radiation field in the management of other tumors, for example flank irradiation in Wilms' tumor, also includes part of the spine. We demonstrated that whole spine irradiation of 27 to 35 Gy will appreciably impair spinal growth.<sup>5</sup> Our most conservative figures indicate that the eventual loss in height is 9 cm when irradiation is given at 1 year of age, 7 cm when given at 5 years, and 5.5 cm when given at 10 years.

Both standing and sitting height standard deviation scores (SDSs) were more negative for children receiving craniospinal irradiation than for those receiving only cranial irradiation (Figure 1, page 8).

### Precocious Puberty

Early puberty occurs in some children receiving cranial irradiation for brain tumors and ALL.<sup>6</sup> Puberty in children with radiation-induced GHD occurs at a significantly earlier chronologic and bone age than in children with spontaneous isolated idiopathic GHD. Both sexes are affected, although the female is more vulnerable to lower radiation doses. Leiper et al<sup>6</sup> reported that the mean age of onset of puberty in 23 girls with early puberty secondary to 18 to 24 Gy of cranial irradiation was 8.8 years, greater than 2 SD from the mean of normal girls. These 23 girls were treated at a mean age of 4 years.



## INDICATIONS FOR GROWTH HORMONE THERAPY

Long-term studies following GH therapy are essential. Ideally, these should include an analysis of the final height and gain or loss in stature (SDSs) from initiation of GH therapy until the end of growth in children with radiation-induced GHD and compared with data from similar patients not receiving GH. Shalet et al<sup>7</sup> and Sulmont et al<sup>8</sup> reported that GH therapy was of significant benefit in children with radiation-induced GHD; however, the height gained, or rather the "height loss" that had been prevented, was disappointingly small and much less than that seen in GH-treated children with idiopathic GHD. A number of factors contributed to the suboptimal growth response, including spinal irradiation, early puberty, an extended interval (mean, 6.0 years) between irradiation and the initiation of GH therapy, and the inadequacy of the GH dosage used in the early studies.

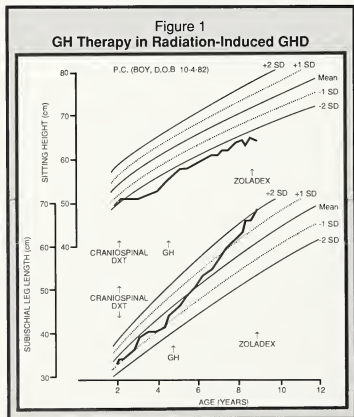
The chances of recurrence of a brain tumor are greatest within 2 years of the primary treatment of the tumor. No evidence exists that GH treatment increases recurrence rates of brain tumors in children with radiation-induced GHD. A reasonable approach, therefore, 2 years after treatment is to consider GH therapy for children with brain tumors treated by standard radiation schedules, including a dose to the HPA in excess of 30 Gy. At this time, cytotoxic chemotherapy is completed. The chance of tumor recurrence is

low, and it has been established that most patients will be GHD. In some centers, endocrinologists offer GH therapy routinely at 2 years without recourse to GH tests or evidence of impaired growth. In other centers, endocrinologists insist on biochemical evidence of GHD and a subnormal growth rate. In our institution, we establish GHD biochemically and then consider GH therapy independent of the growth rate in GHD patients. These guidelines assume that in craniospinal-irradiated children, growth is assessed by leg length velocity and that other causes of poor growth such as radiation-induced hypothyroidism, recurrent tumor, and malnutrition have been excluded.

In practice, our management attempts to match the timing of the introduction of GH therapy with the special circumstances, age, pubertal status, and needs of the individual. Early treatment is particularly suitable for the craniospinally treated young child of short parents, although disproportionate growth may occur (Figure 1).

## ACUTE LYMPHOCYTIC LEUKEMIA

Much confusion and controversy have been generated over the growth patterns and GH requirements of the child with ALL treated with prophylactic cranial irradiation and combination cytotoxic chemotherapy. Some groups report no adverse effects on final height. Others have noted a modest adverse effect on growth.<sup>7</sup> Since final height is unknown in most of the children reported, the possibility of impaired pubertal growth may mean that final height loss is substantial in a minority of children. Kirk et al<sup>9</sup> reported significant retardation of growth. They reported marked slowing of growth 3.0 to 9.5 years after diagnosis in 77 children treated for ALL with chemotherapy plus 24 Gy of radiotherapy as cranial prophylaxis. The Z score, which reflects the deviation of height measurements from the population mean, was used to assess height change. The mean Z score was +0.16 at diagnosis and -1.37 6 years later. Height for age fell by more than 1 SD in 71% of the survivors after 6 years. Younger children and those tall for age at diagnosis were more severely affected. Thirty of 46 patients tested had partial or complete GHD as determined by provocative testing. Analysis of the radiation schedules and chemotherapy protocols used in different centers has led to some understanding of the explanation for the differences in height loss observed between groups. For example, the duration and nature of the combination cytotoxic chemotherapy may influence the growth prognosis. After treatment



with regimens used in the United Kingdom, the effects of cytotoxic chemotherapy are likely to be minor. More intense cytotoxic chemotherapy regimens have had a profound impact on growth.<sup>9</sup>

For many reasons, the need for treatment with GH is more difficult to predict after treatment for ALL than after a brain tumor. One relates to the dissimilar growth patterns observed by different groups in children with ALL.<sup>10,11</sup> The clinical dilemma is how to identify the few who should receive treatment. In general, we suggest that in the presence of biochemical evidence of GHD, those children who are below the 10th percentile, or those whose growth rate is persistently poor after completion of cytotoxic chemotherapy should be considered for a therapeutic trial of GH.<sup>7</sup> GH therapy is usually warranted in short peripubertal children who received 18 Gy of cranial irradiation.

## TOTAL BODY IRRADIATION

Following total body irradiation (TBI), severe growth disturbance is common<sup>12</sup> and may be caused by various etiologic factors, including GHD, thyroid dysfunction, radiation-induced impairment of skeletal growth, or graft-versus-host disease and its treatment. GHD may occur even if the child has not received prophylactic cranial irradiation previously and whether or not the TBI schedule consisted of a single or fractionated dose.

Papadimitriou et al<sup>13</sup> presented preliminary data that suggested a modest benefit followed GH therapy in children with TBI-related growth failure. There was an increase in height velocity adequate to restore a normal growth rate but catch-up growth did not occur. However, the heterogeneous nature of the 13 patients studied confused the issue. Furthermore, the mean age (12.2 years) at which GH therapy was introduced was late.

Graft-versus-host disease and hypothyroidism must be excluded in children who are growing poorly following TBI. Following this, standard provocative tests of GH secretion are required. If the GH responses are subnormal, GH therapy should be offered. If GH responses are normal, a number of questions remain. Is the child growing slowly because of radiation-induced skeletal dysplasia or because of GH neurosecretory dysfunction? Should a 24-hour GH profile be performed to try to establish the latter diagnosis, or should the child receive empiric GH therapy on a trial basis? If GH therapy is instituted, what is the optimum schedule in the possible presence of radiation-induced skeletal dysplasia? As is evident from

these questions, there is a desperate need for more information on the impact of single and fractionated courses of 10 to 13 Gy total body irradiation on the incidence of GHD, on the frequency of GH neurosecretory dysfunction, and on the speed of onset of GHD, as well as on the effect of this dose on the natural history of radiation-induced skeletal dysplasia.

## THYROID DISEASE

The most important complications of radiation to the thyroid gland are hypothyroidism and thyroid tumors.<sup>14</sup> An association between X-ray exposure and thyroid cancer was suggested by Duffy and Fitzgerald.<sup>15</sup> More recently (1989), Ron et al<sup>14</sup> studied 10,834 children who received X-ray therapy for tinea capitis between 1948 and 1960. These were compared with 10,834 nonirradiated controls and 5,392 nonirradiated siblings. Ninety-eight thyroid tumors (~1:100) were identified among the exposed, and 57 (~1:280) among the nonirradiated. An estimated dose of 0.09 Gy was linked to a fourfold increase of malignant tumors and a twofold increase of benign tumors.

### Thyroid Dysfunction

Thyroid dysfunction, after irradiation and cytotoxic chemotherapy in adults with Hodgkin's disease, ranges from frank hypothyroidism with increased TSH and low  $T_4$  concentrations to compensated thyroid dysfunction with raised TSH, but normal  $T_4$  levels.<sup>16</sup> After a radiation dose to the neck of 40 to 50 Gy (fractionated dose) was given to similar patients,<sup>17</sup> approximately 25% of patients showed elevated TSH and low  $T_4$  concentrations, while a further 41% had raised TSH levels in the presence of normal  $T_4$  levels. The time interval between thyroid irradiation and the peak incidence of thyroid dysfunction is unknown. However, the low incidence of thyroid dysfunction (14%) after 1 year rose to a cumulative incidence of 66% 6 years postirradiation.<sup>17</sup>

Children whose thyroid glands are irradiated during craniocervical irradiation for brain tumors, or during TBI before bone marrow transplantation, are vulnerable to thyroid dysfunction. Of significant importance is the finding that the incidence of thyroid dysfunction (16%) following fractionated TBI is much lower than that reported after single fraction TBI (39% to 59%).<sup>12</sup> The incidence may vary with time as recovery of thyroid function has been observed in patients with documented thyroid dysfunction following TBI.<sup>18</sup>

Thirty percent of children treated for brain tumors with cranial or craniocervical irradiation, with or without adjuvant cytotoxic chemotherapy, will develop thyroid dysfunction at some time after

treatment. The most frequent abnormality is compensated thyroid dysfunction. In a high proportion, thyroid function reverts to normal with time. It appears that the combined effect of direct irradiation to the thyroid gland during craniospinal irradiation plus cytotoxic chemotherapy is the most deleterious to the thyroid gland and is associated with the highest incidence of thyroid dysfunction and fastest time to onset of thyroid dysfunction.<sup>18</sup>

### Treatment of Thyroid Dysfunction

In children with frank hypothyroidism, thyroxine replacement is indicated. Our policy has been to also treat with thyroxine those irradiated children with compensated thyroid dysfunction. The elevated TSH level returns to the normal range, which reduces the theoretical risk of thyroid cancer. Long-term thyroxine therapy is not without potential side effects, however, and we recommend that the requirement for thyroxine be reviewed periodically.

### TESTICULAR FUNCTION

Low doses, 3 to 9 Gy, received as a scattered dose to the testes in 20 fractions over 4 weeks during the treatment of pediatric nephroblastoma resulted in oligospermia or azoospermia many years later.<sup>19</sup> Direct testicular irradiation with doses of 24 to 25 Gy completely ablated the germinal epithelium in all.<sup>20</sup> Leydig cell function is affected in most, as indicated by a low testosterone response to an acute bolus of human chorionic gonadotropin (HCG) and/or an increased basal plasma luteinizing hormone (LH) level. Leydig cell failure occurs soon after irradiation, with no evidence of recovery up to 5 years after irradiation. Most of these boys require androgen replacement to enable normal pubertal development to occur and to allow normal sexual function as adults.

Castillo et al<sup>21</sup> reported normal pubertal development following the use of 12- to 15-Gy doses of testicular irradiation for leukemia prophylaxis. Twelve of 13 subjects had normal basal testosterone levels and testosterone responses to HCG stimulation. However, all 7 boys for whom semen analysis was performed were azoospermic.

Lower doses of testicular irradiation may be received by boys undergoing TBI for a bone marrow transplant or scatter irradiation from spinal irradiation administered for some childhood brain tumors. The degree of damage to the germinal epithelium and Leydig cells is dependent on the radiation dose and the age and pubertal stage of the boy.<sup>12,22</sup>

### OVARIAN FUNCTION

There have been few studies of ovarian function following irradiation uncomplicated by the effects of gonadotoxic cytotoxic chemotherapy.<sup>23</sup> Twenty-seven of 38 patients who received whole abdominal irradiation (20 to 30 Gy over 25 to 44 days) for various reasons in childhood failed to undergo complete pubertal development, and an additional 10 later developed premature menopause (median age, 23.5 years).<sup>23</sup> All had elevated follicle-stimulating hormone (FSH) levels and low estradiol levels. Sex steroid replacement was required to induce breast development and prevent subsequent osteoporosis.<sup>19</sup> Less well known is the lack of breast development, even with estrogen replacement, that was reported to occur in 5 of 38 of these patients. These required mammoplasty. In the same study, 15 patients received flank irradiation (20 to 30 Gy). Ovarian function was normal in 14. Three had breast asymmetry.

Morphologic studies following whole abdominal irradiation (20 to 30 Gy) have revealed marked inhibition of follicular growth and severe reduction in oocyte numbers.<sup>24</sup> Recent studies have indicated that the spinal component of craniospinal irradiation for the treatment of brain tumors and TBI before bone marrow transplantation may cause ovarian dysfunction due to radiation-induced ovarian damage.

### RADIATION AND THE UTERUS

In women in whom ovarian function is preserved, but in whom the uterus has been involved in the radiation field, there is evidence that radiation to the uterus often results in failure to carry a pregnancy. In 38 women who received whole abdominal irradiation (20 to 30 Gy) during childhood, studied by Wallace et al,<sup>25</sup> there were 6 conceptions in 4 patients, all ending in second-trimester miscarriages. The majority of these 38 developed radiation-induced ovarian failure following whole abdominal irradiation. The uterine physical characteristics and blood flow were evaluated in some, as was the functional uterine response to exogenous sex steroid replacement.<sup>25</sup> Those who received whole abdominal irradiation in childhood had significantly smaller uteri in length than women with premature ovarian failure not attributable to irradiation. This implies that prepubertal exposure to irradiation may have an irreversible effect on uterine development and vasculature. In addition, the endometrium was unresponsive to physiologic serum levels of estradiol and progesterone, which were given by exogenous administration. Doppler signals from the uterine arteries were absent in most. It is unclear whether there is damage to the

vasculature of the uterus, although this is possible as appropriate vascularization and subsequent growth of the endometrium are essential for implantation and successful continuation of pregnancy. In summary, it is unlikely that women receiving a significant dose of abdominal irradiation in childhood will be able to sustain a pregnancy to term.

## CONCLUSION

The progressive success of radiotherapy in curing various malignancies and/or prolonging life is accompanied by both destructive and stimulatory effects on the endocrine system. We now have adequate information regarding some of these effects to plan irradiation approaches to minimize damaging effects (eg, fractionated doses instead of single large doses) and to monitor the patients for developing endocrine disease. The endocrinologist and oncologist must form a therapeutic team to support the oncology patient.

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## Abstracts From the Literature

### Impaired Pubertal Growth in Acute Lymphoblastic Leukaemia

This extensive, long-term study of 182 children surviving acute lymphoblastic leukemia (ALL) focuses on their growth at the Hospital for Sick Children in London. The children were in first remission, had been off treatment for 2 years or more, and had attained the onset of puberty at the time of the study.

All had received cranial irradiation, usually given within 8 weeks of diagnosis: 2,400 cGy in 93 patients (before 1980, group A), and 1,800 cGy in the 89 others (group B). None had received spinal or gonadal irradiation. All patients were treated with standard chemotherapy, including intrathecal methotrexate in similar dosage regimens in either group. Mean  $\pm$  standard deviation (SD) age at diagnosis or start of treatment was  $4.8 \pm 2.6$  years in group A and  $6.5 \pm 3.3$  years in group B. Patients who received growth hormone and/or an analogue of gonadotropin-releasing hormone were not included in the study, nor were those having dysmorphic syndromes or an abnormal karyotype.

Mean height at diagnosis or start of treatment was  $-0.29$  standard deviation score (SDS) in group A, and  $-0.40$  in group B. Mean final height was  $-0.63$  SDS in group A, and  $-0.53$  SDS in group B, the number of patients having reached final height being larger in group A (44 boys and 33 girls) than in group B (16 boys and 18 girls); the differences were not significant. There was a similar reduction in height SDS for age in both groups during the time of pubertal growth spurt, more important in girls (42 in group A, 47 in group B) than in boys, and also in patients treated before age 7 years than after this age.

The effect of cranial irradiation on the age at onset of puberty was studied in children treated not later than age 7 years. In group A, puberty started at  $12.2 \pm 1.0$  years in boys and  $10.6 \pm 1.0$  years in girls. In group B, surprisingly, it started significantly earlier;  $11.4 \pm 1.5$  years in males and  $9.9 \pm 0.9$  years in females ( $P < 0.01$ ).

**Editor's comment:** This long-term study of children who had undergone cranial irradiation for ALL is not the first but probably the most reliable, since the series of patients is particularly homogeneous, and the methodology for evaluation of growth and puberty particularly accurate. The results differ in some points from those reported in other series of cases. Without discussing these differences, we agree with the authors on their main conclusions, which are that: (1) a dose of 1,800 cGy impairs future growth as much as a dosage level of 2,400 cGy; (2) young age at irradiation is an important factor for later growth insufficiency; and (3) the severe impairment of final height in girls treated at less than 7 years of age probably results from a combination of growth hormone insufficiency and earlier puberty.

These authors did not evaluate the possible effect or role of chemotherapy, which may play an adjunctive role to irradiation in producing growth retardation, as alluded to by Dr. Shalet in his article appearing in this issue.

Since patients who received hormonal treatment were excluded from the study, we await data on the long-term results obtained with growth hormone therapy in the follow-up and care of the survivors of childhood ALL, and then to comparison of their natural history as analyzed by the present study.

Jean-Claude Job, MD

**2nd Editor's comment:** The data in this abstract, and Dr. Job's comments, complement the presentation by Dr. Shalet carried as one of the lead articles in this issue. Age, sex, chemotherapy, and irradiation dose are variables that probably help determine the ultimate height of children treated for ALL with irradiation. Rereading the section regarding ALL in Dr. Shalet's article may be useful.

Robert M. Blizzard, MD

Uruena M, Stanhope R, Chessells JM, Leiper AD. *Arch Dis Child* 1991;66:1403-1407.



## Hemihypertrophy, Uniparental Disomy, and Risk for Cancer Or: Chromosome 11 Uniparental Isodisomy Predisposing to Embryonal Neoplasms

Grundy et al report on a child with hemihypertrophy and congenital adrenal carcinoma in whom Wilms' tumor subsequently developed. It has been known for some time that Wilms' tumor is associated with the inactivation of both alleles of a tumor-suppressor locus on chromosome 11p. Tumor-specific loss of 11p15 sequences also has been demonstrated in adrenal carcinoma. The overgrowth disorder Beckwith-Wiedemann syndrome is associated with the development of Wilms' tumor and other cancers, and it also has been shown to be associated with loss of the same portion of chromosome 11, either through deletion or uniparental disomy (2 copies of chromosome 11p from father). It is always the maternal copy of chromosome 11p that is lost. These results prompted Grundy et al to examine chromosome 11 in this case of hemihypertrophy with Wilms' tumor.

Molecular genetic analysis revealed that the child had a normal-appearing karyotype. However, when restriction fragment length polymorphism (RFLP) analysis was done, it became apparent that he had uniparental paternal isodisomy for chromosome segments 11p13 and 11p15 (ie, both chromosome 11p segments came from the father). This supports the theory that these segments of chromosome 11 are imprinted, ie, they are differentially expressed when inherited from the mother as opposed to the father, and that they play some role in tumorigenesis. (However, because the child had inherited 2 copies of the same chromosome 11, rather than 1 copy from each parent or 2 different chromosomes from the father, it is also possible that the father carried a mutant recessive

tumor-suppressor gene and that the absence of a balancing normal allele in the child has revealed this mutation.) Because the child also had normal kidney and adrenal tissue, the authors conclude that isodisomy cannot represent the final event responsible for oncogenic transformation. Thus, inactivation of an 11p tumor-suppressor locus seems insufficient to cause Wilms' tumor, which they conclude must be a multistep disorder.

The authors comment that their case, with hemihypertrophy and tumors as the only phenotypic abnormality, may or may not represent an incomplete form of Beckwith-Wiedemann syndrome. But it does demonstrate that this type of chromosome 11 aberration can be present without expression of the complete syndrome.

Grundy P, Telzerow P, Paterson MC, et al. *Lancet* 1991;338:1079-1080.

**Editor's comment:** In addition to providing further support for the theory of genomic imprinting, this case also shows us that patients with hemihypertrophy may carry uniparental disomy for particular chromosomal segments, and that this can cause loss of tumor suppression and increase the risk of these patients for developing malignant tumors. These observations may provide a means of identifying those patients with hemihypertrophy who are at risk for malignancy.

Judith G. Hall, MD

## Stimulation of Collagen Synthesis and Linear Growth by Growth Hormone in Glucocorticoid-Treated Children

In this study, the collagen synthesis and insulin-like growth factor 1 (IGF-1) status before and after growth hormone (GH) treatment in children on chronic glucocorticoid (GC) therapy was investigated. Seven children with the following diagnoses were studied: autoimmune colitis, eosinophilic fasciitis, asthma, nephrotic syndrome, and renal transplant recipients for obstructive uropathy, hypoplastic kidneys, or focal segmental glomerulosclerosis. The chronologic age of the patients was between 8 3/12 and 15 7/12 years, the bone age was <10 years for girls and <12 years for boys, and the Tanner staging was prepubertal for all except for one girl, who was Tanner stage III. All had subnormal growth velocity for at least 6 months prior to the study while on stable dosages of GC. Height, weight, IGF-1 activity, glycosylated hemoglobin level, and C-terminal type 1 procollagen levels were measured at baseline and every 3 months thereafter following the initiation of treatment with recombinant human GH (0.3 mg/kg/wk) for 6 to 21 months (mean, 13.1  $\pm$  4.9 months). Skeletal maturation and 2-hour postprandial serum glucose and insulin levels were assessed every 6 months. All patients showed increased growth velocity during treatment with GH. Mean growth velocity increased from 3.43  $\pm$  0.65 cm/yr to 6.72  $\pm$  0.84 cm/yr with GH therapy ( $P < 0.005$ ). SDs corrected for bone age ( $P < 0.005$ ), IGF-1 levels ( $P < 0.005$ ) and C-terminal type 1 procollagen levels ( $P < 0.005$ ) also increased with GH therapy. C-terminal type 1 procollagen levels correlated well with growth velocity ( $r = 0.652$ ), while IGF-1 levels did not ( $r = 0.17$ ). Glycosylated hemoglobin levels rose during GH treatment. It was felt that since no child experienced significant improvement in his or her underlying illness or puberty stage, and since glucocorticoid dosages changed little during the study period (never decreasing below the baseline dose in 6 of 7 children), the improvement in GV

and type 1 collagen synthesis noted were likely the result of GH treatment. It was concluded that both inhibition of IGF-1 effects and collagen synthesis were responsible for the growth-retarding effects of GC therapy.

Allen DB, Goldberg BD. *Pediatrics* 1992;89:416-421.

**Editor's comment:** This study appears to show benefits of GH treatment. It promoted growth and procollagen synthesis in children on long-term GC therapy. This study also offers a biochemical explanation for stunted growth due to GC treatment and for increased growth velocity with GH therapy. This study appears to support the hypothesis that impaired linear growth and skeletal maturation associated with chronic GC therapy results from: (1) inhibited IGF-1 activity and (2) impaired type 1 procollagen synthesis. Additionally, GC may suppress GH secretory response to GH-releasing hormone, but this was not measured by the authors. Each mechanism could potentially be improved by exogenous GH treatment if sufficient dosages are given to overcome these 3 competitive effects of GC. However, the data differ from that reported many years ago by other investigators who showed that GH had no beneficial effects on GC-treated children (*J Clin Invest* 1968;47:436-491). We also have treated several patients with corticosteroid-dependent asthma on GC therapy, and they showed marked improvement in growth after GH therapy. However, the effects of GH therapy were related to the dose of GC given while GH treatment was ongoing (presented at the 73rd Annual Meeting of the Endocrine Society, June 19-22, 1991; abstract No. 1315). GH treatment at the dosage usually employed for treatment of hypopituitarism or for Turner syndrome patients could not overcome the effects of pharmacologic doses of GC.

It is difficult to ascertain from the studies reported by Allen and Goldberg whether improvement in disease activity (not quantitated) and/or alternate-day GC treatment resulted in catch-up growth coincidentally with GH therapy in their patients. Five of 7 patients received 15 to 50 mg/m<sup>2</sup>/d hydrocortisone equivalent on an alternate-day regimen, and the remaining 2 patients received only a physiologic dose of 15 mg/m<sup>2</sup> hydrocortisone equivalent.

The exact amount of GC administered on alternate days necessary to inhibit and/or allow growth is not known. The effect of alternate-day GC treatment on growth was studied by Whittington et al in patients with Crohn's disease (Gastroenterology 1977;72:1338-1344). In that study, the dose of prednisone given was from 52.5 to 157.5 mg/m<sup>2</sup>/d of hydrocortisone equivalent. Despite these pharmacologic doses, the patients showed catch-up growth when GC was given every other day.

In this study, the normal or elevated pretreatment values of IGF-1 were attributed, in part, to the obesity and/or hyperinsulinemia found in many GC-treated patients. Dissociation of serum GH and IGF-1 levels in obese individuals might result from insulin-mediated IGF-1 production and consequent suppression of GH secretion (J Clin Endocrinol Metab 1976;42:370-378 and Endocrinology 1979;73:209-213). Further, it was previously shown that within

hours of oral GC administration, IGF-1 activity falls precipitously while IGF-1 levels remain unchanged (J Clin Endocrinol Metab 1985;61:618-626). This inhibitory effect was reflected by the GC-induced stimulation/potential of circulating IGF-1 inhibitors. Thus, while IGF-1 levels rose with GH therapy in these patients, the poor correlation with growth velocity was not surprising.

The potential beneficial effects of GH treatment in patients receiving GC need to be considered in relation to increasing the risk of side effects when patients are treated with these 2 antagonistic drugs. Particular attention needs to be given to the administration of large doses of GH, which may be needed to overcome the pharmacologic effects of GC. This would potentiate carbohydrate intolerance as well as increase other potential toxicities. Thus, undertaking a trial with GH in growth-inhibited patients receiving GC without an investigative protocol is strongly discouraged. There may be other treatments of potential value to enhance growth while controlling the primary disease that may be of help in corticosteroid-dependent patients, ie, corticotropin therapy. (Acta Paediatr Scand 1990;79:77-83).

Fima Lifshitz, MD

## Osteopenia in Growth Hormone-Deficient Adult Males and Men With Constitutional Delayed Puberty

Finkelstein et al determined bone mineral density in a cohort of 23 men (age 26 ± 2 years) who had a history of delayed pubertal development and who had presented to the Pediatric Endocrine Clinic of Massachusetts General Hospital between 1974 and 1980. Each had a history of the onset of puberty after age 15 years and also a history of height at or below the 5th percentile for chronologic age before the pubertal growth spurt. The findings were compared with those determined in a control group of 21 men (age 24 ± 3 years) who had a history of puberty beginning before 14 years of age. Control subjects at the time of the study were 2 years younger than those in the study group in order to match the groups for duration of exposure to adult levels of gonadal steroids. Forearm bone mineral density was determined by single-photon absorptiometry, and spinal bone density was determined by dual energy X-ray absorptiometry of the first through fourth lumbar vertebrae.

Bone mineral density was significantly lower in the men who had experienced delayed puberty. Multivariate analysis of variance demonstrated that the timing of puberty remained a significant determinant of bone density after accounting for the effects of age, body mass index, exercise, alcohol intake, calcium intake, and serum testosterone. The authors conclude that their data are consistent with the hypothesis that the timing of puberty is an important determinant of peak bone mineral density in males.

Kaufman et al measured bone mineral content by photon absorptiometry in 30 men (age 26.5 ± 1.2 years) with growth hormone deficiency (GHD) (8 with isolated GHD and 22 with multiple pituitary deficiencies) and compared the results with those from 30 male controls of similar age, weight, and body mass index. Bone mineral content was measured at the distal third of the nondominant forearm (proximal site) and close to the carpal joint (distal site) of the same forearm by single photon absorptiometry. Bone mineral content of the lumbar spine (L2 to L4) was determined with dual-photon absorptiometry. All subjects with GHD had received growth hormone (GH) replacement therapy and had reached adult bone age. GH treatment had been interrupted for at least 6 months, but other hormonal replacement was continued. Bone mineral content was significantly lower at both the forearm and the lumbar spine in the subjects with pituitary hormone deficiencies. This was true regardless of whether there were single

or multiple hormonal deficiencies. A 6- to 28-month prospective evaluation of 19 subjects showed no subsequent bone loss. The authors conclude that adult men with childhood GHD have a significant bone mineral deficit as compared with age- and weight-matched controls.

Finkelstein JS, Neer RM, Beverly MK, et al. Osteopenia in men with a history of delayed puberty. *N Engl J Med* 1992;326:600-604.

Kaufman JM, Taelman P, Vermeulen A, Vandeweghe M. Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. *J Clin Endocrinol Metab* 1992;74:118-123.

**Editor's comment:** These 2 papers should be read together. The methodology for each is similar as are the findings. Kaufman et al show that individuals with GHD have lower bone mineral density at adulthood than controls, but they do not exclude the possibility that some of the deficit observed is due to an associated androgen deficiency. Indeed, the majority of their subjects have gonadotropin deficiency. Finkelstein et al conclude that since the only known physiologic abnormality in their subjects is a delay in the onset of puberty, this transient delay in gonadal steroid secretion is important to achieving peak bone mineral density during adolescence. However, Finkelstein's subjects had both delayed puberty and constitutional delay of growth. These patients were at or below the 5th percentile for age and their height before the pubertal growth spurt was at least 3 standard deviations below the mean. Since it is known that gonadal steroids increase GH pulse amplitude during puberty, it is possible that the decrease in bone mineral content associated with pubertal delay is secondary to a relative GH insufficiency during early adolescence. Neither study reported an increased incidence of bone fractures in adults with either GHD or delay of puberty. Such data would be exceedingly important in demonstrating the significance in the findings of either paper. Both authors suggest that their data support a role for early therapy with either androgen supplementation in boys with delayed puberty or GH treatment of males with hypopituitarism.

William L. Clarke, MD

## Abnormalities of Insulin-Like Growth Factor (IGF-1 and IGF-2) Genes in Human Tumor Tissue

The structure and expression of genes coding for insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) were investigated in tissue samples from 37 human tumors: 5 with severe hypoglycemia of  $1.85 \pm 0.7$  mMol/L (range, 0.9 to 2.7 mMol/L), including pleural fibroma, leiomyoma, leiomyosarcoma, lymphosarcoma, and exocrine pancreatic carcinoma; and 32 without known hypoglycemia, including cancers of breast, kidney, and lung (n=17); liver (n=4); Conn's adenoma (n=3); thymoma and pheochromocytoma (n=3); hepatic metastases of 1 pancreatic and 1 colon carcinoma; and 3 embryonic tumors (neuroblastoma, neuroblastoma, and adrenocortical carcinoma). The tumor tissues were removed from patients at surgery, immediately frozen, and stored at  $-80^{\circ}\text{C}$  until biochemical study. Blood samples for extraction of DNA from blood nucleated cells also were obtained from patients.

Three established human tumoral cell lines were studied for comparison: 1 from hepatoma, 1 from colon carcinoma, and 1 from neuroblastoma. Genomic DNA and total RNA were extracted by grinding of frozen tissue samples, separation, and purification.

Specific human genomic DNA probes were used after labeling with  $\alpha^{32}\text{P}$ -ATP. For IGF-1, it was a 667-bp *EcoRI*-*Bam*HI fragment containing the coding region with exons 1,2,3, and 5 between a 163 bp 5' untranslated region and a 44-bp 3' untranslated region. For IGF-2, the coding region was associated with a 99 bp chain from the 3' untranslated region (exons 7,8, and 9) and a 15 bp chain from the 5' untranslated region. A human insulin 2.7-kb DNA fragment and a human calcitonin 827-bp cDNA probe also were used as controls. Analysis was performed by Southern blot.

The extent of DNA methylation was evaluated by comparison between restriction profiles obtained with *Av*all and with other restriction enzymes.

RNA from the samples was studied by northern blot and dot blot methods. Expression of RNA was evaluated on the basis of densitometric analysis, compared with that obtained from normal human adult liver fragments taken as reference samples.

No obvious rearrangement such as deletion or amplification of IGF genes was observed in any of the tumor samples investigated. But the extent of DNA methylation of IGF genes and the level of mRNA expression were extremely variable among these tumoral tissues.

A relationship could be detected between gene demethylation and IGF overexpression in the 5 tumors associated with hypoglycemia: 2 with a great degree of gene demethylation and overproduction of IGF-1 and IGF-2 mRNA, 1 with slightly demethylated IGF-2 gene and large amounts of the corresponding mRNA, and 2 without demethylation or expression of IGF genes.

In the other tumor samples studied, those not associated with hypoglycemia, no IGF-1 demethylation was found. IGF-2 gene demethylation and mRNA expression were highly variable. No relationship was evident.

In the 3 human carcinomatous cell lines analyzed, there was high expression of IGF-2 mRNA without IGF-2 gene demethylation. None expressed any detectable IGF-1 mRNA, although the IGF-1 gene was extensively demethylated in the neuroblastoma-derived cells.

Loss of heterozygosity was found in 3 children with tumor, using parallel investigation of tumoral and normal cells. Family study was possible in 2 and revealed a decrease of the maternal allele for IGF-2 gene. By using an insulin probe, it was shown that in the case of neuroblastoma the allele loss was not IGF-2-specific but extended to a large section of the 11p15 region. In adult patients, no loss of heterozygosity was found in tumor samples or in the blood nucleated cells analyzed. However, an imbalance of the 2 IGF-2 alleles was found in 2 breast and 2 liver carcinomas, raising questions about some role in specific demethylation and expression.

The authors' first conclusion is that in tumors in which IGF-1 and IGF-2 mRNAs are overproduced, there is no detectable DNA deletion or amplification; however, there may exist specific IGF gene demethylation. The second conclusion is that, although embryonic tumors show a loss of heterozygosity in the IGF-2 gene, a different mechanism seems responsible for IGF-2 mRNA overexpression in certain adult tumors.

Schneid H, Seurin D, Noguiez P, Le Bouc Y. *Growth Regulation* 1992;2:45-54.

**Editor's comment:** This extensive work investigates at the genomic level the existence and possible role of IGFs in different types of human tumors, also aiming to determine whether a relationship exists between the tumorigenesis and the structure and expression of IGF genes. The extreme diversity of results suggests that there is no clear or constant relationship between any of the tumoral types or lines studied and abnormalities of IGF-1 and IGF-2 genes or their expression. However, 2 possibly important facts appear. One is the relationship between IGF overexpression and gene demethylation found in tumors associated with hypoglycemia. The second is the confirmation, in some childhood tumors, of a loss of heterozygosity in the 11p15 region coding for IGF-2, completed by demonstration of an imbalance of maternal origin in the corresponding leukocyte alleles. This is a new piece in the complicated field of relationships between IGFs and cancer growth.

Jean-Claude Job, MD

**2nd Editor's comment:** This abstract, the abstract on page 12 entitled "Hemihypertrophy, Uniparental Disomy, and Risk for Cancer," and the abstract entitled "Uniparental Disomy in Beckwith-Wiedemann Syndrome," published in *GGH Vol. 8, No. 2* are interrelated. Chromosome 11 imprinting and disomy seem to be key, but the issues are more complex. Intriguingly, Beckwith-Wiedemann syndrome is characterized by hypoglycemia (probably IGF-1 induced), tumor formation such as Wilms' tumor in high frequency, and overgrowth (possibly a function of IGF-1 or IGF-2). The 11p15 and 11p13 areas have the associated genes to produce the phenomena addressed in these 3 articles. Gene demethylation also appears to play a role—particularly in relation to IGF-1 gene demethylation.

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## Reproducibility of 24-Hour Growth Hormone Profiles in Children

The rate of growth hormone (GH) secretion and the pattern of GH peaks were compared in a group of 9 children during their prepubertal period in repeated 24-hour GH profiles. At investigation, the children were 6 to 13 years old (at first profile, 6 to 11 years old) and of normal height ( $\pm 2$  standard deviations [SD]). Two profiles were obtained per child, with a mean time interval of 1.5 years (range, 0.7 to 3.5 years). The calculated GH secretions of the first and second profiles were compared. As a group, no significant differences were obtained in secreted amount of GH, when the data from second profile was expressed as a percentage of data from the first profile ( $93\% \pm 8\%$ ), number of peaks ( $95\% \pm 7\%$ ), or mean peak amplitudes ( $92\% \pm 11\%$ ). Between the repeated curves of an individual child, maximal difference in secretion, number of peaks, and mean peak amplitudes ranged around 30%, with a mean intraindividual coefficient of variation of 12%. The reproducibility in the peak distribution for all profiles was also analyzed. Reproducibility of the temporal pattern of profiles was analyzed using time-series analysis (Fourier analysis) and showed no difference in rhythmicity between the different occasions.

In conclusion, a high reproducibility of both GH secretion and GH pattern was found for the whole group of prepubertal children. The high degree of reproducibility of the 24-hour GH profiles of the entire group indicated that the information from these curves, in terms of both pattern and total secretion, can be used for clinical as well as for physiologic purposes. The intraindividual reproducibility was less pronounced, however, leading to a sound skepticism when relating biologic phenomena to a single profile of an individual child.

Albertsson-Wikland K, Rosberg S. *Acta Endocrinol* 1992; 126:109-112.

**Editor's comment:** The reproducibility of measurements of GH secretion has been studied and questioned in many previous papers. This study deserves exceptional consideration since it uses extremely accurate methodology and gives all the technical data, including variability of the results of plasma GH radioimmunoassays. The authors conclude that there is a contrast between the excellent overall reproducibility in recording of 24-hour GH secretion in groups of subjects, and these data can be used for clinical and physiologic purposes. However, the extent

of individual variations prompts skepticism about interpreting a single profile in an individual child.

Jean-Claude Job, MD

**2nd Editor's comment:** Martha et al did a similar extensive study that was presented at the American Endocrine Society Meeting in San Antonio in June 1992, under the title "Physiological GH Release is Regulated Over Time Within Characteristic, Individually Determined Limits Which Vary Predictably, But Reciprocally, With Body Mass Index." These authors performed 44 integrated studies in 9 prepubertal, normal-statured boys over 9- to 35-month periods. Among the group data, mean 24-hour integrated concentrations of GH for the individual profiles spanned a 6-fold range (1.1 to 7.0 ng/mL) with an intersubject CV of 46%. In contrast, values of individual subjects exhibited much less variability (mean CV,  $26\% \pm 4\%$ ). Therefore, each individual was consistent in having low, medium, or high integrated GH concentrations, and the mean ICGH level in each of these normal boys correlated strongly and inversely with body mass index (BMI) SD scores.

The authors conclude that during late prepuberty (9 to 12 years of age, Tanner stage I): (1) individual boys regulate daily GH secretion within relatively confined limits, which are characteristic for that individual and much narrower than the broad range present in the larger population; (2) differences in BMI help determine the GH secretion range which characterizes, and is therefore "normal" for, each individual; (3) differences in mean 24-hour GH levels among normally growing boys arise primarily from differences in GH pulse size; and (4) there is no consistent progressive change in mean 24-hour GH release in prepubertal boys before puberty occurs.

In correlation with the paper by Albertsson-Wikland and Rosberg, there is a variation in GH secretion within an individual in respect to quantity, which may vary as much as 75% to 100% between 2 profiles. However, the mean data for the group between profiles is much less variable. In addition to the data presented from Sweden, Martha et al determined that children have significantly different GH secretion from each other on the basis of BMI. GH secretion and BMI vary inversely.

Robert M. Blizzard, MD

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# GROWTH

## Genetics & Hormones

Vol. 8 No. 4

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### Effects of Drugs and Other Chemicals on Fetal Growth

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Prenatal exposure to a number of chemicals can cause growth retardation in humans. Some of these agents also may produce permanent alteration of structure or function and thus are considered to be teratogens.<sup>1</sup> Although most human teratogens are associated with fetal growth retardation, some are not. There also are several agents that can cause fetal growth retardation but are not recognized as human teratogens.

Fetal growth retardation is regularly associated with maternal diseases such as hypertension and severe diabetes mellitus.<sup>2</sup> Growth retardation is a cardinal feature of the embryopathies that result from intrauterine infection with toxoplasmosis, rubella, cytomegalovirus, syphilis, and varicella.<sup>3</sup> Similarly, exposure to high doses of ionizing radiation during embryonic development regularly leads to permanent growth deficiency, microcephaly, and mental retardation.<sup>4</sup> These human teratogens will not be discussed in this paper, which is restricted to consideration of the effects of drugs and other chemicals.

While we usually speak of "teratogens" as if teratogenicity were a property of chemistry alone, it is important to remember that the dose, route, and timing of exposure are as important as the nature of the chemical itself in creating a teratogenic risk. Recognized "human teratogens" are agents for which sufficient conditions of exposure are encountered to produce a teratogenic effect. Studies in experimental animals suggest that many other agents also would have teratogenic

potential in humans if sufficient exposures were encountered.

The same principle no doubt applies to agents that cause growth retardation but not teratogenic effects. The agents that we recognize as having such properties are encountered by pregnant women in doses and circumstances sufficient to permit the growth-retarding effects of these chemicals to be expressed. It seems likely that many other chemicals would have similar potential if sufficient exposure occurred.

#### MEDICATIONS THAT ARE TERATOGENS

Aminopterin is a folic acid antagonist. It has been administered to pregnant women to induce abortion. A related drug, methotrexate, is used as an antineoplastic agent. A rare but strikingly similar pattern of congenital anomalies has been observed among children born after exposure to aminopterin during embryogenesis.<sup>5</sup> Frequent features of this syndrome include growth retardation, delayed calvarial ossification, craniosynostosis, hydrocephalus, abnormal auricles, ocular hypertelorism, micrognathia, and cleft palate. Although developmental delay is seen during childhood, the few affected adults who have been

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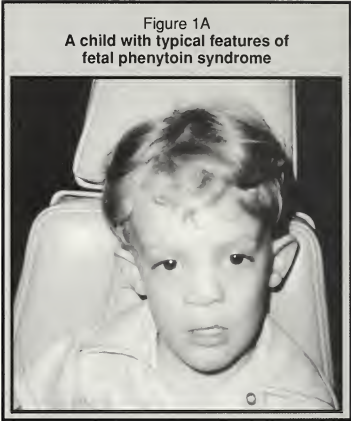
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reported appear to have normal intelligence or only mild mental retardation.

Congenital anomalies have been observed with increased frequency among the children of epileptic women treated with anticonvulsants during pregnancy. This association has been observed with various medications, including trimethadione, phenytoin, valproic acid, and carbamazepine. It is difficult to determine from available data to what extent this increased risk is attributable to the agents themselves as opposed to the seizure disorder or some factor that predisposes to seizures. A characteristic pattern of congenital anomalies, ie, a fetal anticonvulsant syndrome, has been reported with each of these agents.<sup>6-11</sup> Frequent features include growth and developmental retardation and unusual facies (Table 1 and Figure 1A). Although the syndromes associated with all of the anticonvulsants are similar, some features are more commonly encountered with maternal use of a particular agent. For example, distal digital and nail hypoplasia are an especially frequent feature of the fetal phenytoin syndrome (Figure 1B) and spina bifida is most often seen with the fetal valproic acid syndrome.

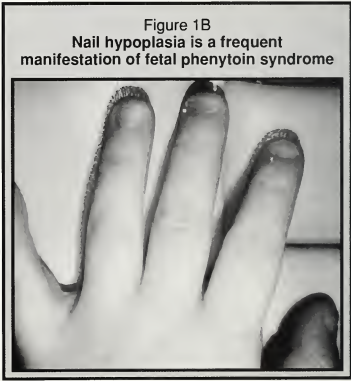
A characteristic pattern of congenital anomalies has been observed in children born to women treated with warfarin during pregnancy.<sup>12</sup> Frequent features of this "warfarin embryopathy" include nasal hypoplasia (Figure 2), stippled epiphyses on radiographs, and growth retardation. No adequate epidemiologic study of pregnancy outcome in women treated with warfarin is available, but on the basis of published experience (an obviously biased sample), it has been estimated that about 10% of infants born alive to mothers who take



warfarin during pregnancy have warfarin embryopathy. Maternal heparin use in gestation has been associated with stillbirth and other complications of pregnancy, but this drug does not cross the placenta.<sup>12</sup>

Captopril and enalapril are antihypertensive agents that act by inhibiting angiotensin converting enzyme. Although maternal treatment with these drugs during early pregnancy is not known to damage the embryo, treatment late in pregnancy is associated

| Table 1<br>Features of Fetal Anticonvulsant Syndromes                       |
|---|
| <b>May be seen with various anticonvulsants:</b>                            |
| Growth deficiency   |
| Developmental delay   |
| Midface hypoplasia  |
| Short nose with broad or flat bridge  |
| Epicanthal folds  |
| Micrognathia  |
| Congenital heart defects  |
| Urogenital anomalies  |
| <b>Associated more with one particular anticonvulsant than with others:</b> |
| Spina bifida (valproic acid)  |
| Distal digital hypoplasia (phenytoin)                                       |
| Nail hypoplasia (phenytoin)   |
| Tracheomalacia (valproic acid)  |
| Talipes equinovarus (valproic acid)   |



with a substantial risk of fetal growth retardation, oligohydramnios, anuria, and perinatal death.<sup>13</sup>

### Recreational Agents and Drugs of Abuse

A pattern of congenital anomalies called the fetal alcohol syndrome occurs in infants born to women with chronic alcoholism during pregnancy.<sup>14,15</sup> This topic was previously reviewed in *GROWTH, Genetics & Hormones* (1988;4[1]:1-3). Prenatal and postnatal growth retardation are characteristic features of this syndrome, and microcephaly is frequent. About 80% of children with severe fetal alcohol syndrome have measured lengths and weights <2 standard deviations (SD) of that expected. Other manifestations include mental retardation, hyperactivity and other behavioral disturbances, poor coordination, and typical facial appearance (Figure 3, page 4). Congenital heart disease and brain malformations are common, but other major congenital anomalies are infrequent. Lower amounts of maternal alcohol consumption during pregnancy (2 to 4 mixed drinks, beers, or glasses of wine per day, on the average) have been associated with milder growth deficiency, intellectual deficits, and behavioral abnormalities.<sup>16,17</sup>

Fetal growth retardation and spontaneous abortion occur with increased frequency among pregnant women who are heavy cigarette smokers.<sup>18,19</sup> Low birth weight is associated with maternal smoking in a dose-related fashion. This effect seems to be due primarily to fetal growth retardation rather than to prematurity. Controversy exists regarding whether the low birth weight seen among infants of women who smoke during pregnancy is caused by smoking or by the other correlated factors. The preponderance of evidence favors the former view. Maternal cigarette smoking may account for up to 40% of fetal growth retardation in advanced countries.<sup>20</sup> Persistent mild reduction of growth and intellectual performance has been observed among the children of women who smoked during pregnancy. Some studies suggest that birth weight also is decreased slightly among the children of nonsmoking women exposed to tobacco smoke in their environment.

The frequency of spontaneous abortion is 20% to 80% higher than expected among women who smoke cigarettes during pregnancy.<sup>18</sup> The risks appear to be greater for heavy smokers than for light smokers. Some studies, but not others, suggest that perinatal mortality and other complications of pregnancy also may be increased among the infants of women who smoke cigarettes during pregnancy. Congenital anomalies do not appear to be unusually frequent among the children of women who are heavy smokers.

An association between maternal coffee drinking during pregnancy and low birth weight has been

observed consistently in epidemiologic studies,<sup>21,22</sup> but in many instances this association is largely due to confounding effects of maternal cigarette smoking. Maternal coffee drinking during pregnancy does not appear to affect the risk of congenital anomalies among the offspring.

Intrauterine growth retardation, perinatal death, and a variety of other perinatal complications have frequently been observed among the children of narcotic-addicted mothers,<sup>23,24</sup> but it is unclear whether these effects are due to fetal exposure to narcotics or to the generally poor health of these women. Subsequent growth of their children appears to be normal in most cases. Malformations are not usually frequent among the infants of narcotic-addicted mothers.

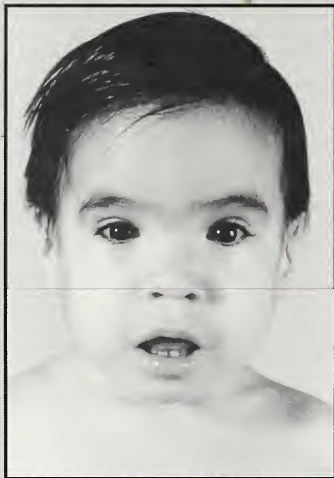
Maternal cocaine use during pregnancy is associated with an increased risk of placental abruption and possibly of congenital anomalies due to vascular disruption.<sup>25</sup> Growth retardation involving weight, length, and head circumference has consistently been noted among infants born to women who use cocaine during pregnancy, but a causal relationship is difficult to establish because of the presence of many confounding factors in these women.

Figure 2  
Nasal hypoplasia and iris dysgenesis in a child demonstrating the effects of warfarin embryopathy





Figure 3  
Facies in fetal alcohol syndrome



(From Little RE, Streissguth AP. Alcohol, pregnancy, and the fetal alcohol syndrome. In: *Alcohol Use and Its Medical Consequences: A Comprehensive Teaching Program for Biomedical Education*. Project Cork of Dartmouth Medical School. Timonium, Md: Milner-Fenwick, Inc.; 1982.)

Maternal inhalation of large amounts of toluene to "get high" during pregnancy may produce growth deficiency, developmental delay, behavioral abnormalities, and minor physical anomalies among the offspring.<sup>26</sup> Women who use toluene in this way often suffer toxic manifestations themselves.

### OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES

Although much concern has been raised about the potential adverse effects of exposure to occupational or environmental chemicals during pregnancy, only 2 exposures of this type (PCBs [polychlorinated biphenyls] and methyl mercury) have convincingly been shown to cause fetal growth retardation in humans. Skin discoloration and growth retardation have been noted among the infants of pregnant women who ate cooking oils that were highly contaminated with PCBs.<sup>27</sup>

Less pronounced effects on fetal growth have been observed among the children of mothers who ate PCB-contaminated Great Lakes fish or were exposed to PCBs in the workplace. Brain damage and consequent cerebral palsy have been seen among the children of pregnant women who ate food that was heavily contaminated with methyl mercury.<sup>28</sup> The frequency of low birth weight correlates with maternal and neonatal blood methyl mercury concentrations in populations whose food supply is contaminated with these compounds.<sup>29</sup>

### PATHOGENIC MECHANISMS

Many processes are involved in normal fetal growth, and interference with any of these processes could result in fetal growth retardation. Cell death or altered cell growth and proliferation are probably important factors in the growth retardation produced by radiation and chemicals such as aminopterin. Fetal vascular compromise, either directly or indirectly by means of an effect on the placenta or maternal vasculature, seems likely to be important with agents such as cigarettes, captopril, and cocaine. Some agents may lead to fetal growth retardation through a combination of effects involving the placenta as well as embryonic or fetal cell proliferation, growth, and death. Alcohol is probably one example.

### PREVENTION OF FETAL GROWTH RETARDATION

Familial factors appear to be the single most important predisposition to fetal growth retardation,<sup>20</sup> but pharmacologic approaches may provide an effective means of prevention in such cases. A recent randomized controlled trial of maternal treatment with low-dose aspirin in the second and third trimesters of pregnancy demonstrated a 225g improvement in birth weight among the infants of women who had had a prior pregnancy complicated by fetal growth retardation, stillbirth, or placental abruption.<sup>30</sup> The beneficial effect was most marked among women who had previously had 2 or more poor pregnancy outcomes. The mechanism by which aspirin improves fetal growth in these high-risk pregnancies is unknown but may involve effects on placental prostaglandins.

Avoidance of cigarette smoking, alcohol drinking, and drug abuse during pregnancy would likely prevent at least 40% of all cases of fetal growth retardation.<sup>20</sup> Many spontaneous abortions, fetal deaths, and other adverse pregnancy outcomes also would be avoided if pregnant women did not use these agents. Despite the importance and apparent simplicity of this approach, it has been difficult to effect in practice. Many of these agents are addictive, and their use is a component of

patterns of social interaction that are very difficult to change. Public education and universal availability of early prenatal care provide the best opportunities for reducing cigarette smoking, alcohol drinking, and drug abuse among pregnant women.

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# Recent Developments in the Study of the Psychosocial Aspects of Short Stature

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While enhanced growth velocity is the well established benefit of GH therapy, potential psychosocial benefits have been suggested by studies of clinical samples of short children. Conclusions based on these early studies are drawn into question because of small samples, diagnostic heterogeneity, and a lack of control observations. Emerging studies, using more sophisticated new research designs, are beginning to challenge and refine the conclusions of earlier reports.

A glimpse of this new wave of studies was recently provided to participants of the Fourth North Coast Conference of the Society of Pediatric Psychology. The meeting, held April 23-26 in Buffalo, New York, included a symposium on the psychosocial aspects of short stature. The studies described focused upon the behavioral adjustment of 4 different populations of children and adolescents with short stature: (1) those in the general population who had not been referred for an evaluation of their growth or height; (2) clinically referred individuals prior to any treatment; (3) those who had received GH; and (4) GH-treated girls with Turner syndrome (TS).

Dr. Michael Vance of the Creighton Medical School in Omaha, Nebraska, reported on a study that investigated the association of short stature to psychological adjustment in children in the general population; a reanalysis of data from cycles II (ages 6 to 11 years; n=7119) and III (ages 12 to 17 years; n=6768) of the National Health Examination Survey.

Measuring such factors as school adjustment, peer relations, aggression, immaturity, and anxiety, Dr. Vance found only very subtle (although statistically significant) differences between children that could be accounted for by their height. Also, change in growth rate over approximately 4 years (as assessed in a subsample of 2177 children) was unrelated to the measures of positive adjustment. These relationships were maintained across subsamples 1 to 3 standard deviations (SD) below the mean for height norms. This study is the only large-scale survey of the relationship between height and psychological functioning conducted in the United States. It suggests that short children in the general population function socially and academically in ways that are reasonably indistinguishable from that of average-statured peers. Based on these findings, Dr. Vance suggested that GH therapy to increase stature for psychosocial reasons is not indicated.

Dr. David E. Sandberg of Children's Hospital of Buffalo reported on the results of a psychosocial screening of 150 children and adolescents consecutively referred to endocrinologists for evaluation of growth and height. The screening, a routine component of the initial endocrine visit, included a brief psychosocial assessment of the patient based on information collected from the parent(s) and the child. The mean standardized height for the group was -2.3 SD, with a range of -4.0 to -1.6 SD. The majority of boys (n=101) and girls (n=49) reported that they were teased because of their size relative to their chronologic age. Parents confirmed these reports. Despite these common stressors, this clinically referred group appeared to be functioning well as reported by both themselves and their

parents when compared with national norms for key standardized instruments used in the screening. Nevertheless, parents reported short boys showed poorer social and academic competencies and greater behavioral problems than boys in the general population, but these differences were modest. The behavior profile of girls was indistinguishable from that of girls in the general population.

The studies by Vance and Sandberg were conducted under very different circumstances, yet the findings are quite similar: short stature, in and of itself, does not appear to be associated with clinically significant disruptions of psychological functioning. These 2 studies do not exclude the possibility that there are children with particular medical conditions that have short stature as 1 feature (eg, bona fide GH deficiency [GHD], TS) who are experiencing significant academic or psychological problems. This possibility was highlighted in the next presentation.

The third speaker, Dr. Brian Stabler of the University of North Carolina at Chapel Hill, summarized baseline data from the Genentech-sponsored, 4-year longitudinal study of academic achievement and psychosocial adjustment of a different clinical population: children receiving GH therapy ( $n=194$ ). Specially trained nurses administered questionnaires and psychometric tests to parents and patients at several endocrine centers around the country. One in 5 children in this group (GHD, TS, and idiopathic short stature [ISS] diagnoses) were experiencing academic achievement problems, which is significantly higher than is observed in the general population. He also stated that behavior problem scores, as reported by a parent on a standardized behavior checklist, were elevated relative to norms among those children diagnosed as either GHD or idiopathic short stature. Girls with TS who were receiving GH therapy did not show elevated behavior problem scores. Both the GHD and TS groups (but not those with ISS) showed poorer social competency than a normative sample as measured by parent questionnaire. The finding that children typically came from well-functioning families (as assessed by another standardized questionnaire) of high socioeconomic status suggests that the problems observed are not likely attributable to factors related to demographic background.

In a separate study of adults with a history of GH therapy during childhood, Dr. Stabler reported that individuals with multiple pituitary hormone deficiencies (MPHDs;  $n=20$ ) showed a muted cardiovascular response (ie, heart rate, systolic and diastolic blood pressure) to psychological stress (public speaking in this case). Those with isolated GHD (IGHD;  $n=5$ ), however, functioned like short healthy comparison subjects ( $n=25$ ). Those with hormone deficiencies were also different in their personality from short healthy adults: "neuroticism" (as measured by the NEO Personality Inventory) was higher in both those with IGHD and MPHD and

"extraversion" was lower in the MPHD group. Finally, the MPHD group was significantly less assertive (on the Rathus Assertiveness Schedule) than short healthy adults, whereas the IGHD group was not. Small sample size in the IGHD group ( $n=5$ ) limits the generalizability of the conclusions related to this subgroup. Detailed findings from this study have recently been published (Stabler B, Turner JR, Girdler SS, et al. *Clin Endocrinol* 1992;36:467).

Dr. Stabler explained how these findings emphasize the importance of comprehensive, multidisciplinary care for individuals with pituitary hormone deficiencies. Short stature by itself did not appear to be the primary factor explaining these results. Some other, as yet unspecified, factor was suggested as being responsible for the poor behavioral and social adjustment, such as a neuroendocrine imbalance.

Dr. Joanne Rovet of the Research Institute at the Hospital for Sick Children in Toronto described a postal survey that assessed the psychological benefits of GH therapy in TS ( $n=46$ ). This ongoing longitudinal study includes 11 centers across Canada and is the only one to maintain a non-GH-treated control group ( $n=40$ ) through to final height. Some of the findings collected up to this time (1.5 years into the study) include: problems of cognitive function that have repeatedly been demonstrated in girls with TS despite average intelligence; problems of sustaining attention in childhood; particular problems in the academic domain of mathematics; poorer social competencies and somewhat higher than expected levels of hyperactive-like symptoms. One and one half years after GH therapy was initiated, changes were observed in the following areas compared with the non-GH-treated group: decrease in hyperactive-like symptoms; poorer math achievement (an unpredicted and unexplained finding); improved social relations, popularity, and self-esteem. There was a trend for improvements in growth velocity with GH to be associated with a reduction in behavioral problems and improved social relations with peers.

These studies of very different nonclinical and clinical populations of children, adolescents, and adults with short stature demonstrate that height by itself may not be the essential factor determining how short individuals function behaviorally, emotionally, or academically. Instead, it may be a particular underlying medical condition—of which short stature is a feature—that may provide a far greater explanation of the variability in psychosocial functioning. The possibility that stature, by itself, predicts behavioral outcomes to only a small degree brings into question the justification of providing GH therapy to all short children to improve psychosocial adjustments. These data allude to the complex interaction between statural deficits, social behavior, and cognitive functioning in short children. Further study of these factors will help to clarify our understanding of such relationships.

# Sleep, Growth Hormone Secretion, and Short Stature

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Considerable investigative effort has been directed toward studying the relationship between physiologic growth hormone (GH) secretion during waking and sleeping hours and growth. Much of this effort has concentrated on children with growth hormone deficiency (GHD) and idiopathic short stature (ISS), but sleep patterns and GH secretion in normal adults and GHD adults have also been reported.

## ONTOGENY OF SLEEP

Sleep appears in virtually all mammalian species, yet we do not understand why we sleep, nor do we know how to measure the outcome of sleeping. We describe sleep by recording its periodicity and its occurrence with respect to the 24-hour light-dark cycle (or circadian rhythm). Periodicity varies in humans such that the neonate may have several sleep-wake cycles during the 24-hour period while most adults have 1 sleep-wake cycle. Adaptation to the light-dark cycle occurs in infants after 2 to 3 months, but the length of time it takes to adapt to sleeping predominantly in the dark phase of the light-dark cycle is variable. In the mature adult it appears that an endogenous "biological clock," with a periodicity of approximately 25 hours, is operative and influenced by external cues serving to keep us on a 24-hour cycle length. It is unknown whether the neonate already has such an endogenous clock.<sup>1-5</sup>

## SLEEP STAGING

Sleep (stages 1 to 3) is staged by electroencephalography (EEG). Electrode placements aid in detecting eye movements (electro-oculography, EOG) and heart rate (electrocardiography, ECG). Using these recordings, the sleep EEG is divided into 2 major phases: rapid eye movement (REM) and non-REM sleep. In REM sleep, there may be increased heart rate (detected by ECG), increased eye movements (detected by EOG), and EEG patterns that are distinctly different than non-REM sleep. Dreams often occur during REM sleep.

In non-REM sleep, there are 4 distinct stages. The last 2 stages (stages 3 and 4) are characterized by the presence of low-frequency, high-amplitude wave forms lasting for extended periods. These stages are called slow wave or delta sleep. Stage 1 sleep is often described as light sleep and is a transition to the more clearly defined stage 2 sleep. Stage 1 is short-lived, usually lasting less than 10% of the total sleep period. Stage 2 sleep is characterized by the presence of clearly defined wave patterns, eg, sleep spindles and K-complexes. The total duration of stage 2 is often greater than 50% of total sleep.

Sleep is further defined by a characteristic transition from light sleep, stage 1 through stages 2, 3, and 4, followed by a period of REM sleep. This non-REM/REM cycle repeats itself throughout the typical night's sleep, although all of the non-REM stages need not occur before a period of REM ensues.

Human sleep stages vary from infancy through childhood to adulthood. Roffwarg et al<sup>6</sup> reported that newborn infants spend nearly 16 hours a day asleep, with half of the sleep being REM sleep. The duration of REM sleep steadily decreases, as does the total sleep period, with age, such that an adolescent sleeps an average of 8 hours a day and adults 50 to 90 years of age sleep an average of <6 hours. After an initial increase in the first decade of life, non-REM sleep time declines from 6.5 to 7 hours to <5 hours in the 50-to-90-year age group. The percentage of non-REM sleep that consists of delta sleep (stages 3 and 4) declines to nearly zero in the latter age group.

## SLEEP-PROMOTING FACTORS (SUBSTANCES)

The discovery of episodic hormone secretion led to an increased understanding of circadian rhythms.<sup>7-9</sup> Sleep-associated rises of GH led to the speculation that sleep and sleep-onset may be influenced by such nocturnal GH elevations. These hypotheses were later proven to be incorrect through research on the causes of sleep, using animal models, during the past 10 to 15 years. Reports have supported speculations that there may be endogenous substances that either

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induce sleep or set into motion a sequence of events leading to sleep.<sup>10,11</sup> Such substances found in the central nervous system (CNS) are short polypeptides (nonapeptides) called delta sleep-inducing peptides (DSIPs) based upon their ability to induce delta (slow wave) sleep (stages 3 and 4). DSIP is a glycoprotein. The exact sequence of glycoside moieties may vary, but the peptide portion appears relatively constant. DSIP is similar to the peptidoglycan sequences found in bacteria cell walls known as muramyl peptides.<sup>12,13</sup> Further studies have revealed that DSIPs, given orally to newborn rats, circulate intact in the blood. Immunoreactive DSIPs can be found in the CNS and some peripheral organs of mammalian species.<sup>14,15</sup> In humans, DSIPs have been found in breast milk, with a circadian secretion pattern consisting of an afternoon peak and an early morning trough.<sup>14</sup>

Other substances apparently have the same effect as DSIPs in promoting sleep. The link with DSIPs could be a common or shared cellular effect that promotes the release of lymphokines. Three major lymphokines, tumor necrosis factor (TNF), interleukin-1B (IL-1B), and interferon-L2 (IFN-L2) have sleep-promoting effects, causing delta sleep (stages 3 and 4).<sup>12,13,16</sup> IL-1B has been shown to release GH in animals. Another substance proposed to have a causal relationship with sleep-onset is prostaglandin D<sub>2</sub>.<sup>12</sup>

## SLEEP AND GROWTH HORMONE SECRETION

Many investigators have described the relationship of sleep and GH secretion. These studies have demonstrated that when sleep—especially delta sleep (stages 3 and 4)—is interrupted, GH secretion is diminished. Despite these observations, it remains unclear whether sleep and GH secretion have a causal interrelationship.<sup>16-17</sup>

GH may be secreted spontaneously during waking hours, during stress, and following provocative stimulation. The mechanism of GH secretion under these circumstances does not involve sleep, although children are often sleepy after administration of clonidine.

This observation led us to evaluate children being challenged with clonidine by monitoring their EEGs.<sup>18</sup> Preliminary data in 12 children with ISS demonstrated the simultaneous occurrence of GH release and EEG-defined sleep after oral clonidine (0.1 mg/m<sup>2</sup>). In 8 of the 12 subjects, EEG-documented delta sleep developed. Their peak GH responses occurred at the same time as the other children who fell asleep but did not enter stage 3 or 4 sleep. The absence of delta sleep in the latter group did not prevent GH secretion, suggesting that neither GH secretion nor delta sleep is the prerequisite of the other.

Finkelstein et al,<sup>19</sup> Prinz et al,<sup>20</sup> and others have described an age-related decrease in GH secretion. Older adults also may have less delta sleep (Table 1).<sup>21</sup> This supports the concept that GH secretion and delta sleep are as closely related in adulthood as in childhood and adolescence.

## Sleep and Growth Hormone Deficiency

Orr and colleagues<sup>22</sup> were among the first to describe sleep in children with GHD. They concluded that GHD children, ages 6 to 8 years, had more delta sleep than older GHD children, ages 14 to 16 years.

Our studies in GHD children suggest differences in stage 1, stage 3, and REM sleep as compared with normal children, but these differences were not statistically significant. We reevaluated these children after GH therapy was instituted and observed that stage 3 sleep decreased significantly

Table 1  
Sleep in GHD and ISS Children

| Study                         | Diagnosis              | Age<br>(Yr. Mo.)       | Sleep Stages<br>(% Total Sleep Time) |      |      |      |        |      | Wake             |
|-------------------------------|------------------------|------------------------|--------------------------------------|------|------|------|--------|------|------------------|
|                               |                        |                        | 1                                    | 2    | 3    | 4    | 3+4    | REM  |                  |
| Wu/Thorpy <sup>24</sup>       | ISS (n=6)              | 4.10 - 16.6            | 6.9                                  | 45.7 | 10.3 | 16.4 | (26.7) | 20.3 | 18.0             |
|                               | GHD (n=7)              | 6.3 - 10.5             | 9.7                                  | 41.0 | 10.0 | 19.7 | (29.7) | 19.5 | 1.0              |
| Astrom/Lindholm <sup>21</sup> | GHD (n=8)              | 18.8 - 28.2            | 6.6                                  | 62.4 | 7.3  | 5.8  | (13.9) | 13.4 | 4.5              |
| Prinz et al <sup>20</sup>     | Normal males<br>(n=14) | 23.0 - 28.0            |                                      |      |      |      | (22.9) |      |                  |
|                               | (n=16)                 | 58.0 - 82.0            |                                      |      |      |      | (10.0) |      |                  |
| Williams et al <sup>3</sup>   | Normal females         | 6.0 - 9.0 <sup>a</sup> | 2.3                                  | 47.8 | 3.1  | 16.8 | (19.8) | 29.3 | 0.7 <sup>b</sup> |
|                               | Normal males           | 5.0 - 9.0 <sup>a</sup> | 2.3                                  | 47.9 | 3.6  | 18.5 | (22.2) | 27.3 | 0.3 <sup>b</sup> |

a) 6.0 - 9.0 year group differed from an older group (10.0 - 12.0), but the difference did not affect comparisons with other studies.<sup>3</sup> b) Calculated from data in Williams et al.<sup>3</sup> REM, rapid eye movement; ISS, idiopathic short stature; GHD, growth hormone deficiency.

( $P < 0.05$ ), while total delta sleep (stages 3 and 4) was unchanged.

Astrom and Lindholm<sup>21</sup> reported that delta sleep is decreased in adults with GHD. These adults, ages 18 to 28 years, had significantly decreased stage 4 sleep. Stage 3 sleep was reported to be approximately 7% of total sleep time. This percentage is comparable to that reported by Taylor and Brook<sup>23</sup> for non-GHD children and our data for GHD and ISS children (pretreatment, see Table 1).<sup>24-26</sup> Astrom and Lindholm further reported no significant differences in the amount of REM sleep corrected for the duration of sleep as compared with age-matched controls.<sup>21</sup>

Astrom et al<sup>27</sup> conducted similar studies in 8 GHD adults, ages 20 to 30 years, and found no change in delta sleep after GH therapy with increased REM and decreased stage 1 and stage 2 sleep. These data suggest that GH may exert its influence on sleep irrespective of delta sleep.

### Sleep and Idiopathic Short Stature

Studying sleep and GH secretion in children with ISS provides an opportunity to evaluate this interrelationship in subjects with normal GH secretion in response to stimuli.

Preliminary data from ISS children reported by Taylor and Brook<sup>23</sup> indicate marked abnormalities in sleep and sleep stages. Their subjects, however, were diagnostically heterogeneous, (eg, genetic short stature, poor nutrition, psychosocial dwarfism, and constitutional delay of growth and puberty). In the subset with psychosocial dwarfism, stage 4 sleep was significantly decreased. REM sleep increased significantly in the subsets with psychosocial dwarfism and genetic short stature. One subgroup of children with severe short stature and behavioral problems (psychosocial dwarfism type 2) had significantly increased waking time. No details on sleep stages were reported, thus comparison with other data was not possible.

Table 2 summarizes our data in GHD and ISS children. Comparable results are demonstrated for REM and sleep stages 1 through 4 for the 2 groups. The percentage of stage 3 sleep in ISS and GHD children was comparable to the percentages

reported by Astrom and Lindholm<sup>21</sup> for young GHD adults. The percentage of stage 3 sleep was significantly higher than that found in normals of the same age range as reported by Williams et al<sup>3</sup> (see Table 1). Our data further show significantly different percentages of waking time between GHD and ISS children (1.0% vs 18.0%, respectively). These observations, along with the reports of others, suggest an association of short stature, neuroregulatory dysfunction of sleep, and GH secretion.<sup>23</sup>

### GH Secretion and Delta Sleep

To test the hypothesis that GH secretion and delta sleep are interdependent, we treated 7 patients with GHD and 11 patients with ISS, recording sleep before and after GH therapy.<sup>26</sup> Five ISS children served as controls. The preliminary results are shown in Table 2. The basic sleep stages, REM cycles, and total sleep times are comparable in the 3 groups. Of particular interest is the similarity of stage 3 sleep in all 3 groups *before* GH therapy. After therapy, there was a significant decrease in stage 3 sleep in the GHD and treated ISS groups ( $P < 0.05$  and  $P < 0.01$  respectively). The decrease in stage 4 sleep for the treated ISS group was not statistically significant.

These preliminary data suggest an interrelationship between exogenous GH and delta (stage 3) sleep. If a sleep-inducing substance such as DSIP is the mechanism for sleep induction and GH release in humans, our data suggest a specific feedback loop triggered by exogenous GH affecting delta sleep. While stage 3 sleep decreased after GH therapy in the treated ISS group, this same group had no demonstrable decline in GH secretion as determined by peak GH concentrations, mean GH levels, and GH secretory rates during sleep when tested 48 hours after the last dose.<sup>28</sup>

The association of GH secretion with sleep appears to be as complex as sleep itself.<sup>8</sup> Careful investigation in children and adults, including normal, ISS, and GHD subjects, is needed to examine age-dependent changes in sleep and GH secretion. Such research will help to elucidate the physiology of sleep and its interrelationship with GH secretion.

Table 2  
Sleep Stages (% Total Sleep Time)<sup>24,25</sup>  
Shown As Before/After GH Therapy

| Diagnosis    | 1        | 2         | 3         | 4         | REM       |
|--------------|----------|-----------|-----------|-----------|-----------|
| GHD (n=7)    | 9.7/11.2 | 41.0/41.4 | 10.0/7.5* | 19.7/20.3 | 19.5/17.9 |
| ISSRx (n=11) | 4.0/6.3  | 38.2/47.7 | 10.0/3.7* | 33.0/22.2 | 15.0/19.4 |
| ISSC (n=5)   | 3.2/6.6  | 53.6/50.7 | 8.0/8.0   | 22.3/25.2 | 12.8/12.3 |

GHD, growth hormone deficiency; Ages 6.3-10.5 (yr.mo). ISSRx, idiopathic short stature, treated; Ages 6.7-11.6 (yr.mo). ISSC, idiopathic short stature, control; Ages 6.0-10.3 (yr.mo). \*  $P < 0.05$ .

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## Abstracts From the Literature

### Aggressive Surgical Management of Craniopharyngiomas in Children

Hoffman et al, from the Hospital for Sick Children in Toronto, report 14-years experience in total excision of craniopharyngiomas in 50 children (22 girls and 28 boys, from 1 year 10 months to 17 years 7 months in age). Headache with a duration of 2 weeks to 4 years was the most common presenting complaint (68%). Thirty-three patients (66%) had some endocrine abnormality at presentation (14%, hypothyroidism; 40%, short stature; 24%, diabetes insipidus; 18%, obesity; and 14%, delayed secondary sexual development). One patient presented with precocious puberty. Visual abnormalities were present in 30% to 60%, with 48% having a field defect, the most common being bitemporal hemianopia.

Computed tomographic (CT) evidence of tumor calcification was observed in all 50 patients. Eighty percent of the tumors had some form of cyst formation. Sellar enlargement and/or blunting of the dorsum sellae was noted in 40%. Half the tumors were prechiasmatic. Hydrocephalus was present in 24 of the 50 patients at the time of surgery and in 74% of the patients with retrochiasmatic tumors. The most common surgical approach was right frontal craniotomy. Forty-five patients, or 90%, were considered by the surgeon to have undergone total tumor excision at the time of surgery.

Follow-up was obtained on 46 patients. One died in the immediate postoperative period, and 2 others died 9 years after initial surgery. Follow-up was from 1 to 14 years and 39% for at least 5 years. Thirty-four percent (16) have experienced tumor recurrence, a third of which were asymptomatic and discovered on routine neuroimaging. Eight presented with headaches, 5 had deterioration in visual acuity, and 1 had an increased need for desmopressin. Among 13 patients with tumor recurrence, 5 had normal postoperative CT findings, but 8 demonstrated either calcification or residual tumor.

Nine had improvement in visual fields, but 16 of those who had no field defect before surgery had deterioration of visual fields. Endocrine deficiencies were observed in all 46 follow-up patients postoperatively. Over 90% required desmopressin, 89% cortisone, 83% thyroid hormone, 31% sex steroids, and 20% growth hormone. Seventy-four percent

required a combination of thyroid hormone, cortisone, and desmopressin. Fifty-two percent were obese at follow-up, but almost 30% of these had been obese prior to surgery.

Twenty-seven children had a formal psychometric evaluation at follow-up. Twenty-six of 27 had intelligence levels at or above average levels. However, memory was impaired in 16 of the 28 children tested. Twenty-four of 39 assessed for educational status were attending regular school.

Quality of life was assessed by categorizing patients into 3 groups based on morbidity. Those in the first group, or those with the "good quality of life," had no tumor recurrence or, if the tumor recurred, it was adequately managed with surgery. In addition, these patients had good control of their endocrine deficiencies and were attending or had attended regular school, and displayed no behavioral or eating disturbances. Sixty-four percent of patients fell into this category. The severely handicapped group included patients with unstable tumor recurrence and poorly controlled endocrine status. They were not attending school because of behavioral problems or major psychological disturbances. Only 9% fell into this group. The remaining 27% fell into the intermediate group.

Hoffman HJ, De Silva M, Humphreys RP, et al. *J Neurosurg* 1992;76:47-52.

**Editor's comment:** This is an interesting and very well compiled follow-up report regarding the outcome of microsurgical management of craniopharyngiomas evaluated by CT. The data have been carefully assessed and should provide useful information for pediatric endocrinologists who are often responsible for diagnosing this tumor and who need to discuss the outcome and prognosis with their patients and their families. It is interesting to note that radiation therapy was used only in patients with tumor recurrences. In 1992 most patients will receive radiation in addition to surgical therapy. Therefore, statistics using data from combined therapy should be improved over those presented here.

William L. Clarke, MD

## Binding Protein for Human Growth Hormone: Effects of Age and Weight

The authors studied the age-related changes in serum levels of high-affinity growth hormone-binding protein (GHBP) measured by gel chromatography on a long (100 cm) column of Sephacryl S200. This GHBP is considered to be identical to the extracellular portion of the hepatic receptor to growth hormone (GH). The method measures the 85-kd complex containing the GHBP, after addition of  $^{125}\text{I}$ -labeled human GH of the 22-kd type in the serum to be studied. The results are expressed as the percentage of radioactivity eluting in the 80- to 90-kd range.

Sera from 250 normal infants and children were studied. GHBP was very low in cord sera, with an average of  $3.3\% \pm 0.7\%$ , and stayed near this level up to age 2 months. Then it sharply increased during infancy and reached  $12.7\% \pm 3.9\%$  at 18 to 24 months of age. Further increase during childhood was slower, with the mean level after age 18 years attaining  $19.7\% \pm 7.1\%$ , and a wide range of individual values between 7% and 40% in children ages 7 to 18 years, with no obvious change at puberty. There was no difference between females and males at any age.

Correlation with age was significantly positive in the younger children ( $r=0.31$ ,  $P<0.005$ ) but below the level of significance in the older group ( $r=0.15$ ). Correlations with height and weight were calculated. Only 2 significant correlations were found: a moderately positive correlation with weight expressed as standard deviations (SD) for age in the patients aged at least 2 years ( $r=0.42$ ,  $P<0.0001$ ), and a weak negative correlation with height SD for age in older children ( $r=-0.17$ ,  $P<0.025$ ). Partial correlation analysis showed no change of the correlation with weight when height was excluded, and a small change of the negative correlation with height when weight was excluded ( $r=0.22$ ).

From these data, the authors point out that nutrition affects GHBP levels. The high serum GHBP found in most overweight children is considered as likely to reflect an increased number of peripheral GH receptors. This could reflect the usually low serum GH concentrations in obesity, contrasting with normal levels of insulin-like growth factor 1 (IGF-1) and normal or sometimes increased growth velocity. The authors comment on other aspects of their results, and compare their data with those previously reported by other authors.

Holl RW, Snehotta R, Siegler B, et al. *Horm Res* 1991;35:190-197.

**Editor's comment:** Among many studies of the main serum GHBP, this one seems of special value since it involves a great number of normal individuals from birth to adulthood, uses a most reliable technique, and calculates partial correlations in order to exclude the "pseudocorrelations" resulting from the physiologic relationships between age, height, and weight during childhood and adolescence. Not only the positive correlation with weight and probable correlation with nutritional status but also the negative correlation with the SD of height are reported. The study in newborns and infants suggests to the authors a relationship between GHBP, probably GH receptors, and the developmental switch from more or less

GH-independent intrauterine growth regulations to GH-dependent postnatal growth mechanisms. The data appear to be a valuable contribution to the presently poor insight we have on early postnatal changes in the regulation of longitudinal growth.

Jean-Claude Job, MD

**2nd Editor's comment:** Dr. Paul Martha, Jr, and colleagues recently presented a paper at the American Pediatric Society/Society of Pediatric Research (APS/SPR) meetings that supplements the data reported by Holl et al. For the purpose of broadening the perspectives regarding GHBP for our readers, the abstract by Martha et al is reprinted here from the program of the APS/SPR meeting.

### A LONGITUDINAL ASSESSMENT OF SERUM GROWTH HORMONE-BINDING PROTEIN IN NORMAL BOYS DURING PUBERTY

Recent studies suggest the high-affinity serum GHBP may serve an important role in regulating normal body growth in humans. Therefore, we evaluated the relationships among GHBP, linear growth, and GH secretion over time in normally growing boys ( $n=11$ ). On 154 occasions over 4.0 to 5.1 years, a physical exam, height and weight measurement, 24-hour GH study, and serum GHBP measurement were performed. GHBP levels varied widely among the group and spanned a 12-fold range (40 to 504 pmol/L; coefficient of variation [CV] = 51%). However, individual subjects' values varied within much more narrow limits (intrasubject mean CV = 30%, mean range 3.1-fold,  $P<0.05$ ). The individual subjects' overall mean GHBP correlated inversely with the overall mean 24-hour GH level ( $r=-0.61$ ,  $P<0.05$ ) and correlated directly with mean body mass index SD score ( $r=0.69$ ,  $P=0.018$ ). For individual subjects, GHBP did not correlate with growth velocity or age. GHBP levels (pmol/L) according to age (years) at the time of study were as follows:

| Age  | <11    | 11-11.9 | 12-12.9 | 13-13.9 | 14-14.9 | 15-15.9 | 16-16.9 |
|------|--------|---------|---------|---------|---------|---------|---------|
| GHBP | 208±24 | 241±39  | 196±36  | 175±24  | 161±27  | 217±29  | 200±39  |

No statistical differences were detectable among groups, therefore these data indicate that although serum GHBP concentrations for each child fluctuate over time during puberty, they do so within relatively narrow limits more characteristic of the individual child than of the larger population. The maintenance over time of a positive correlation between GHBP to body mass index SD score and an inverse relationship to mean GH secretion level lends further support to the concept that these factors are intimately and inextricably interrelated to normal growth and development. The data do not support the existence of an increase in GHBP levels confined specifically to the period of active pubertal development.

Robert M. Blizzard, MD



## Effects of Prolonged Growth Hormone Administration in Rats With Chronic Renal Insufficiency

Surprisingly, growth hormone (GH) administration to children and rats with chronic renal failure significantly increases the growth velocity (GV) over short periods. The long-term effect on GV and kidney anatomy, physiology, and histology is not yet known. Therefore, Allen et al sought to determine the long-term effects on rats. Four groups of young rats were placed in the study: GH-treated and untreated 75% nephrectomized rats and GH-treated and untreated sham-operated rats. GH 1.0 mg SC was administered *tw*. The rats were examined at 9, 15, and 25 weeks. GH was administered during weeks 4 through 12. The following results were determined:

1. Uremic GH-treated rats grew significantly more than untreated rats. The lengths of treated uremic rats were comparable to untreated sham-operated rats at all times.
2. Sham-operated rats treated with GH were longer than untreated sham-operated rats.
3. Weights of uremic GH-treated rats equaled those of untreated sham-operated rats at 15 weeks.
4. Glomerular filtration rate was markedly and comparably reduced in all uremic rats. GH therapy did not affect glomerular filtration rate in either group.

5. Diminished food efficiency of uremic rats was not improved significantly with GH treatment.

6. Both mean glomerular area and sclerotic index were increased in GH-treated rats.

7. Mortality from chronic renal failure was 8 of 19 (42%) in uremic GH-treated rats versus 4 of 13 (31%) in untreated uremic rats.

Allen DB, Fogo A, El-Hayek R, et al. *Pediatr Res* 1992;31:406-410.

**Editor's comment:** Studies of this sort are very much needed to sort out the beneficial versus detrimental effects of GH treatment in children with chronic renal failure and those posttransplantation (GGH 1991;7[2]:13-14). Collaborative efforts among several centers are in progress to determine the effects of long-term GH treatment in chronic renal failure. In the meantime, GH should not be used for growth retardation in renal disease unless under an approved Investigational Review Board (IRB) protocol.

Robert M. Blizzard, MD

## Growth of Infants With Neonatal Growth Hormone Deficiency

There are limited data regarding the growth patterns of infants with neonatal growth hormone deficiency (GHD), and differences of opinion exist regarding the need for growth hormone (GH) to produce normal growth in the first 6 months of life. The authors studied 15 patients (8 males, 7 females) with GHD as well as other tropic hormone deficiencies in an attempt to answer this question. The patients were categorized as having GHD in the newborn period because of the presence of at least 1 of the following in association with GHD: hypoglycemia (13 of 15), micropenis (7 of 8 males), and/or jaundice (13 of 15). Breech delivery occurred in 5 of the 15.

Five had a birth length less than 2 standard deviations (SD) below the mean for gestational age. The mean birth length of the 15 was -1.5 SD below the normal average length. Eight patients had a growth curve with a downward deviation from birth onward, and 7 had no marked lateral deviation from the normal percentile curves up to 9 months.

The conclusion made was that the data are compatible with the hypothesis that (1) some infants with neonatal panhypopituitarism do not have total GHD at birth but develop such deficiency in the ensuing months. In support of this, 2 infants had shown peaks of normal GH release to provocative tests shortly after birth, but the peaks decreased later; (2) GH is necessary for growth immediately after birth; (3) it is uncertain whether, on the basis of the data presented, GH plays a part in prenatal growth; and (4) the ICP (infancy-childhood-puberty) growth model is dependent upon the presence of GH throughout all phases and is not independent during the

infantile phase, as postulated by some clinical investigators.

Wit JM, van Unen H. *Arch Dis Child* 1992;67:920-924.

**Editor's comment:** These data and conclusions are confirmatory as they support, in part, conclusions made some years ago. Brasel et al (*Am J Med* 1965;38:484) reported that 39.5% of 39 patients with idiopathic GHD had growth failure during the first year of life. Of the 36 infants born at term, only 4, or 11%, had birth weights less than the third percentile, which is in contrast to the data of Wit and van Unen. Brasel et al concluded that GH was necessary for growth in the first year of life but probably not necessary in utero. The latter also is deducible from the studies of GHD infants born to GHD mothers where the infants are of normal birth weight and length. Interestingly, the GH insensitive patients of the Laron-type often are small at birth. Laron (*Adv Intern Med* 1984) reported that 10 of 16 were more than 2 SD below the mean birth weight of normals. These data suggest that absence of the pituitary receptor may be more important in normal uterine growth than GH itself.

A clinical point made by Brasel et al, which is not appreciated by many, is that one third of 33 patients with adequate dental records had delayed eruption of the primary teeth, and 75% of 36 GHD patients with adequate dental records had delayed eruption of the secondary teeth.

Robert M. Blizzard, MD

## Psychosocial Growth Failure: A Positive Response to Growth Hormone and Placebo

Boulton et al report their study of a double-blind placebo crossover trial of growth hormone (GH) in 7 children (3 males, 4 females, ages 3.6 to 11.6 years; bone age range of 2 to 9.5 years) with the diagnosis of psychosocial growth failure. Six of these children had a disorder of attachment dating from infancy with recurrent depression in 3. The other child had reactive depression from current family stress. All children were measured with a Harpenden stadiometer. Growth velocity and weight were converted to standard deviation scores (SDS). All children had heights <3rd percentile (-2 SDS), growth velocity <25th percentile (-0.09 SDS), and had been monitored at least 1 year at 3-month intervals. All were prepubertal at the start of the trial. GH secretion was measured at 20-minute intervals during the first 3 hours of sleep and the results analyzed using the PULSAR program. Dietary intake was assessed by computer analysis of 4-day food diaries.

Mean GH concentration during sleep was  $10.9 \pm 4.4$  mU/L with a mean peak level of  $19.6 \pm 6.7$  mU/L. All subjects had a maximum peak of 20 mU/L or greater. Mean peak interval was  $147 \pm 108$  minutes. The mean ( $\pm$  SEM) insulin-like growth factor 1 (IGF-1) was  $1.08 \pm 0.31$  U/mL. The mean ( $\pm$  SEM) SDS growth velocity prior to treatment was  $-2.32 \pm 0.122$ , for the placebo period  $-0.6 \pm 0.69$ , and for the GH treatment period  $+4.66 \pm 1.88$ . Significant differences in velocity between each of the 3 periods were demonstrated by analysis of variance ( $P < 0.0001$ ). The greatest difference between growth velocities was between the pretrial and GH periods ( $P < 0.001$ ). The order of treatment did not affect the growth response. The mean ( $\pm$  SEM) IGF-1 did not change significantly during GH treatment ( $1.24 \pm 0.34$  U/mL at the end of treatment versus  $1.09 \pm 0.31$  U/mL pretreatment). The mean daily food energy intake was similar for the trial, pretrial, and posttrial periods.

Boulton TJC, Smith R, Single T. *Acta Paediatr* 1992;81:322-325.

**Editor's comment:** This is an interesting report, but it is not clear that the children studied had classic psychosocial dwarfism. While in their adverse environment, as defined by Powell et al, children with this syndrome often have reversible GH deficiency with abnormal GH responses to pharmacologic stimuli. GH secretion in the children in the present study was normal. Therefore, they were not shown to have GH secretory dysfunction. The authors acknowledge this, and suggest that the GH secretion and growth response of these children are similar to those of children with constitutional delay of growth.

However, the presence of a significant placebo effect suggests that the intervention may have altered family dynamics in some manner. Even though these children do not necessarily fit the original criteria for the definition of psychosocial dwarfism, they clearly had psychological dysfunction and significant growth retardation that responded to GH administration. Thus, these children, and other similar children, may be potential candidates for GH treatment. Whether or not such treatment may alter their psychological status is left for speculation.

William L. Clarke, MD

**2nd Editor's comment:** The topic of psychosocial short stature (PSS) has been of great interest since we (Powell et al, *N Engl J Med* 1967) reported a group of children with the syndrome who had reversible hyposomatotropism. Recently, I have written 2 reviews of this topic; the first in *Pediatric Endocrinology: A Clinical Guide*, edited by F. Lifshitz (1990), and the second in a text entitled *Bailliere's Clinical Endocrinology and Metabolism. Growth Disorders*, edited by J. Bierich (1992). These references are given for readers who may wish to read further concerning the topic.

The report by Boulton et al is of importance because of the response of depressed children with growth failure to pharmacologic doses of GH ( $1.2$  U/kg/wk), which is significantly more than the physiologic replacement dose ( $0.3$  U/kg/wk) reported by Fraiser and Rallison (*J Pediatr* 1972;80:603) to not increase the growth of patients with PSS. As pointed out by the authors and by Dr. Clarke in his editorial commentary, these children are different from most children reported with PSS in that they secreted normal amounts of GH. The authors, unfortunately, did not comment on the behavioral characteristics of these children, except for depression. The children described by us and others with PSS often had polyphagia, polydipsia, and encopresis; ate and drank from bizarre places such as dog dishes, gorging themselves to the point of vomiting; and were emotionally rejected by their parents. Hopefully, Boulton et al will write a follow-up article or write a letter to the Editor of *GROWTH, Genetics & Hormones*, providing the psychological and emotional characteristics of the children reported and of their parents. The paper by Boulton et al is an important paper and we need to be able to place it in a better context in relation to other papers published on the topic.

Robert M. Blizzard, MD

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#### Fragile X Syndrome: Review and Current Status

by David L. Nelson, PhD

#### Prenatal Evaluation of Growth by Ultrasound

by Douglas Wilson, MD

#### The Use of Fluorescence in situ Hybridization to Identify Human Chromosomal Anomalies

by Beverly S. Emanuel, PhD

#### The Importance and Methods of Using Animal Models to Study Human Disease

by Robin M. Winter, MD

#### Relevance of Developmental Genetics to Human Malformations

by Golder N. Wilson, MD, PhD

## Pituitary Evaluation and Growth Hormone Treatment in Prader-Willi Syndrome

Angulo et al evaluated the spontaneous growth hormone (GH) secretion and GH responses to clonidine, levodopa, and insulin-induced hypoglycemia in 11 obese and 4 nonobese Prader-Willi syndrome (PWS) patients, 1.5 to 15.5 years of age. Ten patients (3.7 to 15.5 years of age) were treated with GH at 0.1 mg/kg tiw for 6 months.

Integrated concentrations (ICs) of GH using a Cormed-Kowarski constant withdrawal pump, with the specimens being collected over 24 1-hour periods, ranged between 1.0 to 2.1  $\mu\text{g/L}$ , which the authors state was markedly deficient in all.

The responses to insulin, clonidine, and levodopa were variable. Only 1 patient had a serum GH level above 8.0  $\mu\text{g/L}$ , following 150  $\mu\text{g/m}^2$  po of clonidine. Values of serum GH following levodopa were above 8.0  $\mu\text{g/L}$  in 3 patients (12.6, 11.4, and 11.4  $\mu\text{g/L}$ ). The response to insulin (0.1 U/kg IV) was 8  $\mu\text{g/L}$  or above in 8 patients. The authors conclude that GH deficiency probably was present in all patients. The somatomedin-C (Sm-C) determinations were <1.0 U/mL and compatible with GH deficiency in 9 of 15 patients. Bone age (BA) determinations were not less than -2 standard deviations (SD) for chronologic age (CA) in any patients.

GH treatment over 6 months increased the mean growth velocity (GV) from  $2.0 \pm 2.3$  cm/yr to  $5.3 \pm 1.5$  cm/yr. GV doubled in all patients except in a 15.5-year old male with a BA of 14.5 years, a BA at which the average male has only 5% of his ultimate height yet to be gained. The patients gained little weight. Sm-C levels routinely increased to above 1.2 U/mL with a mean of  $1.5 \pm 0.2$  U/mL.

The authors conclude that "short stature associated with obesity, hypotonia, decreased energy expenditure, delayed skeletal maturation and failure to respond to GH stimuli makes PWS children potential candidates for GH therapy. Further studies, however, are necessary to investigate the safety and long-term effects of this form of therapy."

Angulo M, Castro-Magana M, Uy J. *J Pediatr Endocrinol* 1991;4:167-172.

**Editor's comment:** The authors performed a well-designed study. The data provide both the stimulation and basis for future investigation of GH secretion and response to GH therapy by PWS patients. The data, as presented, support the concept that these patients may have GH deficiency. Responses of GH release secondary to pharmacologic testing with 3 stimulating agents certainly appear to be low. Seven of the patients had no peak of GH >8.2 ng/mL in any of the tests (insulin, clonidine, levodopa). Only 2 patients in the entire group of 15 had a value >8.0 ng/mL in more than 1 of the 3 tests. The IC values of GH were <2.1 ng/mL in all which logically prompts one to suspect GH deficiency. Unfortunately, the authors have not provided data of IC for children of normal size. Their control data are taken from the literature, which is always suspect because of the variability of studied groups and the different assays employed, and from studies they have published for children with delay of growth and pubertal development (*J Pediatr Endocrinol* 1989;3:225). In that study, the IC of GH for 44 of 49 short patients was  $2.2 \pm 0.6$  ng/mL. Although some investigators have shown that children with similar short stature have normal ICs of GH, as compared with children of normal size, others have not. Therefore, Angulo et al need to publish concerning their study controls of normal size to diagnose GH deficiency in the PWS patients by using the IC of GH. The response to GH treatment by PWS patients is encouraging, although the study of GH treatment was for only 6 months.

It must be noted that the dose of GH used (0.1 mg/kg tiw) is a pharmacologic dose, and may be expected, therefore, to often produce an increased GV in both children of normal and short stature. However, the fact that increased growth rate in PWS patients results from pharmacologic doses does not diminish its potential therapeutic application. I use the word "potential," as the effect on final height is the ultimate criterion for GH treatment in these patients. The authors appropriately conclude that further studies are necessary to investigate safety and long-term effects. All of us will follow with interest the subsequent periods of treatment.

Robert M. Blizzard, MD

## Effects of Insulin-Like Growth Factor on Linear Growth, Head Circumference, and Body Fat in Patients With Laron-Type Dwarfism

Five children with Laron-type growth hormone insensitivity syndrome (LTS) were treated with recombinant insulin-like growth factor 1 (IGF-1) injected SC once daily at an initial dose of 150  $\mu\text{g/kg/d}$ . The dose then was adjusted according to serum IGF-1 concentration. Striking changes in growth occurred from the first month, with a growth velocity corresponding with 8.8 to 13.6 cm/yr. Body fat, measured by subscapular skin-fold, decreased in the same time.

In 2 of these LTS patients, continuation of treatment for 10 months induced important morphologic changes, characterized by maximal limb growth and, unexpectedly, a striking and early increase of head circumference, even at age 13 to 14 years. There were no undesirable side effects, particularly metabolic. This suggests to the authors a possible effect on brain growth. Though preliminary, these results are encouraging for long-term treatment of LTS and probably other growth hormone insensitivity syndromes.

Laron Z, Anin S, Klipper-Aurbach Y, et al. *Lancet* 1992;339:1258-1261.

**Editor's comment:** This is the second report that IGF-1 increases the growth of LTS individuals. The first was by Walker et al (*N Engl J Med* 1991;324:1482). IGF-1 has the potential to be as important a therapeutic agent as growth hormone. We can anticipate in the next few years reading many studies designed to test the effectiveness of IGF-1 in metabolic disorders as well as in growth disorders. Fortunately, it has been demonstrated that IGF-1 can be given to humans with minimal concern of producing hypoglycemia from its insulin-like action. (Takano, et al. *Growth Regulation* 1991;1:23-28.)

Jean-Claude Job, MD

## Expression and Regulation of IGF-1 in Cartilage and Skeletal Muscle

Isgaard presents a mini-review of this topic of great importance. The major questions to be answered relate to the roles that growth hormone (GH) and insulin-like growth factor 1 (IGF-1) play individually and collaboratively on the acute and long-term growth of cartilage and skeletal muscle.

Because of the length and complexity of the review, only the introduction and concluding remarks are reproduced here, along with a brief editorial comment. Those who are interested, and there should be many, will wish to obtain and read the entire article.

### INTRODUCTION

A number of studies have demonstrated that both GH and IGF-1 have important roles for skeletal growth. Although IGF-1 was originally considered to be produced mainly in the liver, it is now generally recognized that IGF-1 is synthesized in numerous organs and tissues of many animal species. It appears that IGF-1 synthesis in most tissues is regulated by GH, and autocrine and paracrine functions of IGF-1 have been suggested as important components of GH action. Moreover, several studies have revealed enhanced expression of IGF-1, both on the messenger and protein level, during tissue regeneration and repair. The present review is mainly focused on recent studies of IGF-1 and their relevance to possible *in vivo* effects during growth and regeneration of skeletal tissues.

### CONCLUDING REMARKS

Accumulating evidence indicates an important role of IGF-1 in the promotion of tissue growth and repair. However, the relative importance of autocrine/paracrine versus endocrine actions remains to be fully clarified and matters of opinion differ. It has been suggested that the autocrine/paracrine actions of IGF-1 play a minor role compared to endocrine effects, which would account for approximately 80% of the total accumulated GH-IGF-1 dependent postnatal height in humans. These investigators base their assumption on the fact that local administration of GH into the growth plate or via the arterial blood supply of one hindlimb of hypophysectomized rats produces only 12% to 22% of the maximal growth that can be achieved with systemic administration of GH. However, it is improbable that the conditions during these experiments are comparable to those when GH is administered systemically. Therefore, it is highly unlikely that local administration of GH

could produce effects of the same order of magnitude as systemically administered GH. It would also be reasonable to assume that locally produced IGF-1 is of importance during tissue hypertrophy and repair, when high levels of IGF-1 mRNA are expressed without a concomitant rise in circulating IGF-1. Moreover, the stimulatory effect of locally administered GH on the growth plate of hypophysectomized rats was completely abolished if antibodies to IGF-1 were infused with GH. This observation argues for the fact that locally produced IGF-1 is essential for the growth-promoting effect of GH *in vivo*.

The role of GH in the regulation of IGF-1 expression in peripheral tissues other than the growth plate is less clear. IGF-1 synthesis appears to be regulated by GH in most tissues, since the levels of both IGF-1 and IGF-1 mRNA decrease in a large number of tissues after hypophysectomy. On the other hand, during tissue regeneration following injury, increased expression of IGF-1 has been demonstrated in the regenerating tissue, both in intact and hypophysectomized animals. It is conceivable that GH regulates the synthesis of IGF-1 in tissues during normal growth and development, in contrast to emergency situations such as tissue injury or loss of tissue, during which this GH dependence may be uncoupled. Precisely which are the factors that regulate the synthesis of IGF-1 during tissue repair have yet to be clarified.

Isgaard, J. *Growth Regulation* 1992;2:16-22.

**Editor's comment:** This paper reviews the data known on the role of IGF-1 in cartilaginous and muscular growth and repair. *In vitro*, as well as *in vivo*, studies show that it is an important factor. However, the respective effects of endocrine GH-dependent and local paracrine/autocrine IGF-1 are not yet clarified, and the regulation of local IGF-1 by GH is still controversial. Some experiments quoted in this review, based on the expression of IGF-1 and the measurement of its mRNA in cartilage and muscle under influence of GH, suggest that the endocrine and/or GH-dependent effects are predominant under normal conditions. It can thus be speculated by Isgaard that GH regulates the synthesis of IGF-1 in tissues during normal development, in contrast to emergency situations, during which this dependence to GH may be uncoupled.

Jean-Claude Job, MD

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Expression and Regulation of Insulin-Like Growth Factor 1 in Cartilage and Skeletal Muscle

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# GROWTH

## Genetics & Hormones

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### Prenatal Evaluation of Growth by Ultrasound

**R. Douglas Wilson, MD, MSc**

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Abnormal growth patterns are associated with an increased risk of perinatal morbidity and mortality. Therefore, the ability to evaluate growth at a very early age in utero utilizing ultrasound techniques is a major advancement in minimizing fetal and perinatal morbidity and mortality.<sup>1</sup> Understanding the applications of ultrasound to evaluate normal and abnormal intrauterine growth enhances significantly the understanding of postnatal growth. Unfortunately, pediatricians have had limited opportunity to become acquainted with these applications. The purpose of this article is to help fill that void.

Prenatal growth is divided into 2 periods: embryonic and fetal. Prenatal ultrasound allows specific measures of both embryonic and fetal structures, and comparison to normal values permits evaluation of

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growth patterns. Prenatal evaluation is usually possible 3 weeks postconception.

#### EMBRYONIC DEVELOPMENT AND GROWTH

Embryonic development and growth starts with fertilization and progresses through blastogenesis (postconception days 0 through 21) and organogenesis (days 21 through 60). In humans, fusion of the eyelids (days 56 through 60) is regarded as an arbitrary end of the embryonic period (Table 1).<sup>2</sup>

**Table 1: Human Embryonic Development and Growth**

| PERIOD        | CONCEPTION <sup>1</sup><br>(d) | LMP <sup>1</sup><br>(d) | CROWN-RUMP<br>LENGTH (mm) | EXTERNAL<br>CHARACTERIZATIONS           | Staging <sup>2</sup> |                                       |
|---------------|--------------------------------|-------------------------|---------------------------|---|----------------------|---------------------------------------|
|               |                                |                         |                           |   | Jirasek              | Streeter<br>Carnegie <sup>3</sup>     |
| Blastogenesis | 0-14                           | 0-28                    | 0-0.4                     | Unicellular to bilaminar plate          | 1-4.3                | 1-6b <sup>3</sup>                     |
|               | 15-21                          | 29-35                   | 0-4.2                     | Trilaminar embryo to open neural groove | 5.1-6.1              | 7-8 <sup>3</sup><br>ix-x <sup>3</sup> |
| Organogenesis | 22-35                          | 36-49                   | 2-8                       | Neural tube closure to limb buds        | 6.2-7.2              | xi-xiii <sup>3</sup>                  |
|               | 36-60                          | 50-74                   | 8-35                      | Limb growth to fused eyelids            | 7.3-8.2              | xiv-xxii <sup>3</sup>                 |
| Fetal         | 61-266                         | 75-280                  | 35-350                    | Fetal maturation                        | 9-10                 | -                                     |

<sup>1</sup>Embryonic development is dated from conception.

<sup>2</sup>Prenatal growth evaluation by ultrasound is dated from day of last LMP (last menstrual period). This is termed "gestational age."

<sup>3</sup>Adapted from Jirasek JE. In: Sciarra JJ, ed. *Gynecology and Obstetrics*. Philadelphia, Pa: Lippincott Co; 1991:2.1.

While embryonic development is dated from conception, prenatal growth evaluation by ultrasound is dated from the first day of the last menstrual period, which is termed "gestational age," and this is the term and time relationship used subsequently in this article.

Normal embryonic and early fetal growth occurs spontaneously and is not affected by secondary factors such as uterine size and placental function, as is growth in the second and third trimesters. Normal growth<sup>3</sup> in the last 2 trimesters is roughly linear, with some slowing from approximately 38 weeks gestation until delivery. However, this slowing may start earlier in twin gestations and in different ethnic populations. Following this normal deceleration in late pregnancy, there is an acceleration after birth and growth similar to in utero growth during the second and third trimesters. Normal embryonic and fetal growth allows an embryo weighing approximately 2 g at 7 to 10 weeks to grow to a fetal mean weight of 3240 g at 42 weeks.

## PRENATAL EVALUATION OF GESTATIONAL AGE

In order to evaluate prenatal growth, it is necessary to accurately estimate the gestational age of the embryo and fetus. Ultrasound measurements used for this estimation are: the mean gestational sac diameter; the crown-rump length (CRL); the biparietal diameter (BPD); and femur length (Table 2).

A gestational sac<sup>4</sup> can usually be identified at 5 weeks and is an early indication of an intrauterine pregnancy. A gestational sac does not confirm a viable pregnancy. Ultrasound evaluation of the embryo can be summarized as follows<sup>5</sup>:

1. At 6 weeks gestational age, embryonic structures and heart activity are almost always visible.
2. At 7 weeks, the embryo is 10 mm at a minimum and fetal heart activity should be visible in 100% of viable pregnancies.

3. At 8 weeks, fetal structures are visible and the yolk sac is identified as a circular structure measuring 5 mm in diameter. The detection of a yolk sac excludes the diagnosis of a blighted ovum since a viable embryo is necessary for yolk sac development.
4. An empty gestational sac with a mean diameter greater than 30 mm with no visible embryonic structures means a nonviable pregnancy (blighted ovum) exists.
5. At 9 to 11 weeks, progressive ossification occurs with major centers in the calvaria and ilium.

The CRL is measured from the outer edge of the cephalic pole to the outer edge of the fetal rump. This measurement predicts the gestational age with an error of  $\pm 3$  days (90% confidence limits) after 7 to 10 weeks. The error increases to  $\pm 5$  days between 10 and 14 weeks gestation. Fetal flexion may decrease maximal CRL length by 5%.

The BPD is maximally accurate for estimation of gestational age between 12 and 20 weeks. It is measured at the level of the thalamus from the outer table of the proximal skull to the inner table of the distal skull. Changes in skull shape, ie, flattening or rounding, can be identified by the cephalic index (CI).<sup>6</sup> This is the ratio of the BPD divided by the occipital frontal diameter (OFD). A normal ratio is 0.75 to 0.85. After 20 weeks gestation, the BPD is less reliable for gestational dating due to changes in shape, growth disturbances, and individual variation.

Femur length is an excellent parameter to determine fetal age. The femur can be measured as early as 10 weeks gestational age.<sup>7</sup> Normal percentile charts are available for the femur and for other long bones, including the humerus, ulna, radius, tibia, and fibula.<sup>8</sup>

Fetal BPD and femur length for gestational age dating have a confidence interval of  $\pm 1$  week from 12 to 22 weeks,  $\pm 2$  weeks from 22 to 32 weeks, and  $\pm 3$  weeks from 32 to 41 weeks.

Table 2: Measurements of Gestational Age by Various Parameters

| MEAN<br>GESTATIONAL AGE<br>(wk)* | MEAN<br>GESTATIONAL SAC<br>DIAMETER (mm)** | EMBRYO<br>CROWN-RUMP<br>LENGTH (mm) | FETAL BIOMETRY<br>BIPARIETAL<br>DIAMETER (mm) | FETAL BIOMETRY<br>FEMUR (mm)† |
|----------------------------------|--|-------------------------------------|---|-------------------------------|
| 5+0                              | 2  | -                                   | -   | -                             |
| 6+0                              | 10   | 6                                   | -   | -                             |
| 7+0                              | 18   | 10                                  | -   | -                             |
| 8+0                              | 26   | 10                                  | -   | -                             |
| 9+0                              | -  | 25                                  | -   | -                             |
| 10+0                             | -  | 33                                  | -   | -                             |
| 11+0                             | -  | 10                                  | -   | 6                             |
| 12+0                             | -  | 55                                  | 17  | 9                             |
| 13+0                             | -  | 68                                  | 20  | 12                            |
| 14+0                             | -  | 85                                  | 25  | 15                            |

\*From 1st day of last menstrual period

\*\*Daya et al, 1991

† Jeanty, 1983

## OTHER FACTORS TO BE CONSIDERED IN THE PRENATAL EVALUATION OF FETAL GROWTH

The accuracy of gestational age estimation using ultrasound needs to be considered. In determining this, one must understand that all parameters used to measure fetal growth have ranges of normal values, which vary with gestational age. It also is important to have an appropriate set of normal data for the population under study. Establishment of one's own normal data base for comparison with published standard curves is highly desirable. At a minimum, each reproductive center must have collected enough data known to be accurate to ascertain that the published data apply to the use of their equipment, technology, and experience.

Birth weight distribution is non-Gaussian as the birth weight of a significant portion of newborns falls outside the expected distribution. Small for gestational age (SGA) is defined as a birth weight less than the 10th percentile. Therefore, the SGA group includes a majority of normal but small infants. Accelerated catch-up growth in the first 3 months of life usually occurs if intrauterine growth problems are independent of fetal genetic factors. Unfortunately, this postnatal observation is not available during prenatal assessment when a fetus is SGA. Large for gestational age (LGA) is defined as a birth weight greater than the 90th percentile. This LGA group also will include normal but large infants.

Fetal weights can be estimated by using established charts comparing the BPD and the abdominal circumference (AC).<sup>9,10</sup> Alternatively, the head circumference (HC) can be compared with the AC. The HC exceeds the AC until 38 weeks gestation, when they become equal. The AC subsequently exceeds the HC. Estimations of fetal weight also can be obtained by using average fetal size percentiles and comparing these measurements to a birth weight-gestational age table. These birth weight-gestational age tables will vary for different populations. The estimated fetal weight using BPD and AC has an error range of approximately  $\pm 15\%$ .<sup>9</sup> There is a tendency to overestimate fetal weights, and the estimate is less accurate when fetal weights are more than 4000 g. The accuracy of the estimated fetal weight is improved in the SGA fetus below the 5th percentile. The calculated weight estimation has decreased sensitivity but fewer false-positive results. Differences in fetal sex also will influence fetal weight. Females have a 3% to 8% lower weight at the same gestational age. Race and parity also may affect normal fetal growth.

The use of BPD and AC gives only minimally greater accuracy than using AC alone, but there is a much higher predictive value in a growth-retarded population.<sup>11</sup>

AC measurement correlates most strongly with overall size of the fetus.<sup>12</sup> The AC is a cross-sectional measurement of the upper fetal abdomen at the level of the liver (fetal portal venous system) and stomach.

The fetal liver is relatively large and significantly affected by growth retardation. However, the AC estimates only whether the fetus is large or small. The sensitivity and predictive value of a positive test increases with gestational age. Optimal screening is at gestational age  $34 \pm 1$  week.

Age-independent ratios also have been considered. Femur length divided by AC after 24 weeks remains at  $0.22 \pm 0.02$ .<sup>13,14</sup> This is more useful when accurate gestational age is unknown. The ratio for growth retardation is greater than .235; for macrosomia the value is less than .205.

Another age-dependent ratio is HC:AC, which may allow classification into symmetric and asymmetric growth patterns, if HC or AC is outside the normal range.

Recommendations<sup>15,16</sup> for ultrasound measurements of fetuses at risk for growth abnormalities usually include dating the pregnancy at 10 to 12 weeks. This is followed with serial growth assessment by ultrasound every 6 weeks between 18 and 30 weeks, and every 3 weeks from 30 weeks to delivery. Longitudinal studies in fetuses at risk for growth retardation indicate that the growth pattern in affected fetuses is more erratic than that seen in normal pregnancies, and may show periods of normal and abnormal growth rates. As the growth problem becomes more serious, the abnormal growth rate persists.

In general, multiple abnormal parameters indicate a more serious problem than a single abnormal parameter, and the risk for a bad outcome significantly increases when the weight estimate and/or HC become abnormal.

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## Letter From the Editor

Dear Colleagues:

In 8 years of publication of *GROWTH, Genetics, & Hormones*, the Editorial Board has been instrumental to the successes of the publication. Editorial Board Members have been picked because of their capabilities as teachers, communicators, authors, and investigators. In addition to those who currently serve, Dr. William Clarke, Dr. Judith Hall, Dr. William Horton, Dr. Jean-Claude Job, and Dr. Fima Lifshitz, whose capabilities and participation I admire and appreciate, there are others who have rotated off the Board and deserve equal recognition. These are Dr. Jürgen R. Bierich, Dr. David L. Rimoïn, Dr. Alan D. Rogol, and Dr. James M. Tanner.



Dr. Allen W. Root, Professor of Pediatrics and Molecular Biology, All Children's Hospital, University of Florida, joined the Board on January 1, 1993. He is eminently qualified as he has broad academic and practical experience in pediatric endocrinology and has written extensively in leading journals and textbooks for almost 30 years. Dr. Root's acceptance of an appointment to the Editorial Board will unequivocally enhance the quality of *GROWTH, Genetics, & Hormones*.

Sincerely,  
Robert M. Blizzard, MD

## Letter To the Editor

This letter is written in follow-up to the abstract and editorial comments concerning our paper entitled "Psychosocial Growth Failure: A Positive Response to Growth Hormone and Placebo" published in *GROWTH, Genetics, & Hormones*, Vol. 8, No. 4.

I welcome the opportunity to elaborate on the characteristics of the children who were included in our study and who had psychosocial growth failure (PSGF) or psychosocial short stature (PSS). I will comment regarding the differences between these patients and those you have described with PSS.

We have a steady referral of classic cases of emotionally damaged children (often with physical abuse) who have all the textbook features of PSGF with impaired growth hormone (GH) secretion, who bounce back to normal once they are placed in foster care. This type of case was excluded from those presented in our article which Dr. Clarke abstracted for *GGH*.

The children in this series were typical short, slow-growing children who had normal GH testing during both sleep and pharmacologic stimulation.

The psychologic assessment revealed anxious attachment. Their depressive symptoms were typical: acting out and/or withdrawal, sad affect, and oversensitivity to minor crises.

They *did not show* lack of discrimination in relationships, nor did they display the self-destructive behavior, pain agnosia, or bizarre eating and sleeping disorders seen in classic PSS. In addition, the parents were not indifferent and rejecting, as are those of patients with typical PSS. The parents had insight into their problem, and several felt guilty and/or had depression. In the classic PSS situation, as you and your colleagues have described it, the parents are

usually considered untreatable because of their own personality damage and entrenched rejection of the child.

The children underwent a battery of psychologic instruments. These included ratings for depression, from which the results were added to those of the diagnostic interview. The assessment process was intense, focusing in large part on relationships with the family. The individual assessments allowed many of the children to discuss concerns that had not previously surfaced. These factors, together with the relative emotional health of the parents in our group, appeared to generate a therapeutic effect from the assessment process itself. This was not predicted when the study initially began. Anecdotally, the parents reacted much more favorably to their children; they all reported greater appetite, even though objective measurements discounted that! This positive outcome remained.

This level of attachment disorder is probably common, underrecognized, and well-camouflaged in middle-class families. Its identification needs an independent psychiatric evaluation, separate from the child's own specialist. I recognize that many people will remain skeptical about the diagnosis of depression in short children. However, the placebo effect was a real surprise. I had not wanted to include it. The Ethics Committee recommended it, and the results are hard to explain, apart from a major change in family function.

I certainly do not recommend GH (or placebo injections!) be used as a cure-all. In fact, our rate of new cases undergoing GH therapy has leveled off in the past year or 2. We have 65 children on GH, for a referral population of 110,000 children aged 0 to 16 years. However, I do think that these observations are a salutary reminder that even if we often deal with

rare and complex diseases with expensive treatment, family issues are the bread and butter of pediatrics, and close collaboration with our liaison child-psychiatrist colleagues can help identify those parents and children who need more expert help.

Yours sincerely,

T.J.C. Boulton, MD  
Professor and Chairman  
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John Hunter Hospital  
Newcastle, NSW, Australia

**Editor's comment:** Dr. Boulton was very kind to write and clarify the characteristics of the patients reported in the paper written by him and his colleagues (abstracted in GGH 1992; 8[4]:13). The observation that children with nonclassic PSGF may respond to GH, and to some extent placebo injections, is most interesting and important.

The description of the patients and their parents reported by Boulton et al requires emphasis that there are several different types of PSS. I suggest that we now use 3 different groupings for PSS, specifically:

Type 1 - Children under 2 years of age whose mothers are overburdened with responsibilities, often neglectful, and who fail to provide adequate calories and stimulation. These patients secrete GH and

respond to increased caloric intake with increased growth. Nutritional failure is the primary etiology of growth failure in this group (Krieger I. Clinical Pediatrics 1974;13:127-133).

Type 2 - Children 2 years of age or older whose parents psychologically abuse and reject them. The majority of these children have diminished GH secretion and some or all of the classic characteristics—polyphagia, polydipsia, encopresis, severe shyness or aggressiveness, pain agnosia—and may or may not be underweight for height (Powell GF et al. N Engl J Med 1967;276:1271-1283. Blizzard RM, Bulatovic A. Psychosocial short stature: a syndrome with many variables. In: Bierich J, ed. Baillieres' Clinical Endocrinology and Metabolism. Volume 6, Number 3. Philadelphia, Pa: WB Saunders Co [Bailliere Tindall Ltd]; 1992.) These children have many physical characteristics in common with GH-deficient patients.

Type 3 - Children over 2 years of age who are depressed, whose parents are not rejecting but who may have emotional problems themselves. The children do secrete GH in normal amounts, but otherwise have many characteristics in common with GH-deficient patients (Boulton TJC et al. Acta Paediatr Scand 1992;81:322).

May I also suggest that we will broaden the spectrum of PSS as our knowledge increases.

Robert M. Blizzard, MD

#### Please Send Correspondence to:

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#### Special Announcement

Back issues of *GROWTH, Genetics, & Hormones* are available. To request copies of back issues of *GGH*, Volumes 1 through 8, Numbers 1 to 4, please write to:

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#### In Future Issues

##### **Fragile X Syndrome: Review and Current Status**

by Douglas Nelson, MD

##### **The Relevance of Developmental Genetics to Human Malformation**

by Golder Wilson, MD

##### **Overgrowth Syndromes and Disorders: Definition and Classification**

by David Weaver, MD

##### **The Overgrowth Syndromes: An Update**

by Kenneth L. Jones, MD

##### **Adrenarche and Its Variants**

by Songya Pang, MD

##### **The Importance and Methods of Using Animal Models to Study Human Disease**

by Robin Winter, MD

##### **Contiguous Gene-Deletion Syndromes**

by Frank Greenberg, MD

##### **Clinical Significance of Urinary GH Measurements**

by Margaret MacGillivray, MD

# The Use of Fluorescence In Situ Hybridization to Identify Human Chromosomal Anomalies

**Beverly S. Emanuel, PhD**

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During the 20 years since the initial discovery of chromosome banding,\* the clinical relevance of chromosomal analysis has become firmly established for genetic diagnosis and evaluation of tumor-specific chromosomal alterations. Global surveillance of the entire cellular genome\* by routine and high-resolution cytogenetic studies permits detection of numerical and structural chromosomal abnormalities, allowing the visual diagnosis of alterations of single chromosomal bands on the order of approximately 5 to 10 million base pairs.\* Smaller changes, those involving less than 1 million and up to several million base pairs of DNA, are difficult or impossible to detect using standard cytogenetic methods. Chromosomal alterations with indistinct banding patterns, such as marker chromosomes,\* de novo unbalanced translocations,\* and abnormally banded regions in somatic\* or tumor cells, also are a problem to identify. Other drawbacks to standard cytogenetic techniques arise from the fact that to perform chromosome analysis the cells must be dividing and the chromosomes must be arrested in metaphase. This means that the process requires a significant investment of time and labor to generate enough dividing cells to perform the study. Finally, cell selection may occasionally cause the results of such studies to be misleading because cells that proliferate in vitro may not be representative of the original population. This is particularly a problem when dealing with tumor specimens.

Many of the aforementioned difficulties have been circumvented with the introduction of fluorescence in situ hybridization\* (FISH) technology into clinical diagnostic laboratories. This relatively new technology provides an important adjunct to classic cytogenetics because of its unique ability to simultaneously assess molecular and cytologic information. This has led directly to numerous clinical applications, such that FISH methods have been developing at a rapid pace for the purposes of high-resolution gene\* dosage analysis and chromosomal abnormality detection.

The ability to detect and characterize chromosomal abnormalities using FISH has been greatly enhanced by the rapidly increasing availability of numerous

chromosome-specific probes.\* In addition, it is possible to accomplish a diagnostic assay within 24 to 48 hours, in contrast to the greater amount of time required for some cytogenetic analyses or for performing standard Southern blotting\* to assess DNA sequence copy number. Several cytogenetically-based disorders are more easily assessed by FISH than by other routine cytogenetic studies. The recent studies by Kuwano et al, Altherr et al, Goodship et al, and others suggest that submicroscopic or cryptic translocations, which occur in association with the Miller-Dieker and Wolf-Hirschhorn syndromes, for example, are better assessed with FISH because there is more complete diagnostic capability than with other techniques. This allows more accurate determination of risks of recurrence of a defect.

## FLUORESCENCE IN SITU HYBRIDIZATION

FISH permits determination of the number and location of specific DNA sequences\* in human cells (Table 1A), both in interphase nuclei and directly on metaphase chromosomes. The FISH procedure relies on the complementarity between the 2 strands of the DNA double helix. Probe DNA molecules are nonisotopically labeled by incorporation of a chemically modified nucleotide that is subsequently detected with a fluorescently

Table 1  
FISH Assays

### A) Permit

- Copy number of specific chromosomes (aneuploidy detection)
- Copy number of specific chromosomal regions (duplication or deletion detection)
- Identification of unknown or derivative chromosomes
- Analysis and diagnosis of nonrandom chromosomal translocations

### B) Procedure Steps

- Label the probe DNA
- Prepare and denature the sample or target DNA (metaphase chromosomes or interphase nuclei)
- Hybridize the denatured, single-stranded probe to denatured target DNA
- Wash away unbound or weakly homologous labeled probe DNA
- Detect the resulting probe DNA:target DNA hybrid molecules

\*Please reference *Genetics Glossary* insert.

tagged reporter molecule (Table 1B). Most often, nucleotides\* substituted with biotin-dUTP or digoxigenin-dUTP are enzymatically incorporated by nick-translation\* into probe DNA in place of thymine. These are usually employed because of the high sensitivity of DNA-DNA hybrid detection and the commercial availability of the appropriate labeling and detection reagents. Taking advantage of the biotin-avidin affinity reaction, biotin can be detected with fluorescently tagged avidin, whereas digoxigenin is detected with fluorescently tagged antidigoxigenin antibodies.

The target cells or metaphase chromosomes to be tested are prepared on glass microscope slides. The cells are usually derived from specimens similar to those that would normally be employed for standard cytogenetic studies. Metaphase spreads from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes or from dividing cells obtained from bone marrow aspirates or from cultured cells recovered from skin or tumor biopsy specimens are usually used. Specimens appropriate for prenatal diagnosis can be retrieved from amniotic fluid, chorionic villi, or fetal blood obtained by fetoscopy or percutaneous umbilical blood sampling (PUBS). However, and most importantly, FISH does not require preparation of metaphase chromosomes to be successful, in contrast to more standard cytogenetic approaches. Hence, for rapid diagnosis, as would be preferred for prenatal and tumor specimens, one has the option of directly fixing and immobilizing interphase nuclei of nondividing cells retrieved directly from the tissue source or even tissue sections. Since each individual chromosome occupies a moderately discrete territorial domain within the interphase nucleus,\* it is possible to determine chromosomal or regional copy number by counting the number of signals present. Thus, using the immobilized interphase nuclei as the hybridization\* target, one is able to diagnose chromosomal abnormalities. This approach, termed interphase cytogenetics, speeds diagnosis and avoids the problems encountered with cell selection upon extended cell growth.

The probe and target DNAs are denatured, and the modified probe DNA is hybridized to the target at a temperature below the melting point of the DNA duplex. This allows the modified probe to bind to its complementary sequences in the target. Many DNA probes contain repetitive DNA sequences, of which there are numerous copies throughout the genome. Thus, to suppress nonspecific hybridization or binding to the repetitive sequences in the target DNA, the repeat sequences in the probe DNA are blocked by addition of competitor DNA to the hybridization mixture. This is accomplished by the addition of either total genomic\* DNA or Cot-1 DNA, which is a DNA fraction selectively enriched in highly repetitive sequences. Probes are

hybridized for 3 to 18 hours, and then unbound probe is washed off the target, and fluorescent detector molecules are added to and combine with the modified probe hybridized to the specimen. Fluorescent detection is most often accomplished with conventional epifluorescence microscopy; if, however, the fluorescent signal is weak, digital imaging techniques can be used.

## PROBES

Several different types of probes are available for use in the detection of chromosomal abnormalities. The choice of probe will vary with the particular application in question. In general, probes fall into 3 classes: (1) locus\*-specific probes; (2) aliphoid or centromeric repeat probes; and (3) whole chromosome or chromosomal region "painting" probes. In addition, total genomic DNA can be used as a probe to determine the human component in human/rodent somatic cell hybrids\* (Figure 1A, page 8).

**Locus-Specific Probes:** Applications directed at aneuploidy\* detection, which is detection of duplication or deletion of specific chromosomal regions, and detection of tumor-associated translocation breakpoint detection rely on the availability of probes that are specific for and that reliably generate a bright fluorescent signal at a unique chromosomal locus in metaphase or in an interphase nuclei. These DNA probes range in size from 15 to 500 kilobases\* and are used to diagnose a specific cytogenetic abnormality. They are generated by identification and propagation of a locus-specific DNA segment cloned into a large insert phage,\* cosmid,\* or yeast artificial chromosome (YAC)\* vector. For example, hybridization signals seen on the metaphase chromosomes from a normal individual after hybridization with 2 unique cosmids that map to chromosome 22, 1 to the proximal long arm and 1 to the distal long arm, are demonstrated in Figure 1B (page 8). Figure 2 (page 9) also is a diagrammatic representation of a single-locus probe hybridization for aneuploidy detection. Numerous locus-specific markers are becoming readily available for significant chromosomal loci as a result of the intensive mapping efforts under way in association with the Human Genome Project.\*

**Aliphoid or Centromeric Repeat Probes:** Satellite or repetitive DNAs constitute approximately 10% to 20% of all human DNA. In particular, chromosomes carry from  $10^5$  to more than  $10^6$  base pairs of centromeric and pericentromeric short, tandemly repeated DNA sequences. The monomeric units that form these alpha satellite, beta satellite, or other satellite DNA sequences vary, such that many are chromosome-specific. Hence, chromosome-specific, repeat sequence probes have been isolated and cloned for the majority of human chromosomes, and many of these



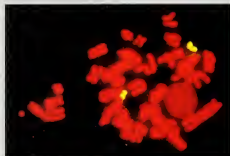
probes are commercially available. These repeat probes produce signals that are very intense in interphase nuclei and on metaphase chromosomes (Figures 1C, 1D, and 2).

**Whole Chromosome (Painting) Probes:** FISH using a probe mixture composed of numerous different DNA sequences with homology to many sites along a single chromosome permits the entire chromosome to be "painted" or "decorated" both in metaphase or in the interphase nucleus. Painting probes have been produced from chromosome-specific, flow-sorted libraries\*

and by polymerase chain reaction (PCR)\* amplification\* of DNA derived from either monochromosomal somatic cell hybrids or flow-sorted chromosomes. Another approach to preparation of chromosome- or region-specific probes has been microdissection of chromosomes or chromosomal regions from metaphase spreads followed by PCR amplification of the microdissected material (Meltzer et al, 1992). This PCR-amplified material can then be painted back to normal metaphase chromosomes to determine the origin of the microdissected material.

### Figure 1 Fluorescence In Situ Hybridization

Shown below are the results of FISH using probes of different degrees of complexity. In all cases, chromatin is counterstained with propidium iodide (red fluorescence). The target regions, hybridized with biotinylated probe DNA, are detected with fluorescein isothiocyanate (FITC)-avidin, which appears with yellow fluorescence in these photographs.



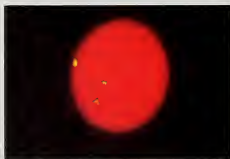
(A) A metaphase spread from a human X hamster somatic cell hybrid (EYEF3A6) with chromosome 22 as its major human component. The metaphase is hybridized with total human genomic DNA, which identifies the human material in the metaphase spread. Chromosome 22 is seen as an intact brightly fluorescent chromosome and there is a fragment of another human chromosome translocated to a hamster chromosome.



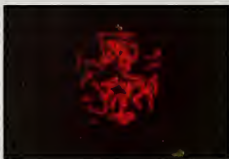
(B) Hybridization of metaphase chromosomes from a normal male with 2 labeled cosmid probes (~40 kb/probe). The cosmids recognize the DiGeorge critical region (DGCR) near the centromere and a locus on the distal long arm of the chromosome.



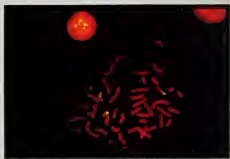
(C) Metaphase spread and interphase nuclei from a patient with karyotype of 46,XX/47,XX,+r. Phenotypic features were consistent with mosaic trisomy 8. Hybridization with the chromosome 8 centromere-specific probe (ONCOR; Gaithersburg, Md) demonstrates the presence of 3 discrete signals in metaphase spreads containing the ring chromosome and mosaicism for the ring in interphase nuclei.



(D) Interphase nucleus from a pediatric glioma hybridized with a chromosome 7 centromere-specific probe (ONCOR; Gaithersburg, Md), demonstrating the presence of trisomy for chromosome 7. The aneuploidy was not detected in metaphase chromosome spreads.



(E) Microdeletion detection in a patient with velocardiofacial syndrome using the cosmids described in Panel B (above center). One of the chromosome 22 homologues is positive with both probes, whereas the deleted chromosome is labeled only for the probe at the telomere.



(F) Metaphase spread from a patient with karyotype of 46,XX,15p+. Parental chromosomes were normal. The 15p+ chromosome was distamycin-DAPI-negative and centromere 15 probe-negative, suggesting the centromere was not from chromosome 15. The G-band pattern resembled 17p, and the Miller-Dieker syndrome probe cocktail, which labels the centromere of chromosome 17 and 17p13 (ONCOR; Gaithersburg, Md), was hybridized to metaphase spreads. Fluorescent signal is detected on 3 chromosomes, the 2 normal 17s and the 15p+.

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# GROWTH

## Genetics & Hormones

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Dear Colleagues:

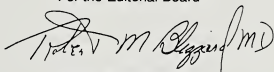
The field of genetics is where the action is today. The members of the Editorial Board have been aware for some time that the fields of genetics, pediatric endocrinology, nutrition, and growth are intimately intertwined. This knowledge prompted establishment of *GROWTH, Genetics, & Hormones* to stimulate and facilitate intellectual exchange of important knowledge among these disciplines. Drs. William Horton and Judith Hall have been key in representing members of the genetic subspecialty on our Editorial Board. The glossary that you are holding in your hands results from their efforts to simplify and interpret terms that recently have appeared in the vocabulary of geneticists. This they have done to permit us to more readily understand that which we read. We thank them for their effort and contribution.

We also thank Genentech, Inc. for the additional funds placed in our educational grant so this glossary can be brought to you.

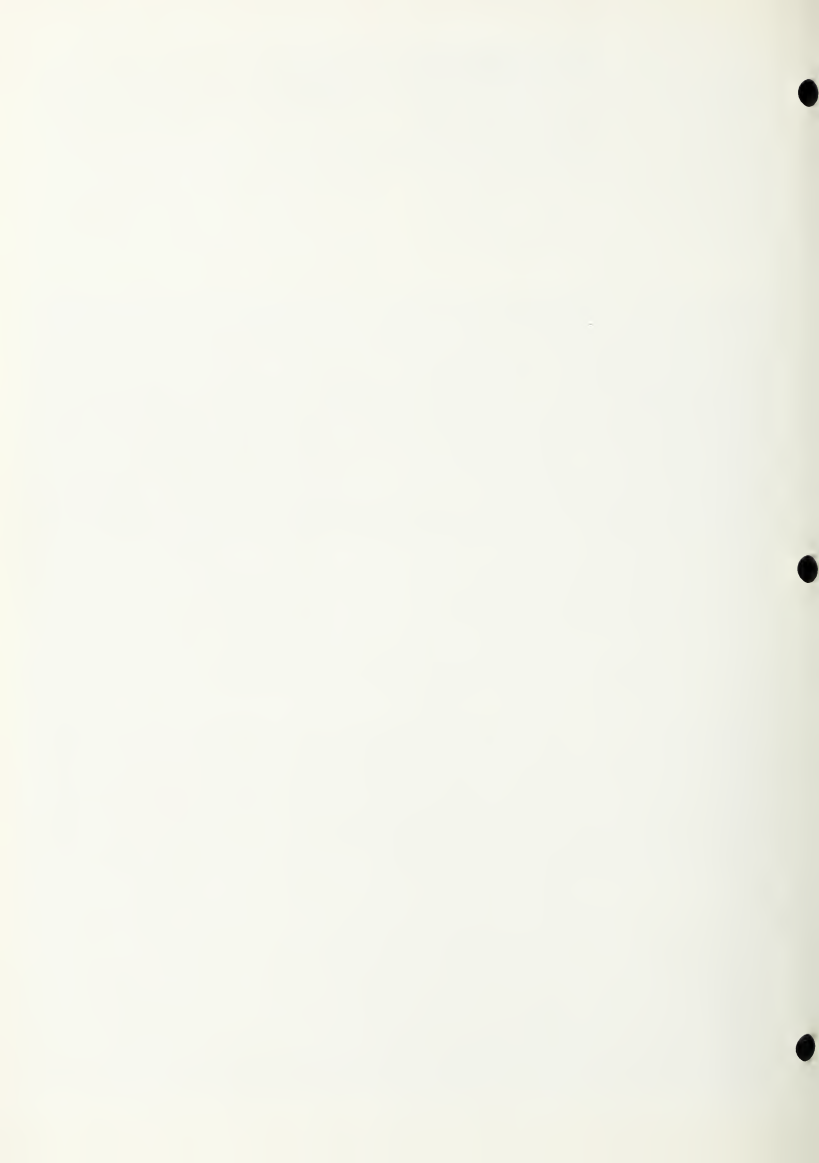
Please note that this glossary is physically separate from the remainder of the publication. This is by design to permit you to readily access the information in the glossary when you need it in interpreting the articles that you will read in the future, both in *GROWTH, Genetics, & Hormones* and elsewhere.

We hope this endeavor constructively assists you in quickly understanding more fully the important and pertinent articles that will be appearing in future issues of *GROWTH, Genetics, & Hormones*.

Respectfully,  
For the Editorial Board

A handwritten signature in black ink, appearing to read "Robert M. Blizzard MD", with a stylized flourish at the end.

Robert M. Blizzard, MD  
Editor



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# GROWTH

## Genetics & Hormones

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### GENETICS GLOSSARY

**allele** An alternative form of a gene at a given locus. Being diploid organisms, humans may have 2 alleles at a given locus, ie, a normal and a mutant allele.

**allelic disorders** Disorders, which may be phenotypically different, that are due to mutations in the same gene.

**Alu repetitive sequence** Repetitive sequence found about 500,000 times in human genome. The sequence contains a recognition site for the restriction enzyme *Alu* I and is around 300 base pairs in length.

**amplification** An increase in the number of copies of a particular DNA fragment. Can occur under natural circumstances, eg, amplification of a repeat sequence as in fragile X syndrome, or during laboratory procedures such as cloning or polymerase chain reaction.

**annealing** See hybridization.

**aneuploid** A chromosome number that is not an exact multiple of the haploid number. Usually refers to an absence (monosomy) or an extra copy (trisomy) of a single chromosome.

**anticipation** Phenomenon in which the severity of a genetic condition appears to become more severe and/or arise at an earlier age with subsequent generations.

**antisense strand (of DNA)** The noncoding strand of the DNA double helix that serves as the template for mRNA synthesis.

**autosome** Any chromosome other than the X or Y. Humans have 22 pairs of autosomal chromosomes.

**bacteriophage (phage)** Bacterial virus used as a vector for cloning segments of DNA.

**band (chromosomal)** A chromosomal segment defined by staining characteristics. Both lighter and darker segments are called bands, and are numbered from the centromere outwards, with smaller bands classified by a second number. Example - (p=short arm; q=long arm; ie, 3p25=short arm of chromosome 3, second band out from the centromere, fifth band outward within the second large band.)

**base pair (bp)** In the DNA double helix, a purine and pyrimidine base on each strand that interact with each other through hydrogen bonding. The number of base pairs is often used as a measure of length of a DNA segment, eg, 500 bp.

**base sequence** The order of nucleotide bases in a DNA molecule. Length is usually defined as the number of base pairs.

**breakpoint** Refers to sites of breakage when chromosomes break (and recombine).

**CCAAT box** Sequence that occurs 70 to 90 base pairs upstream from the initiation start site of a gene. The sequence is thought to be involved in regulation of transcription.

***Caenorhabditis elegans* (*C. elegans*)** Round worm used as an experimental model, especially in developmental biology.

**carrier** A clinically unaffected individual who may have clinically affected offspring. The term traditionally refers to an individual who is heterozygous at a given autosomal locus for a normal and a mutant gene (which causes disease only in the homozygous state) or a female who is heterozygous at an X-linked locus for a normal and a mutant gene (which causes disease in the hemizygous state in males). More recently used to describe unaffected individuals who carry unstable or dynamic mutations that can expand and cause a genetic condition in offspring.

**cDNA** Most often implies (complementary) DNA synthesized from RNA that corresponds to expressed sequences of genomic DNA. The term complementary DNA also may refer to DNA that is complementary to a particular DNA sequence.

**cDNA library** A collection of clones containing inserts of cDNA fragments representing expressed sequences (mRNA). cDNA libraries differ from 1 tissue or cell type to another.

**centimorgan (cM)** Measure of genetic distance defined in terms of recombination frequency. Two genetic loci are 1 cM apart if there is a 1% chance of recombination due to crossing over in a single generation. In humans, 1 cM corresponds to approximately 1 million base pairs.

**centromere** A specialized chromosome region to which spindle fibers attach during cell division. Appears as a distinct "waist" by microscopy.

**chimera** An organism comprised of cells from 2 or more zygotes.

**chorionic villus sampling (CVS)** Procedure used to obtain fetal cells for prenatal diagnosis; involves biopsy of the placental membranes.

**chromatid** Chromosomal strands produced during meiosis when a chromosome divides.

**chromatin** The composite of DNA and proteins that comprise chromosomes.



**chromosome** A highly ordered structure composed mainly of chromatin that resides in the nucleus of eukaryotic cells.

**cis** (1) Historically implies on the same chromosome. (2) In molecular biology refers to an effect on a gene directed by the sequence of that gene in contrast to trans effects, which are produced by other factors, such as transcription factors encoded by other genes. The terms are commonly used to describe factors that influence gene expression.

**cloning** (1) Production of genetically identical cells (clones) from a single ancestral cell. (2) Cloning is utilized in molecular biology to propagate single or discrete DNA fragments of interest.

**cloning vector** A DNA segment capable of autonomous replication, ie, a plasmid or phage, that is used to carry the desired DNA segment for replication.

**codon** A triplet of bases in DNA or RNA that specifies a single amino acid.

**codon usage** Given the degeneracy of the genetic code, refers to the preference of codons used to specify particular amino acids. Often differs among species and among different genes and proteins.

**compound heterozygote** An individual who has 2 different mutant alleles at a given locus.

**consanguinity** Relationship of 2 individuals by descent from a common ancestor. A consanguineous mating is one in which the mates are related, ie, first cousins.

**consensus sequence** A minimum nucleotide sequence found to be common (although not necessarily identical) in different genes and in genes from different organisms that is associated with a specific function. Examples include binding sites for transcription factors and splicing machinery.

**conserved sequence** Base sequence in a DNA molecule (or an amino acid sequence in a protein) that has remained essentially unchanged throughout evolution.

**contig map** Genetic map showing the order of (contiguous) DNA fragments in the genome.

**contiguous gene syndrome** Syndrome due to abnormalities of 2 or more genes that map next to each other on a chromosome; most often caused by a deletion that involves several contiguous genes.

**cosmid** Vector used to clone moderate-sized fragments of DNA (up to 45 kilobases). See plasmid.

**CpG island** Short DNA sequence having a high content of cytosine and guanine (CG) dinucleotides. CpG islands are often found near the transcription start sites of genes.

**CVS** See chorionic villus sampling.

**degeneracy (of the genetic code)** Different codons code for the same amino acid.

**DNA (deoxyribonucleic acid)** The polymeric, double-stranded molecule that encodes genetic information. The strands are held together by hydrogen bonds between nitrogenous bases that constitute the code: adenine (A) and thymine (T), which pair with each other, and guanine (G) and cytosine (C), which pair with each other.

**DNA marker** A DNA sequence variation that is easily detectable; examples include restriction fragment length polymorphisms, dinucleotide and trinucleotide repeat polymorphisms.

**DNA methylation** Attachment of methyl groups to DNA, most commonly at cytosine residues. May be involved in regulation of gene expression. Also may prevent some restriction endonucleases from cutting DNA at their recognition sites.

**DNA polymerase** Enzyme responsible for replication of DNA.

**DNA sequence** The relative order of base pairs.

**domain** A discrete portion of a protein (and corresponding segment of gene) with its own function. A protein may have several different domains and the same domain may be found in different proteins.

**dominant mutations** Mutations that produce an abnormal clinical phenotype (disorder or trait) when present in the heterozygous state.

**dominant negative mutations** Heterozygous mutations in which the product of the mutant allele interferes with the function of the product of the normal allele.

**downstream** A DNA sequence is written from the left, or 5', direction or to the right, or 3', direction. Downstream refers to the 3' direction, ie, the stop codon for a gene is downstream (3') of the coding sequences of that gene.

***Drosophila melanogaster* (*drosophila*)** Fruit fly used for classic genetics studies and utilized as an experimental model by developmental biologists.

**dysmorphology** Study of abnormalities of morphologic development.

**electrophoresis** An analytical method used to separate nucleic acid, peptide, or protein fragments based on size and charge of the molecule; typically smaller fragments travel farther through the media (gel) in which separation is carried out.

**enhancers** DNA sequences that increase transcription of a nearby gene; they can act in either orientation, may be either upstream (5') or downstream (3') to the gene or within an intron.

***Escherichia coli* (*E.coli*)** Common bacterium extensively used in cloning.

**euchromatin** The chromatin that is thought to contain active or potentially active genes. Light (vs dark) bands on G-banding.

**exons** The sequences within a eukaryotic gene that code for protein, in contrast to introns, which do not.

**F1, F2, etc** The first (F1), second (F2), etc, generations of progeny of a mating.

**FISH** See fluorescence in situ hybridization.

**fluorescence in situ hybridization (FISH)** A physical mapping technique in which fluorescein-tagged DNA probes are hybridized to chromosomes.

**fragile site** Gap or defect noted in the continuity of a chromosome when stained, eg, fragile X site. Many are apparent only when cells are cultured under special conditions.

**frameshift mutation** A mutation that alters the normal triplet reading frame so that codons downstream from the mutation are out of register and not read properly.

**Giemsa banding (G-banding)** Method of staining chromosomes that produces light and dark bands characteristic for each chromosome.

**gamete** Mature reproductive cell (sperm or ovum); contains a haploid set of chromosomes (23 for humans).

**gene** The fundamental unit of heredity. Functionally defined by its product. Structurally defined as an ordered sequence of nucleotides located in a particular position on a particular chromosome that includes regions involved in regulation of expression and regions that code for a specific functional product.

**gene targeting** Artificial modification of a gene in a specific and directed fashion. Typically refers to substituting one DNA sequence for another to inactivate a gene or introduce or correct a mutation in a gene.

**genetic locus** A specific position or location in the genome.

**genome** The complete genetic information of an organism, usually described as total number of base pairs; human genome contains  $3 \times 10^9$  base pairs.

**genomic DNA** DNA from the genome containing all coding (exon) and noncoding (intron and other) sequences, in contrast to cDNA, which contains only coding sequences.

**genomic library** A collection of clones containing DNA inserts of DNA fragments representing the entire genome of an organism.

**genotype** The genetic constitution of an individual or organism.

**germ cell** See gamete.

**germ line mosaicism** Presence of 2 or more cell lines that differ in genetic makeup among germ cells; implies risk of transmission of mutations present in the gonads to offspring.

**gonadal mosaicism** See germ line mosaicism.

**heterochromatin** Chromatin composed of repetitive DNA; stains as dark (vs light) bands in G-banding.

**heterozygosity** The presence of different alleles at a given genetic locus.

**histones** Proteins associated with DNA in chromosomes in the nucleus of the cell.

**homeobox domain** A short DNA sequence common to the genes of many DNA binding proteins.

**homeobox (HOX) genes** Family of genes conserved throughout evolution that share a common DNA binding domain and encode DNA binding proteins involved in regulation pattern formation during early embryologic development.

**homologies** Similarities found in DNA or protein sequences of individuals of the same or different species.

**homologous chromosomes** Chromosomes containing the same linear gene sequences. In a normal mating, 1 of a pair of homologous chromosomes is derived from each parent. Humans normally have 22 pairs of homologous chromosomes and 2 X chromosomes or 1 X and 1 Y chromosome.

**homologous recombination** Substitution of a segment of DNA by another that is identical (homologous) or nearly so. Occurs naturally during meiotic recombination; also used in the laboratory for gene targeting to modify the sequence of a gene.

**homozygosity** Presence of the same allele at a given genetic locus.

**housekeeping genes** Genes that encode proteins necessary for basic cellular functions. They are expressed in virtually all cells.

**Human Genome Initiative (Project)** Collective name for several projects designed to map and eventually sequence the human genome.

**hybridization** The artificial pairing of 2 complementary strands of DNA or 1 each of DNA and RNA to form a double-stranded molecule. One strand is often labeled and used as a probe to detect the presence of the second strand.

**imprinting** Phenomenon in which an allele at a given locus is altered or inactivated depending on whether it is inherited from the mother or father. Implies a functional difference in genes inherited from the 2 parents.

**initiation codon** The trinucleotide (AUG) that signals the start of translation of a protein.

**in situ hybridization** Use of a nucleic acid probe to detect the presence of a DNA sequence in chromosome spreads or in interphase nuclei or an RNA sequence in cells. It is used to map gene sequences to chromosomal sites and to detect gene expression.

**insert** In molecular genetics, refers to a DNA sequence of interest that has been inserted into a cloning vector such as a plasmid or bacteriophage.

**insertion** Type of mutation in which a DNA sequence of variable length is inserted into a gene, disrupting the normal structure of that gene.

**intervening sequences** See introns.

**introns** DNA sequences that interrupt the protein-coding sequences of a gene. They are removed during processing of mRNA. Introns may contain sequences involved in regulating expression of a gene.

**kilobase (kb)** 1,000 base pairs of DNA sequence.

**knockout** Term commonly used to describe inactivation of a gene by gene targeting.

**library** Collection of clones in which genomic DNA or cDNA fragments have been inserted into a cloning vector.

**linkage** The close proximity of 2 or more genetic loci on a chromosome. The loci can be genes responsible for certain traits or inherited diseases or DNA markers. Closely linked loci are usually inherited together since the closer 2 loci are to each other the lower the likelihood of recombination during meiosis.

**locus** The position on a chromosome, usually that of a gene, but may refer to a DNA marker.

**lod score** Literally refers to the log of the odds. A statistical term applied to a set of linkage data to indicate if 2 loci are linked or unlinked. A lod score of +3 (1,000:1 odds) or more is commonly accepted to show linkage and a score of -2 (100:1 odds against) or less excludes linkage.

**marker** A detectable physical location on a chromosome. It can be a restriction enzyme cutting site, gene, minisatellite, or microsatellite (ie, dinucleotide or trinucleotide repeat or variable number tandem repeat nucleotide) polymorphism whose presence and inheritance can be monitored.

**maternal inheritance** Inheritance pattern displayed by mitochondrial genes that are propagated from one generation to the next through the mothers; the mitochondria of the zygote come almost entirely from the ovum.

**megabase (Mb)** One million base pairs of DNA sequence; roughly equal to 1 centimorgan of genetic distance.

**meiosis** The type of cell division that occurs during gamete formation and results in the halving of the diploid number of chromosomes so that each gamete is haploid and contains 1 of each chromosome pair.

**messenger RNA (mRNA)** Processed RNA that serves as a template for protein synthesis or for synthesis of cDNA.

**methylation** See DNA methylation.

**microsatellites** Highly polymorphic DNA markers comprised of mononucleotides, dinucleotides, trinucleotides, or tetranucleotides that are repeated in tandem arrays and distributed throughout the genome. The best studied are the CA (alternatively GT) dinucleotide repeats. They are used for genetic mapping.

**minisatellites** Highly polymorphic DNA markers comprised of a variable number of tandem repeats that tend to cluster near the telomeric ends of chromosomes. The repeats often contain a repeat of 10 nucleotides. They are used for genetic mapping.

**missense mutation** Mutation that causes one amino acid to be substituted for another.

**mitochondrial (mt) DNA** DNA distinct from nuclear DNA in that it is mostly unique sequence DNA and codes for proteins that reside in mitochondria.

**mitosis** The type of cell division that occurs in somatic cells in which a cell duplicates itself and its genetic material.

**mosaicism** Condition in which an individual harbors 2 or more genetically distinct cell lines; results from a genetic change after formation of a zygote, ie, postzygotic event.

**motif** Three-dimensional structure of gene product (protein) with known or implied function, eg, DNA binding, membrane spanning. A motif is often inferred from cDNA sequence.

**mutation** A permanent and heritable change in genetic material. Types of mutations include point mutations, deletions, insertions, and changes in number and structure of chromosomes.

**nick translation** Method used to introduce  $^{32}\text{P}$  into a DNA probe so that the probe can be detected.

**nonsense mutation** Mutation that changes a codon for an amino acid to a termination or stop codon and leads to premature termination of translation.

**northern blot** Method by which RNA is analyzed. RNA is separated by size, transferred to a membrane (blotted), and detected by a complementary labeled probe that hybridizes to a specific species of RNA, revealing information about its identity, size, and abundance.

**nucleosome** The basic structural unit of chromatin, in which DNA is wrapped around a core of histone molecules.

**nucleotide** A purine or pyrimidine base to which a sugar (ribose or deoxyribose) and 1, 2, or 3 phosphate groups are attached.

**nucleus** The organelle in eukaryotic cells defined by the nuclear membrane that contains the chromosomes.

**oligonucleotide** A short fragment of single-stranded DNA, typically 5 to 50 nucleotides.

**open reading frame (ORF)** A sequence of DNA following an initiation codon that does not contain a stop codon. Detection of an open reading frame in DNA implies the presence of a gene that codes for a protein.

**ORF** See open reading frame.

**pax (genes)** Paired-box containing genes found in many species that are involved in regulation of early embryogenesis. Pax genes code for (DNA binding) transcription factors. The paired box refers to a particular conserved DNA sequence that is shared by the different members of the pax gene family.

**PCR** See polymerase chain reaction.

**penetrance** Refers to clinical expression of a gene or mutation of a gene. If a mutation produces a recognizable phenotype in a patient, the mutation is said to be penetrant. Reduced penetrance means that individuals who harbor a mutation do not always manifest the mutant phenotype clinically.

**phage** See bacteriophage.

**phenotype** The appearance (physical, biochemical, and physiologic) of an individual that results from the interaction of environment and genotype. Often used to define the consequences of a particular mutation.

**physical map** A map of physical landmarks on a DNA fragment or chromosome measured in base pairs. Landmarks include restriction endonuclease recognition sites, DNA sequence, and chromosomal bands.

**plasmid** Small, circular extrachromosomal DNA molecule capable of autonomous replication within a bacterium. Commonly used as a cloning vector for small pieces of DNA (typically 50 to 5,000 base pairs) by insertion into the plasmid.

**poly A RNA** RNA transcript that contains a tail of poly A residues at its 3' end; implies that an RNA sequence is mRNA.

**polyamines** Compounds with many amino groups that are associated in the cell with nucleic acids.

**polymerase chain reaction (PCR)** A method to amplify a DNA sequence using a heat-stable polymerase and 2 sets of primers that define the sequence to be amplified. Several variations have been developed for specific needs. May be combined with reverse transcription of mRNA to cDNA to amplify an mRNA, so called RT-PCR.

**polymorphism** The occurrence of 2 or more genetically determined forms. Applied to many situations ranging from genetic traits or disorders in a population to the variation in the sequence of DNA or proteins.

**positional cloning** Strategy for identifying and cloning a gene based on its location in the genome rather than the biologic function of its product. Usually involves linking the gene locus of interest to one that has already been mapped.

**premutation** A permanent and heritable change in a gene that does not have phenotypic consequences (does not cause disease) but predisposes to a "full" mutation that may.

**primary transcript** The initial RNA transcript of a gene, before processing to mRNA; it contains introns as well as exons.

**primer** Short single-stranded oligonucleotide that anneals to a nucleic acid template and promotes copying of the template starting from the primer site.

**proband** The proband (or probanda), or index case, that brings the family to medical attention.

**probe** Single-stranded DNA or RNA molecule of specific base sequence, labeled either radioactively or by other means, that is used to detect a complementary base sequence by hybridization.

**promoter** A sequence on a gene that is upstream (5') to coding sequences to which RNA polymerase binds and initiates transcription of the gene.

**pseudogene** Sequence of DNA that is very similar to a normal gene but has been altered slightly so that it is not expressed.

**reading frame** Register in which translation machinery reads the genetic triplicate code.

**recessive mutations** Mutations that produce an abnormal clinical phenotype when present in the homozygous or hemizygous state. Heterozygosity for the mutation, ie, carrier state, may often be detected in persons whose clinical phenotype is normal.

**recombinant DNA molecules** DNA molecules of different origins that are combined and manipulated in the laboratory.

**recombinant DNA technologies** Laboratory procedures used to manipulate DNA fragments, eg, cut, modify, ligate, etc, and introduce them into an organism so that their number can be amplified as the organism replicates, ie, cloning.

**recombination** The formation of new combinations of linked genes by crossing over between their loci during meiosis.

**restriction enzyme, restriction endonuclease** Bacterial-derived enzyme that recognizes specific, short nucleotide sequences and cuts DNA at that site.

**restriction fragments** DNA fragments that result from digestion of DNA with restriction enzymes.

**restriction fragment length polymorphism (RFLP)** Genetic variation resulting from a difference in DNA sequence that affects the recognition sequence for restriction enzymes. When DNA is digested by a particular enzyme, the fragment sizes will differ, depending on the presence or absence of the proper recognition sequence for the enzyme.

**restriction map** A map of a DNA sequence with restriction enzyme recognition sites serving as landmarks.

**restriction site** Shortened term for restriction endonuclease recognition sequence.

**retroviruses** RNA viruses that encode the enzyme reverse transcriptase so that their RNA can be transcribed into DNA in the host cell; modified retroviruses are used as vectors to introduce genes (or portions thereof) of interest into eukaryotic cells.

**reverse transcriptase** An enzyme that catalyzes the synthesis of DNA from an RNA template.

**RFLP** See restriction fragment length polymorphism.

**RNA polymerase** Enzyme that synthesizes (transcribes) RNA from a DNA template.

**RNA splicing** Process by which introns are removed from primary RNA transcripts, leaving only exons that encode the amino acid sequence of a protein.

**sequence** Order of bases in DNA or RNA or of amino acids in a protein.

**sequence-tagged sites (STSs)** Short sequences of genomic DNA for which the base sequence is known. Polymerase chain reaction can be used to amplify the known sequences, which can serve as physical landmarks for mapping.

**sequencing** Determination of the order of nucleotides in a DNA or RNA fragment, or the order of amino acids in a protein.

**sequencing gel analysis** Electrophoretic technique by which nucleotide size differences as little as a single base pair can be discerned.

**somatic cell hybrid** A hybrid cell line derived from fusion of cells from different sources. Human/rodent hybrids containing a small amount of human genetic material, such as a single chromosome, are used in human gene mapping.

**somatic cells** All cells in the body except gametes and their precursors.



**somatic mosaicism** Presence of 2 or more cell lines that differ genetically in somatic (non-germ line) cells.

**Southern blot (hybridization)** Method by which DNA is analyzed that was originally described by E. M. Southern. DNA is fractionated by electrophoresis, transferred to a membrane (blotted), and detected by a complementary labeled probe that hybridizes to the DNA, revealing information about its identity, size, and abundance.

**splicing** See RNA splicing.

**STSs** See sequence-tagged sites.

**synteny** Refers to the presence of 2 or more loci on the same chromosome; they may or may not be linked closely. For example, 2 gene loci that map to the distal and proximal locations on the long arm of chromosome 1, respectively, would not be linked but would exhibit synteny.

**tandem repeat sequences** Multiple copies of the same base sequence on a chromosome. When the number of repeats varies in the population, they are useful as DNA markers.

**TATA box** A conserved sequence 25 to 30 base pairs upstream from the start site of transcription, in many but not all genes; binding site for general factors involved in initiation of transcription.

**telomeres** Refers to the ends of chromosomes that contain characteristic repetitive DNA sequences.

**termination (or stop) codon** One of the 3 codons (UAG, UAA, or UGA) that causes termination of protein synthesis.

**trans** (1) Historically implies on a different chromosome. (2) In molecular biology, refers to an effect on a gene caused by a factor distinct from the sequence of that gene, in contrast to cis effects, which are encoded in the sequence of the gene. Cis and trans are commonly used to describe factors that influence gene expression.

**transfection** Transfer of a DNA fragment into prokaryotic or eukaryotic cells.

**transcript** Refers to an mRNA molecule that encodes a protein.

**transcription** The synthesis of an RNA molecule (transcript) from a DNA template in the cell nucleus catalyzed by RNA polymerase.

**transcription start site** Site within a gene where transcription of RNA begins.

**transgenic** Containing foreign DNA, eg, transgenic mice contain foreign DNA sequences in addition to the complete mouse genome.

**translation** Assembly of amino acids into peptides based on information encoded in mRNA, ie, mRNA sequence of

bases is translated into sequence of amino acids in a peptide or protein. Occurs on ribosomes.

**translocation** The exchange of genetic material from one chromosome to another nonhomologous chromosome, usually through a reciprocal event at meiosis.

**uniparental disomy** Situation in which an individual has 2 homologous chromosomes (homologues) or chromosomal segments from 1 parent and none from the other. May be heterodisomy if 1 copy of each of the homologues from the single parent are present or isodisomy if 2 copies of the same homologue are present.

**unique sequence DNA** Nonrepetitive DNA that potentially codes for mRNA and protein.

**upstream** A DNA sequence is written from the left, or 5', direction or to the right, or 3', direction. Upstream refers to the 5' direction, ie, regulatory elements of a gene are typically located upstream (5') of the coding sequences of that gene.

**variable number tandem repeat (VNTR)** A type of DNA marker. See marker.

**vector** See cloning vector.

**western blot** Method by which proteins are analyzed. Terminology based on convention of Southern (DNA) and northern (RNA) blots. Proteins are fractionated by electrophoresis, transferred to a membrane (blotted), and detected by a labeled probe, usually an antibody. Provides information about size, abundance, and identity of the protein.

**X-inactivation** The inactivation of most of the genes on 1 of the X chromosomes in female somatic cells during early embryonic development.

**YAC** See yeast artificial chromosome.

**yeast artificial chromosome (YAC)** A vector used to clone large DNA fragments. The inserts can be much larger than those accepted by other vectors, such as plasmids or cosmids.

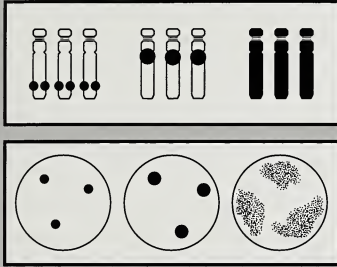
**zygote** The diploid cell resulting from the union of the haploid male (sperm) and female (ovum) gametes.

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Figure 2

## Diagrammatic Representation of Trisomy 21 Detection by FISH

Unique Locus  
ProbeAlphoid or  
Centromeric  
Repeat ProbeChromosome-  
Specific  
Painting Probe

(A) The hypothetical appearance of chromosome 21 in a metaphase spread when hybridized with each of the appropriate probe types: Black indicates probe-specific fluorescent signal. Thus, a locus-specific probe gives a sharp, discrete signal at its relevant position. An alphoid or centromeric repeat probe gives a large, more diffuse signal near the centromere. A painting probe decorates the entire chromosome. The copy number of the specific chromosome can be determined with any of the probe types.

(B) The appearance of G1 interphase nuclei after hybridization with each of the probe types: The locus-specific probe gives the best resolution and also would detect duplication of the Down syndrome critical region as a result of translocation. The alphoid repeat probe gives a bright and discrete signal but would not necessarily detect a translocation. Because of the diffuse nature of the chromosomal domain in interphase, overlapping domains visualized with the painting probe can make chromosome enumeration more difficult.

## CLINICAL APPLICATIONS

Clinical applications of FISH technology take advantage of molecular probes that are specific for defined regions of cytogenetic interest. The methodology widens the scope of what is diagnosable by the clinical cytogenetics laboratory because it is based on microscopic visualization to determine the copy number of specific DNA sequences in the target. Since the technique permits visualization of changes at a sensitivity beyond what can be seen on banded chromosomes with the light microscope, many additional cases of constitutional cytogenetic abnormality are identifiable, and appropriate recurrence risks and prognostic information for the associated disorders can be effectively established for the family (Table 2).<sup>1</sup> Specific karyotypic abnormalities for which FISH is useful are discussed below.

**Aneuploidy:** An example of the use of FISH to document autosomal trisomy is diagrammatically represented in Figure 2. A hypothetical unique locus probe, specific for the Down syndrome critical region on the distal long arm of chromosome 21, has been used as an example for detecting trisomy 21 by hybridization to metaphase chromosomes as shown in Panel A or to

interphase nuclei as shown in Panel B. As is shown in the remainder of Figure 2, similar studies could be accomplished with the use of an alphoid probe specific for the centromeric region of the chromosome, or by painting the entire chromosome with a chromosome 21 painting cocktail. These studies also can be effectively used to rapidly assess the presence of all clinically significant trisomic conditions during prenatal diagnosis<sup>2</sup> or during the newborn period, and when questions of clinical management require timely information regarding the karyotype of the fetus or the neonate. In addition, one can envision numerous applications using interphase nuclei to assess tumor specimens for ploidy or for additional copies of a specific chromosome. An interphase nucleus from a pediatric glioma hybridized with a chromosome 7 alphoid sequence that demonstrated 3 copies of chromosome 7 is shown in Figure 1D. Extra copies of chromosome 7 have been reported in adult gliomas, but trisomy 7 was not demonstrable in metaphase preparations from the pediatric tumor presented in Figure 2. Thus, FISH may be more sensitive than standard cytogenetic analysis for detection of aneuploidy in tumors.

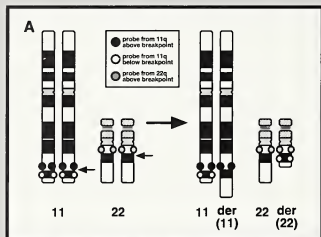
**Translocations:** The identification of nonrandom translocations in neoplastic cells using FISH is a rapidly expanding field. The molecular description of these tumor-associated translocations has enabled the development of FISH-based assays for clinical evaluation of the appropriate leukemias, lymphomas, and solid tumors. Although cytogenetic analysis is unmatched in its ability to define the full constellation of chromosomal changes in a tumor, sequence-based assays utilizing FISH offer several advantages for detection of specific chromosomal rearrangements. These advantages include greater sensitivity, decreased time and cost per assay, small sample size, and obviation of cell culture.

Table 2  
FISH Technology

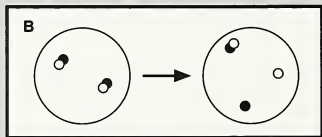
## Clinical Applications

- Prenatal diagnosis of cytogenetic abnormality
- Assessment and management of the critically ill newborn with suspected aneuploidy
- Tumor karyotype identification at initial diagnosis
- Assessment of minimal disease

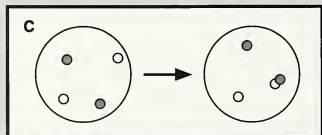
**Figure 3**  
**Diagrammatic Representation of Ewing's**  
**Sarcoma t(11;22) Translocation**  
**Breakpoint Detection by FISH**



(A) An ideogrammatic representation of the t(11;22) showing the location of 3 single copy probes that could be used to detect the translocation: The small arrows to the right of the chromosomes indicate the position of the translocation breakpoints on chromosomes 11 and 22. Probes are used in pair-wise combinations. Either a pair of probes that flank the breakpoint on 1 of the 2 involved chromosomes (for example ● and ○ on chromosome 11) or a pair of probes that are below the breakpoint on 1 of the involved chromosomes and above the breakpoint on the other (for example the ○ probe from chromosome 11 and the ● probe chromosome 22).



(B) Diagrammatic representation of results of FISH with breakpoint-flanking probes from chromosome 11 on normal (left) and t(11;22)-positive Ewing's sarcoma interphase nuclei (right): The fluorescent signals become separated in the interphase nucleus as a result of the translocation.



(C) Diagrammatic representation of results of FISH with translocation breakpoint-related probes (for example ○ from below the breakpoint on chromosome 11 and ● from above the breakpoint on chromosome 22): The chromosome 11 and chromosome 22 probe signals that are normally separated in interphase (left) are brought into juxtaposition (right) as a result of the translocation.

For example, an application of FISH technology for t(9;22) rearrangement detection in interphase nuclei has been described. Simultaneous hybridization of differentially labeled probes for *bcr* and *abl* to chronic myelogenous leukemia (CML) bone marrow nuclei detects co-localization of the 2 genes as a result of the translocation.<sup>3</sup> Similar studies have been accomplished for the t(11;22) translocation of Ewing's sarcoma, a solid tumor that is difficult to diagnose.<sup>4,5</sup> The assays for such tumor rearrangements use either a pair of probes derived from one of the translocation partners, which co-localize in normal cell nuclei and are distinctly separated in tumor nuclei as a result of the translocation, or, alternatively, a pair of probes from both translocation partners, which are separated in normal nuclei and co-localize in tumor nuclei as a result of the translocation. This is shown diagrammatically in Figure 3.

Once a suitable pair of probes is identified, FISH of interphase nuclei from tumors can be utilized as a rapid assay for the translocation. This assay should eventually have applicability to a variety of clinical material. Since this assay can be accomplished much more quickly than the standard cytogenetic analysis, FISH analysis of biopsy material will be clinically advantageous in difficult diagnostic situations.

**Microdeletion Syndromes:** One of the most challenging tasks in human clinical cytogenetics is the identification of cytogenetically undetectable microdeletions in association with phenotypic features of known deletion syndromes. Several examples are: Wolf-Hirschhorn (4p-), cri du chat (5p-), Langer-Gideon (8q-), Prader-Willi and Angelman (15q-), Miller-Dieker (17p-), Alagille (20p-), and DiGeorge and velocardiofacial (22q-) syndromes. Analysis of small deletions of human chromosomes is best approached by hybridizing with probes that are targeted to the critical deletion region that is responsible for the syndrome. An example of such an approach for velocardiofacial syndrome is shown photographically in Figure 1E (page 8) and diagrammatically in Figure 4. In the photograph, a cosmid for the DGS critical deletion region has been hybridized to metaphase chromosomes from a possible DGS patient. The metaphase chromosome demonstrates the microdeletion as absence of hybridization to 1 of the chromosome 22 homologues. This is shown diagrammatically in Figure 4, which also demonstrates that the interphase nucleus would show a single signal.

**Marker Chromosomes:** Small additional marker chromosomes in the human karyotype are impossible to identify because of their paucity of banding landmarks. Numerous researchers have recently taken advantage of the centromere-specific alphoid satellite probes to determine the origin of such marker

chromosomes.<sup>6</sup> This technique provides information regarding the origin of the marker chromosome, which has significance for patient management. In addition, numerous patients with sex chromosomal abnormalities have a karyotype characterized by the presence of a single X chromosome while the second sex chromosome is replaced by a small marker chromosome, presumed to be derived from the missing sex chromosome. Identification of the origin of the small marker chromosome is easily accomplished by FISH using probes for the centromeric region of the X and Y chromosomes.

**Sex Chromosomal Assessment:** Probes that identify the centromeric regions of the X and Y chromosomes also are useful for monitoring patients who have had sex-mismatched bone marrow transplants. With FISH one can easily identify residual host cells in marrow aspirate by their sex chromosome content. In this instance, interphase cytogenetics may prove to be extremely useful during therapy, when there is a paucity of cells available on which to perform standard cytogenetic analysis. Sex chromosome identification also may prove useful in prenatal diagnosis of X-linked disease.

**Gene Amplification:** Oncogene amplification is associated with a poor prognosis for several tumors. This is particularly true for *N-myc* amplification in neuroblastoma and *erb B2* amplification in breast tumors.<sup>7</sup> These amplified sequences can be readily detected in meta-

phase and interphase using FISH. Probes for these markers are commercially available for use in tumor staging and management.

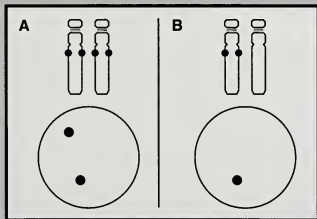
**De Novo Additions:** The de novo addition of unidentifiable chromosomal material to a recognizable human chromosome represents a dilemma for the cytogeneticist. Such material is often identified in a dysmorphic newborn whose parents' karyotypes are normal; questions are then raised regarding prognosis and management based on the identification of the additional material. The results of such a study are seen in Figure 1F (page 8). In this patient, de novo additional material on the short arm of one chromosome 15 was suspected of being derived from chromosome 17. FISH with cosmid probes designed to detect the Miller-Dieker deletion on 17p (ONCOR; Gaithersburg, Md) was successful in allowing identification of the origin of the extra chromosomal materials.<sup>8</sup> In addition, numerous tumor specimens contain chromosomes with additional material that is difficult to identify. It would be useful to determine the composition of such aberrant chromosomes. In many cases, the approach is chromosome-specific probe hybridization or chromosome painting using locus-specific or composite paint probes judged to be the most likely candidates from the banding pattern. The other approach to such a situation, although not as readily available, would be microdissection followed by PCR and hybridization of the amplified microdissected material back to normal human metaphase chromosomes. This is a very powerful technique, one probably best handled in a limited number of reference laboratories rather than in typical diagnostic cytogenetic laboratories.

In summary, the genetic and oncologic diagnostic applications of FISH are increasing steadily with the availability of chromosome- and disease-specific reagents. The parallel development of sophisticated microscopy and detection systems, in addition to their greater accessibility, is enhancing the capability of routine clinical diagnostic laboratories to perform such analyses, allowing for easier and more accurate assessment of specimens. The technology has enormous potential and critical implications for the future of cytogenetics. With FISH, the enumeration and localization of the DNA sequences underlying a cytogenetic abnormality can be accomplished by direct visualization at the microscope with both sensitivity and accuracy.

#### ACKNOWLEDGEMENT

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**Figure 4**  
**Diagrammatic Representation of Deletion**  
**of the DiGeorge Syndrome or**  
**Velocardiofacial Syndrome Critical**  
**Regions (DGCR) Detected by FISH on**  
**Metaphase Chromosomes**  
**and Interphase Nuclei**



(A) Hypothetical appearance of the chromosomes 22 hybridized with a probe from the DGCR in a normal individual and the appearance of the G1 interphase nuclei.

(B) Hypothetical appearance of the chromosomes 22 hybridized with a probe from the DGCR in an affected individual and the appearance of the G1 interphase nuclei.



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## Abstracts From the Literature

### Comparative Assessment of Dual-Photon Absorptiometry and Dual-Energy Radiography

Dual-energy bone densitometry can be performed with 2 types of scanners, dual-photon absorptiometry (DPA) and dual-energy radiography (DER). DPA uses an isotope source (gadolinium-153; <sup>153</sup>Gd) and was developed in the 1960s and 1970s. DER, which was developed in the late 1980s, uses an incorporated X-ray tube. DER also is known as DRA (dual-energy radiographic absorptiometry), QDR (quantitative digital radiography), and DEXA (dual-energy X-ray absorptiometry). Glüer et al, authors of this paper, suggest that DER be used exclusively.

Comparison of the basic principles, advantages, and disadvantages of DPA and DER, as presented in the current paper, are tabulated in the accompanying table. Comparison was made for both bone mineral density (BMD) and bone mineral content (BMC). Normal healthy adults and females with and without osteoporosis were studied.

The authors discuss that the underlying concepts of DPA and DER are very similar in that they both are based on dual-energy projection (3-dimensional) scanning. Development of DER relates to improvements in technology, particularly to the enhancement of X-ray beam intensity by replacement of the <sup>153</sup>Gd isotope source with an X-ray tube and, in some machines, the incorporation of internal calibration devices. The authors state that comparative assessment of the 2 densitometers demonstrates that these technologic improvements have resulted in marked progress in a number of clinically important performance characteristics. For example, the results reveal a significant improvement in precision with DER. The marked enhanced resolution accounts for the significantly increased precision. This improvement was found to

be of particular importance for the analysis of spinal BMD.

In clinical practice, the scanning time of 6 to 7 minutes for DER, as compared with 20 to 45 minutes for DPA, represents important progress. Errors caused by patient movement are reduced, better utilization of the equipment is achieved, and, therefore, cost is reduced.

In conclusion, the precision, spatial resolution, and scanning time of DER are significant improvements over those of DPA. Fortunately, normative data obtained by using DPA can be used for DER studies. The bone mineral values for individual patients can be corrected by the average differences between DER and DPA.

Glüer CC, Steiger P, Selvidge R, et al. *Radiology* 1990;174: 223-228.

**Editor's comment:** Although this article was published in 1990, its importance to those who are becoming involved in measurements of BMD and BMC prompts our abstracting it for GGH.

This article can serve as a foundation for those who have an interest in extending their knowledge of DPA and DER. Finally, the improvements to DER permit changes in BMD and BMC to be detected over a period of months instead of years, when therapeutic agents are tested.

Robert M. Blizzard, MD

#### Editorial Board

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|                         | Dual-Photon Absorptiometry (DPA)  | Dual-Energy Radiography (DER)   |
|-------------------------|---|---|
| <b>Basic Principles</b> | 1. <sup>153</sup> Gd isotope source<br>2. Photon-counting detector<br>3. Multiple accessible bone sites | 1. X-ray tube source<br>2. Alternating X-ray generator voltage with integrating detector  |
| <b>Advantages</b>       | 1. Low X-ray dose<br>2. Clinically adequate accuracy  | 1. Reduced scanning time (6-7 min)<br>...patient comfort, equipment utilization<br>2. Improved resolution<br>...reduced precision error |
| <b>Disadvantages</b>    | 1. Long scanning time (20-45 min)<br>2. Limitations in precision (up to 6%)                             |   |

## Magnetic Resonance Imaging in the Diagnosis of Growth Hormone Deficiency

In this study, 46 patients (29 male) with idiopathic growth hormone (GH) deficiency were examined by magnetic resonance imaging (MRI) at a mean ( $\pm$ SEM) age of  $9 \pm 1$  years (range, 15 days to 20 years). At the time of evaluation, 37 patients were prepubertal, 5 had spontaneous pubertal development, and 4 were receiving supplementary testosterone or estrogen-progesterone therapy. MR images were obtained before therapy with human GH (hGH) ( $n=28$ ), during hGH therapy ( $n=13$ ), or after hGH therapy ( $n=5$ ). The diagnosis was confirmed by a GH peak response  $<8$  ng/mL after 2 pharmacologic stimulation tests.

In all cases, T1-weighted images, 3 mm thick, were obtained in the sagittal and coronal planes. The maximal height of the pituitary gland was measured in a plane perpendicular to the floor of the sella turcica. Ischemic lesions of the hypothalamus and basal ganglia were looked for on 5 mm thick T2-weighted coronal images.

The patients were classified into 2 groups according to MR images: group 1 ( $n=29$ ) had pituitary stalk interruption syndrome (PSIS) and group 2 ( $n=17$ ) had normal pituitary anatomy. PSIS was diagnosed based on the following criteria: lack of visible pituitary stalk, lack of the normal posterior lobe hypersignal into the sella turcica, and presence of a hyperintense nodule in the region of the infundibular recess of the third ventricle. All patients with PSIS had a pituitary height  $<2$  standard deviations (SD) for age; 3 had no visible anterior pituitary lobe. The pituitary height was less than normal in 10 patients (60%) with normal pituitary anatomy.

The group with PSIS had the first symptom of GH deficiency at an earlier age ( $2.8 \pm 0.6$  years vs  $5.5 \pm 1.2$  years;  $P<0.001$ ), were of smaller stature ( $-4 \pm 0.2$  SD vs  $-3 \pm 0.2$  SD;  $P<0.01$ ), and had lower GH peak response to provocative testing ( $3 \pm 0.4$  ng/mL vs  $5 \pm 0.5$  ng/mL;  $P<0.001$ ) than the group with normal pituitary anatomy. Their pituitary gland also was shorter ( $2.5 \pm 0.2$  mm vs  $3.5 \pm 0.2$  mm;  $P<0.01$ ). All the patients with multiple pituitary deficiencies except 1 ( $n=19$ ) belonged to this group. There was a higher incidence of multiple pituitary deficiencies (65% vs 5%) in the PSIS group ( $P<0.001$ ). There also were more perinatal abnormalities (24% vs 17%), associated congenital abnormalities (17% vs 12%), microphallus (15% vs 11%), and hypoglycemia (14% vs 0%) in the PSIS group compared with the group with normal pituitary as determined by MRI.

The authors concluded that the evaluation of the shape and height of the pituitary gland by MRI is an additional tool for the diagnosis of PSIS-related GH deficiency. The presence of pituitary stalk interruption confirms this diagnosis and is predictive of multiple anterior pituitary deficiencies.

Argyropoulou M, Perignon F, Brauner R, et al. *J Pediatr* 1992;120:886-891.

**Editor's comment:** This study clearly demonstrates that MRI is of value (when performed using the appropriate equipment and personnel) for the evaluation of patients with GH deficiency. MRI studies in hypopituitarism often add information to confirm the diagnosis. The authors showed that MRI is the best clinical tool to document PSIS, which cannot be diagnosed with certainty by any other clinical or radiologic methods. MRI may even contribute data regarding the duration and long-term prognosis of the pituitary deficiencies.

A decreased pituitary height and/or the presence of pituitary stalk interruption as demonstrated by MRI will assist in the interpretation of the biochemical and hormonal data. For example, MRI abnormalities would help elucidate the depressed, spontaneous,

or stimulated GH levels in an obese short child. It also may help the clinician in interpreting the GH levels utilized as a cutoff level for diagnosis of GH deficiency. The demonstration of MRI abnormalities of the pituitary stalk also should alert the clinician to the possibility of multiple hormonal deficits.

However, MRI findings do not necessarily correlate with pituitary function.<sup>1</sup> In one study, the height of the anterior lobe of the pituitary gland in 17 of 22 children with idiopathic GH deficiency was less than 3 mm.<sup>2</sup> Although anatomic abnormalities of the hypothalamic-pituitary axis are more commonly observed in patients with multiple pituitary defects, there is overlap between these patients and those with isolated GH deficiency.<sup>1</sup> Thus, MRI cannot obviate a complete endocrine workup. The authors of this study did not comment on the presence of ectopic pituitaries as a possible distinct entity from PSIS. The authors did not state in what number of patients MRI demonstrated the presence of a hyperintense nodule in the third ventricle, with or without evidence of pituitary stalk alterations. Evidence suggestive of an "ectopic" posterior pituitary gland in children with idiopathic anterior hypopituitarism was shown in previous MRI studies.<sup>3</sup> This, and numerous other studies, confirms that MRI is expanding the capabilities of endocrinologists to interpret suspected pathophysiology.

Fima Lifshitz, MD

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## Erratum

In *GROWTH, Genetics, & Hormones* Vol. 8, No. 4 (December 1992) an error on page 7 under the section entitled Sleep Staging, (the first sentence) incorrectly identifies "(stages 1 to 3)". This should have been provided as references 1 to 3.

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## Growth Hormone and Tumour Recurrence

Ogilvy-Stuart et al report data on the recurrence of CNS tumors in children in the northwest region of England who were treated with human growth hormone (GH). Included in the analysis were 207 children with brain tumors between 0.5 and 14.4 years of age (median, 6.7 years); 47 of these (29 boys) received GH. The median length of time from diagnosis to initiation of therapy was 4.5 years, and the median duration of therapy was 3.2 years. Serving as a comparison group were 160 children who had not been treated with GH. Each child had received cranial irradiation (median dose, 3000 cGy); 36 children had received a tumor-site booster dose (median dose, 1500 cGy). All were evaluated for GH deficiency at approximately 2 years postradiotherapy, a time when tumor recurrence is most likely to occur and also a time at which tumor-induced GH deficiency may be readily identified. The dose of GH was 12 IU/wk prior to 1989 and 0.5 IU/kg/wk after 1989.

Five of the 47 children (11%) who were treated with GH had a clinical relapse associated with recurrence of brain tumor. In 2, relapse occurred after the completion of the GH treatment, while in 3 patients relapse occurred from 0.5 to 3.3 years after starting therapy. Forty-two of 160 children (26%) who did not receive GH

relapsed. Thus, the authors conclude that there is no association between GH and tumor recurrence.

Ogilvy-Stuart AL, Ryder WDJ, Gattamaneni HR, et al. *Br Med J* 1992;304:1601-1605.

**Editor's comment:** This is another reassuring study for pediatric endocrinologists assessing children who have received cranial irradiation and who have growth retardation. It is noteworthy that 10 of 44 children with brain tumors who had computed tomography scans performed at the beginning of GH therapy showed residual tumor. Thus, there does not appear to be a clear association between tumor growth and GH treatment. The authors point out that as more children are successfully treated for CNS malignancy, more of these children will be presenting to pediatric endocrinology clinics for possible GH therapy. It is important that similar registry data be continued to ensure that children treated with GH do not show an increased risk of tumor recurrence.

William L. Clarke, MD

## Elevated Growth Hormone Secretory Rate in Premature Infants

Wright et al studied 5 premature infants (gestational age, 24 to 34 weeks) and 6 full-term infants for growth hormone (GH) secretory characteristics by drawing blood every 15 minutes for 6 hours for determination of GH concentrations. Deconvolution analyses were done to study the GH secretory characteristics in both groups.

The authors confirmed their own previous work that: premature infants have higher GH concentrations than full-term infants ( $18,100 \pm 800 \mu\text{g/L}$  vs  $10,200 \pm 2700 \mu\text{g/L}$ ;  $P=0.067$ ); the half-life of circulating GH for both groups was similar to that reported for normal adult men (18.9 minutes); and premature infants had significantly higher secretory burst amplitudes than full-term infants, as well as higher production rates. The insulin-like growth factor 1 (IGF-1) values were lower in premature infants than in full-term infants.

When these data are interpreted in conjunction with other known data, eg, premature infants have lower levels of IGF-binding protein 3 and GH-binding protein than full-term infants, the authors conclude that the increased GH secretory activity in premature infants reflects an increase in hypothalamic GH-releasing hormone activity and/or reduced somatostatin tone.

Wright NM, Northington FJ, Miller JD, et al. Elevated growth hormone secretory rate in premature infants: deconvolution analysis of pulsatile growth hormone secretion in the neonate. *Pediatr Res* 1992; 32:286-290.

**Editor's comment:** The authors are to be commended for performing a tedious task and deriving valuable data while drawing only 2.8 mL of blood. The findings provide further understanding of the pulsatile characteristics of GH secretion at a relative early gestational age (24 to 34 weeks).

One must realize, however, that the characteristics of GH secretion are probably not related to fetal growth, as GH is not required for normal or near-normal fetal growth. The factors stimulating fetal growth are probably multiple (see GGH, 8[1]:1), but probably do not include GH, human chorionic somatomammotropin, prolactin, or IGF-1. Regardless, the data reported by Wright et al are valuable for the reasons stated above.

Robert M. Blizzard, MD

## The Birth Injury Theory of "Idiopathic" Growth Hormone Deficiency

At the beginning of his article, Dr. Itsuro Hibi gives an historical survey of the origin of the birth injury theory in respect to the etiopathogenesis of idiopathic growth hormone deficiency (GHD). The pathologist Simmonds (1919) was the first to describe a severe atrophy of the adenohypophysis, probably due to birth trauma, in a 21-year-old sexually infantile dwarf. Prader (1960) reported on the birth histories of 25 children with idiopathic GHD. Complicated births were ascertained in 18 cases. In 1962, both Bierich and Van der Werff ten Bosch reported that two thirds of their patients with idiopathic GHD were born in noncephalic positions, mainly by breech deliveries. It has been known for a long time that such births are connected with high mortality and morbidity. In the majority of Bierich's patients (1965), perinatal asphyxias and convulsions also were recorded. In the years

following, these findings were confirmed by numerous authors, including Hibi and Tanai (1979).

Dr. Hibi has considered the following questions: Does GHD result from or cause breech delivery? If GHD can be caused by breech delivery, why is spinal cord injury not found more frequently? If breech delivery causes GHD, why are only a small proportion of children who are born by breech delivery affected by hypopituitarism? How can male preponderance of GHD be explained in relation to the association of breech delivery with GHD?

After studying 95 siblings of 70 idiopathic GHD children born by breech delivery and finding that none of the 20 siblings also born by breech delivery had idiopathic GHD, Dr. Hibi concluded that idiopathic GHD in these 70 patients resulted from the breech delivery.



The author states that there are reports associating spinal cord injury and GHD, and concludes that this supports the thesis that GHD results from breech delivery. However, he readily admits that most patients with GHD and born by breech delivery do not have spinal cord problems.

The author only briefly considers why such a small portion of children born by breech delivery have GHD, and provides no satisfactory answer to this question.

Most intriguing in this paper are the data pertaining to the sex ratio. From these reports concerning 523 patients with idiopathic GHD, the following are retabulated.

| Delivery | No. Patients | Male:Female Ratio |
|----------|--------------|-------------------|
| Breech   | 316          | 5.1 - 7.8*        |
| Vertex   | 207          | 1.1 - 1.7*        |

\* According to various series.

The author concludes that the reason why more male than female infants with GHD are born by breech delivery is unclear.

In closing, Dr. Hibi notes that the percentage of patients born by breech delivery among GHD patients in Japan between 1986 and 1988 is very low (6% of 6,357 GHD patients). Of course, mild GHD is much more frequently diagnosed than previously, and it may be that only severe GHD is found in association with breech delivery.

Hibi I. *Clin Pediatr Endocrinol* 1992;1(1):1-3.

**Editor's comment:** Dr. Hibi's article is the first article in a new journal, *Clinical Pediatric Endocrinology*. This is the official English journal of the Japanese Society for Pediatric Endocrinology, and it will be published twice yearly.

Because Professor Jürgen Bierich published extensively regarding this topic, I have asked him to comment. We are proud to note that he is a former Editorial Board member of GGH. His reply is as follows:

Hibi and Tanaka (1979) investigated 95 siblings of 70 breech-born patients with idiopathic GHD. Twenty of these 95 also were born by breech delivery. Therefore, the author drew the conclusion that the GHD of the initial 70 patients was the result of the breech delivery — an event that leads to GHD in only a minority of cases. The question why only a small fraction of the breech-born children acquire hypopituitarism remains unanswered in Dr. Hibi's paper. In my opinion, the reason lies in the fact that breech deliveries exhibit rather variable courses. Usually, but by no means in all of the cases, they are connected with additional complications, eg, prematurity, prolapse of the umbilical cord, and early abruption of the placenta. Whether hypothalamic injury occurs depends on the obstetric situation in toto.

To seriously consider the pathophysiologic basis of GHD possibly associated with breech delivery, one must consider the anatomy that has been found in association with isolated GHD. The few autopsy records of patients with idiopathic GHD have shown severe atrophy of the adenohypophysis with loss of the chromophilic cells. During the last 15 years, several investigations with modern imaging techniques were performed in patients with idiopathic GHD; these showed rather small pituitary glands with no demonstrable stalks. Dr. Hibi concludes that transections of the stalk, or ischemic alterations of the pituitary, represent the typical morphologic correlate of idiopathic GHD. However, older investigations favor the assumption that vaginal breech deliveries cause predominantly cerebral hemorrhages. During the last 15 years, numerous endocrinologic studies have demonstrated that idiopathic GHD in the

majority of cases represents a hypothalamic disorder and rests primarily upon a growth hormone-releasing hormone deficiency. The atrophy of the pituitary is a secondary phenomenon resulting from inadequate central stimulation. Actual spinal cord lesions, in particular syringomyelias, have been observed in patients with idiopathic GHD (Fujita et al, 1992). However, many of these escape the pediatrician's detection because they manifest themselves relatively late, eg, in the second decade of life. It is possible that the incidence of spinal cord lesions is increased in children with idiopathic GHD in association with breech delivery, but the tabulations to evaluate the association have not been determined because the studies were done early in life.

Dr. Hibi states that the reason why more male than female infants with idiopathic GHD are born by breech delivery is unclear. The author discusses the so-called male disadvantage, ie, the generally enhanced susceptibility of male infants to perinatal damage. With regard to mortality, the male:female ratio in the large series of Naye et al (1971) was 1.7:1. For cerebral birth trauma, Prader (1960) found a sex ratio of 2:1 in the children of the Kinderspital Zurich. I am in accordance with Hibi that the male disadvantage can explain the slight preponderance of the male sex in children born in vertex position, but not the extraordinarily high ratio (ranging from 5.1 to 7.8) in the breech-born infants. In order to arrive at a plausible interpretation of these findings, 2 questions require clarification: first, whether breech presentations occur more frequently in male than in female infants; and second, whether breech deliveries are more likely to cause cerebral lesions in boys than in girls. The follow-up study of Manzke (1978) speaks to this question. Among breech-born infants who were reinvestigated at age 6, the male patients exhibited worse neurologic and mental test results than the female ones. The differences were statistically significant, but small. Certainly, investigations of larger cohorts are necessary in order to come to definite answers.

Thank you for inviting my comments.

Jürgen Bierich, MD

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**2nd Editor's comment:** Although breech delivery occurs more frequently with male children with idiopathic GHD than with female children with idiopathic GHD or than with male children without idiopathic GHD, the answers to Dr. Hibi's questions remain elusive. We thank Dr. Bierich for providing his insight into the problem. He also believes the questions still remain unanswered, and suggests what must be done to supply answers to the questions—if possible.

Robert M. Blizzard, MD



## MEETINGS CALENDAR

**April 15-18, 1993** Intl Immunol and Diabetes Wkshp (IDW), Orlando, FL. Info: Dr NK Maclaren, Dept of Pathol, Univ of FL, Box 100275, JHMC, Gainesville, FL 32610. Tel: 904-392-6840; Fax: 904-392-6249.

**May 3-6, 1993** Amer Pediatr Soc/Soc for Pediatr Research/Ambulatory Pediatr Assoc, Washington, DC. Info: Elk Grove Village, IL. Tel: (SPR) 708-427-0205, (APS) 708-427-1205; Fax: (both) 708-427-1305.

**May 26-29, 1993** Wkshp on Non-Conventional GH Therapy - ISGD Course in Therapeutic Aspects of Childhood Diabetes, Siena, Italy. Info: Dr F Chiarelli, Dept of Paediatr, Univ of Chieti, 11 Via Nicolini, 66100 Chieti, Italy. Tel: 39-871-412-72; Fax: 39-871-63-669.

**May 27-June 1, 1993** 7th Intl Clin Genet Sem on "Dysmorphology" and "Genetics of Cardiovascular Disorders" in Samos, Greece. Info: Dr C Bartsocas, Dept of Paediatr, "P & A Kyriakou" Children's Hosp, GR-11527 Athens, Greece. Tel: +30-1-7709316; Fax: +30-1-7796461.

**May 30-June 3, 1993** Amer Soc for Biochem and Molecular Biol, San Diego, CA. Info: M Sternburg, 9650 Rockville Pike, Bethesda, MD 20814. Tel: 301-530-7010; Fax: 301-550-7014.

**June 1-2, 1993** Symp on "Male Sexual Differentiation." Info: Dr CJ Migeon, CMSC 3-1000, The Johns Hopkins Hosp, Baltimore, MD 21205. Tel: 410-955-6463; Fax: 410-955-9773. S Raiti, MD, 5805 Stony Run Drive, Baltimore, MD 21210-1330.

**June 3-6, 1993** 3rd Intl Wkshp on Fetal Genetic Pathol, Perugia-Bosco, Italy. Info: Dr G Neri, Istituto di Genetica Medica, Universita Cattolica, Roma, Italy. Tel: +6-3054449; Fax: +6-3050031.

**June 3-7, 1993** 4th Joint LWPES/ESPE Mtg, San Francisco, CA. Info: For LWPES members: MM Grumbach, MD, Univ of CA-San Francisco, c/o Extended Programs in Medical Education, Room LS105, Box 0792, San Francisco CA 94143. Tel: 415-476-4251; Fax: 415-476-0318. For ESPE members: Dr M Ritton, Dept of Paediatr Endocrinol, Karolinska Hospital, S-104 01, Stockholm, Sweden. Tel: 46-8-729-2465; Fax: 46-8-729-5128.

**June 9-12, 1993** 75th Ann Mtg of The Endocrine Soc, Las Vegas, NV. Info: C Huck, The Endocrine Soc, 9650 Rockville Pike, Bethesda, MD 20814. Tel: 301-571-1835; Fax: 301-571-1869.

**September 2-6, 1993** 19th Ann Mtg of the Intl Study Group of Diabetes in Children and Adolescents (ISGD), Athens, Greece, on board MTS "Arcadia." Info: Dr C Bartsocas, Dept of Paediatr, "P & A Kyriakou" Children's Hosp, GR-11527 Athens, Greece. Tel: +30-1-7709316; Fax: +30-1-7796461.

**September 6-8, 1993** Frontiers of Paediatr Neuroendocrinol, London, Eng. Info: Dr MO Savage, Dept of Endocrinol, St Bartholomew's Hosp, London EC1A 7BE, UK. Tel: 44-71-601-8487; Fax: 44-71-601-8505.

**September 12-15, 1993** Ann Mtg of the Eur Soc for Paediatr Research (ESPR), Edinburgh, Scot. Sci Info: Prof N McIntosh, Dept of Child Life and Health, Univ of Edinburgh, 17 Hatton Pl, Edinburgh EH9 1UW, Scot, UK. Tel: 44-31-667-2617; Fax: 44-31-668-2605. Genl Info: ESPR '93, Edinburgh Conf Ctr, Heriot-Watt Univ, Riccarton, Edinburgh EH14 4AS, Scot, UK. Tel: 44-31-449-5111; Fax: 44-31-451-3199.

**September 13-15, 1993** Conference on "Glycobiology: New Perspectives on Human Disease." Info: G Holt, Natl Inst of Health, Bldg 10, Rm 9S242, 9000 Rockville Pike, Bethesda, MD 20892. Tel: 301-496-9101; Fax: 301-402-0234.

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# GROWTH

## Genetics & Hormones

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### Fragile X Syndrome: Review and Current Status

David L. Nelson, PhD

*Institute of Molecular Genetics  
Baylor College of Medicine*

In the past year, a surprising new class of mutations involving unstable triplet nucleotide repeats has been found associated with 3 human genetic diseases. This article reviews recent findings in fragile X syndrome, the first of this type of unstable mutation to be described. Similarities and differences with myotonic dystrophy and spinal/bulbar muscular atrophy (Kennedy disease) are also noted.

#### Letter From the Editor

The **fragile X syndrome** has been an endocrine and genetic enigma. New and exciting gene findings prompt us to feature a lead article concerning the genetics of the syndrome and 3 abstracts concerning clinical findings. These all complement each other, which should enlighten you, our reader. You may wish to review Dr. Judith Hall's perspective, "The Strange Case of Fragile X Syndrome: Increased Mutation Frequency, Fragment Size, and/or Genomic Imprinting?" (*GGH* 1991;7[4]:9-10) before reading this issue. Dr. Hall's article sets the stage for the material contributed by Dr. David Nelson and the 3 abstracted journal articles. Please use your glossary included with the last issue of *GGH*. The asterisks (\*) in Dr. Nelson's article indicate when you may be helped by referencing the glossary.

Robert M. Blizzard, MD

#### FRAGILE X SYNDROME

Fragile X syndrome is the most frequently encountered form of inherited mental retardation in humans, with a frequency estimated to be 1/1,250 males.<sup>1,2</sup> Fragile X syndrome segregates as an X-linked dominant condition with incomplete penetrance\* since either sex, when carrying the fragile X mutation, may exhibit mental deficiency. Sherman et al<sup>3,4</sup> showed that approximately 30% of carrier females are affected and that 20% of males carrying the fragile X chromosome are phenotypically normal but may transmit the disorder and have affected grandsons. In addition to the mental retardation, which is variable in severity, affected males exhibit other manifestations, including macroorchidism and distinctive facies.<sup>5</sup> Fragile X syndrome, as implied by the name, is associated with a fragile site,\* expressed as an isochromatid gap in the metaphase X chromosome, at position Xq27.3.<sup>6</sup>

#### GENETICS OF FRAGILE X

Inheritance of the fragile X syndrome is quite complicated, although the features of the mutation at the DNA level (see below) are beginning to provide an explanation for the unusual genetics found in this disorder. The most striking aspect of fragile X is its incomplete penetrance in both males and females. This is particularly interesting in the case of normal transmitting males (NTMs), who transmit the mutation to grandsons but are unaffected themselves. More complicated are the probabilities of mental deficiency based on the affected status of relatives. This has become known as the Sherman paradox.<sup>3,4</sup> It states that the probability of mental retardation is increased by the number of generations through which the mutation has passed. The probability is higher for both sons and daughters of affected females or females with affected sibs. The recent identification of the fragile X mutation, and its characterization as an unstable DNA fragment, provides a rational basis for this phenomenon.

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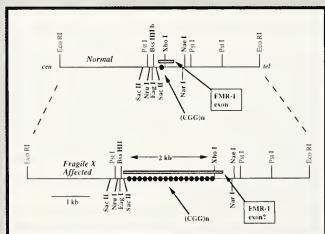
## THE FRAGILE SITE

In May of 1991, 3 groups reported identification of the fragile X site in Xq27.3 (Figure 1) using positional cloning\* strategies.<sup>7-9</sup> All made use of yeast artificial chromosome (YAC) clones containing this region to define a small section of the chromosome broken in a series of somatic cell hybrid lines.\*<sup>10</sup> These hybrid cell lines contained chromosomal breakpoints suspected to be at the fragile X site. All groups found mutations in this chromosomal region in families with the fragile X syndrome. Somatic mosaicism in affected individuals was determined by inspection of DNA using Southern hybridization.\* Two general classes of mutation were seen. These became known as *premutation* and *full mutation* and were first defined by increases in the size of fragments observed by Southern hybridization. Premutations are found in all NTMs and many carrier females, and involve increases in the length of this region by 50 to 500 bp. Full mutations are found in all affected individuals, male or female, and in some carrier females. The full mutation alleles show increases of 600 to 3,000 bp in length and are usually heterogeneous within an individual demonstrating somatic instability of the mutant allele.

The observation that a cluster of restriction sites in the fragile X region were differently methylated\* in normal and fragile X males provided another clue to the location of the fragile site.<sup>11,12</sup> Restriction enzymes that do not cut DNA when cytosines in their recognition sequences are methylated were used to demonstrate the differences.

Figure 1

Map of the 5.2-kb Fragment in Xq27.3 Produced by Digestion With Restriction Enzyme *EcoRI*



The fragment contains the CGG repeats (●) mutated in fragile X syndrome in normal and fragile X-affected forms. Restriction sites for other enzymes and the exon of FMR-1 are indicated. Restriction sites in bold type are sensitive to methylated cytosine residues in CGP dinucleotides. Cen refers to the centromere and tel to the telomere portion of the chromosome.

This cluster comprised a tract of CG (CpG) dinucleotides known as a CpG island. CpG islands are often found near the transcription start sites\* of genes. The specific methylation differences are observed in full mutations but not in premutation alleles.

## THE FMR-1 GENE

Verker et al<sup>7</sup> also reported the isolation of a cDNA\* from the region of the fragile X mutation. It was derived from a gene denoted FMR-1 (fragile X mental retardation-1). A 4.8-kb mRNA transcript from this gene was found in a variety of tissues, with brain tissue showing highest levels of expression. Additional unpublished results indicate that testes, uteri, and placenta all have similarly high levels of the transcript. Comparisons of the predicted amino acid sequence of FMR-1 have not revealed significant relationships with other known proteins. Related sequences have been observed by Southern hybridization in a variety of other species of mammals, as well as in yeast and *Caenorhabditis elegans*,\* indicating that the FMR-1 gene has been highly conserved through evolution. The functions of FMR-1 are still unknown.

The most interesting feature of FMR-1 was the identification at the 5' end of a cDNA clone of the sequence (CGG)<sub>5</sub>AGG(CGG)<sub>5</sub>AGG(CGG)<sub>10</sub> (expressed hereafter as CGG repeats). In the reading frame\* of FMR-1, this sequence would predict a run of 30 consecutive arginine residues. Such a sequence is unprecedented in known proteins, although there are a number of proteins with stretches of up to 10 arginine residues. These generally have DNA-binding activities (histones\* and polyamines\*). It is as yet unclear whether this sequence is translated into protein. However, this repeat sequence is the site of the fragile X mutations and accounts for their unstable nature.

Pieretti et al<sup>13</sup> reported complete loss of expression of FMR-1 RNA in 80% (16/20) of fragile X males studied. The 4 cases with RNA expression demonstrated partial methylation of the CpG island, while the 16 cases with no expression showed complete methylation of the island. Thus, the expression of FMR-1 appears to be dependent upon the methylation status of the adjacent CpG island, and a likely mechanism of the fragile X phenotype involves expansion of the CGG repeats (see below) followed by methylation of the region, which causes loss of expression of FMR-1 RNA. The 4 cases with partial methylation were not detectably less severely affected. However, given the somatic mosaicism observed for the mutation, it is possible that the methylation pattern of blood cells studied was irrelevant since it did not reflect the pattern in cells actually responsible for the phenotype.

\* Terms marked with an \* are defined in the *GGH Genetics Glossary*, Volume 9, Number 1.

## CGG REPEATS AND FRAGILE X MUTATION

While 2 of the 3 original reports noted this repeated sequence and suggested it may have a role in the unstable mutation, Kremer et al<sup>14</sup> demonstrated that the site of mutation, as well as the instability, was within the CGG repeats, and that the size variation of restriction fragments observed by Southern hybridization was due to changes in the number of copies of the CGG repeats. Fu et al<sup>15</sup> characterized the repeats in more detail, finding them to be polymorphic in the normal population, with the most frequent allele having 29 repeats and a range of sizes from 6 to 54 repeats. In fragile X premutations, the smallest number of repeats reported was 52, while the largest was 193; the majority were in the 75 to 120 range.

Precision of measurement of allele sizes in different persons was achieved by polymerase chain reaction (PCR)\* amplification of the alleles followed by sequencing gel analysis for size discrimination. One provocative finding was the observation of instability of the premutation alleles during meiotic transmission. In every case examined (n=67), the number of repeats was found to be altered from parent to offspring. Thus, the mutation frequency of premutation alleles is ~10%. This is an unprecedented frequency. Mutations tend to increase the number of repeats in each generation; the number of repeats tends to grow upon passage from parent to offspring. Only 3 of 67 transmissions had decreases in size.

## THE SHERMAN PARADOX

Fu et al also studied the frequency with which the premutation was altered to the full mutation from parent to child. With father-to-daughter transmission, the premutation expanded to the full mutation in none of the 4 examples. This also was seen in Southern analysis<sup>9,16</sup> and fits with the observation that daughters of NTMs are never found to be affected.<sup>3,4</sup> Premutation to full mutation changes are found only in offspring of females.

### Recognition Award

With the support of Genentech, Inc. and the Editorial Board, *GROWTH, Genetics, & Hormones (GGH)* adopted the utilization of an environmentally conscious paper stock in 1991. We are pleased to share our Recognition Award from Mohawk Paper Mills, presented at the 1993 Editorial Board Meeting, for the utilization of 50/10 recycled stock. *GGH* was judged for this award against a multitude of publications with formats as varied and unique as the individual publications themselves. It is an honor to receive this award and we wish to thank our readership for your continued interest and to Genentech, Inc. for its generosity in supporting *GGH*.

The *GGH* Editorial Board

The risk of expansion to the full mutation varies with the size of the premutation in the mother. This finding, along with the observation of premutation and full mutation alleles, and the tendency of alleles to increase in size with subsequent generations, elucidates the Sherman paradox. The risk of retardation to an individual is dependent upon the number of repeats in his/her mother's allele. If a mother is affected, she already has an expanded full mutation allele (roughly one half of females with the full mutation are found to be affected).<sup>16</sup> If this mother has an affected brother, she may have a premutation with a repeat number at the high end of the premutation range, or a full mutation that resulted in no phenotypic expression. In pedigrees with documented NTMs, however, the risks of retardation were found to be considerably smaller and the empiric data of Sherman fit nicely with the findings of Fu et al regarding escalatory risks of expansion to the full mutation.

## HERITABLE UNSTABLE DNA

Finding heritable unstable triplet repeats at the fragile X site led to speculation that similar repeats might be found in other conditions showing unusual inheritance patterns.<sup>17</sup> One prime suspect was myotonic dystrophy, which has been mapped to a small region of chromosome 19. Myotonic dystrophy exhibits a phenomenon known as anticipation,\* whereby the severity of the disease increases with subsequent generations. While there has been considerable debate about the existence of anticipation, the clue provided by the fragile X mutation prompted a search for triplet repeats in the relevant region of chromosome 19.

### The Human Growth Foundation Announces a Grant Program

The Human Growth Foundation announces a Grant Program for investigation of human growth and its disorders. Special consideration will be given to new investigators and ideas new to the field. Research dealing with all aspects of normal and abnormal growth such as biologic, psychologic, educational, and dietary will be considered. One or more grants in the amount of \$7,500 to \$10,000 will be awarded.

An NIH-type biographic sketch and 2-page letter of intent should be sent to the address below by July 1, 1993. Applicants selected to submit a complete application will be notified by August 1, 1993, and completed applications will be due October 1, 1993.

Send correspondence to:

Human Growth Foundation  
7777 Leesburg Pike, Ste. 202S  
Falls Church, VA 22043



Numerous reports describing unstable DNA based on Southern hybridization,<sup>18-20</sup> and the subsequent identification of a triplet repeat (CTG in the 3' untranslated sequence of an mRNA likely encoding a protein kinase) as the basis of the instability have recently been published.<sup>21-23</sup> The severity of the phenotype in muscular dystrophy correlates well with the number of repeat units in the mRNA; and the number of repeats generally increases with subsequent generations, providing a molecular basis for the anticipation phenomenon. As in fragile X syndrome, the repeat is polymorphic. It ranges from 5 to 27 residues in normal individuals and grows in length to at least 50 repeats, from which it can expand dramatically in length. However, unlike fragile X, the repeat expansion in muscular dystrophy is found in offspring of both male and female parents.

A third human genetic disorder involving triplet repeats has been found. In spinal/bulbar muscular atrophy, the mutation has been localized to an increased number of CAG codons in a region of the androgen receptor gene on the X chromosome.<sup>24</sup> This region encodes a polyglutamine repeat in the receptor. It ranges from 17 to 26 residues in normal individuals, but exceeds 40 residues in affected individuals. Evidence for instability of the triplet repeat has not yet been published. As yet, no large expansions have been identified. The CAG repeat in spinal/bulbar muscular atrophy is the complement of the CTG repeat in muscular dystrophy; thus, these are the same repeats.

The identification of a mutation that confers increased mutability onto itself is a rather astounding finding, offering insight into several previously mysterious phenomena in genetics. The idea that DNA is not necessarily inherited in the same form as the parents' or that it can be significantly altered from tissue to tissue within an individual is radical, and calls for reexamination of some of the principles of genetics. It is a rare delight when fundamentally new phenomena are uncovered in genetics, and heritable unstable DNA represents such a new principle. We can only hope that the continuing inquiry into the nature of our genes will yield more such insights.

## CLINICAL TESTING FOR FRAGILE X SYNDROME

Advances in molecular testing for fragile X mutations have complicated the clinical evaluation of a child in whom the syndrome is suspected. Important questions surround the accuracy and reliability of both cytogenetic and molecular assays, especially with regard to the rare exceptions, such as deletions in the FMR-1 gene, that are not detected cytogenetically. Cytogenetic analysis certainly has a role in evaluating an isolated case of developmental delay, since other chromosomal abnormalities may be detected. However, in families with known fragile X mutations, it is difficult to justify the cost; DNA-based analyses may be

more accurate if they are available. There is still considerable debate about the merits of different types of DNA-based testing, ie, Southern hybridization-based versus PCR-based assays, and technical improvements continue to be made in many research and diagnostic laboratories. However, it is clear that DNA-based testing will be the method of choice for some time to come.

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## In Future Issues

**The Relevance of Developmental Genetics to Human Malformation**  
by Golder Wilson, MD

**Overgrowth Syndromes and Disorders: Definition and Classification**  
by David Weaver, MD

**The Overgrowth Syndromes: An Update**  
by Kenneth L. Jones, MD

**Adrenarche and Its Variants**  
by Songya Pang, MD

**The Importance and Methods of Using Animal Models to Study Human Disease**  
by Robin Winter, MD

**Clinical Significance of Urinary GH Measurements**  
by Margaret MacGillivray, MD

## Standard for Selected Anthropometric Measurements in Males With the Fragile X Syndrome

Butler et al prepared anthropometric data on 185 white males (ages 0 to 26 years) with fragile X syndrome confirmed by chromosome analysis. Height, weight, head circumference, ear length, and testicular volume were measured; similar control data were collected and utilized for comparison. Standards were then developed for the 5th, 50th, and 95th percentiles of both groups. At least 7 individuals were measured at each 1-year age interval.

The curves produced showed remarkable similarity in height and weight between fragile X subjects and controls, with the exception of a slight tendency for obesity in the affected individuals at approximately 12 years of age. Head circumference was slightly increased at all ages. Ear length at the 5th, 50th, and 95th percentiles was consistently above the respective values for normals at all ages (see Figure 1), as were testicular volumes. The 50th percentiles for testicular volumes approximated the control 95th percentile until 6 years of age, after which both the 50th and 95th

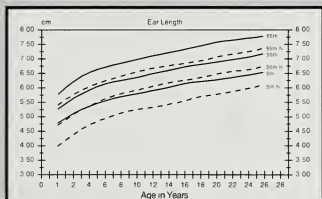
percentiles were markedly greater than the control 95th percentile (see Figure 2).

Butler MC, Brunschwig A, Miller LK, et al. *Pediatrics* 1992;89:1059-1062.

**Editor's comment:** The author states that fragile X syndrome is the most common genetic cause of mental retardation in males except for Down syndrome. The fragile X syndrome accounts for 30% to 50% of families with male mental retardation. The data presented in this article, in particular ear length and testicular volume, may be useful in identifying individuals for whom diagnostic chromosomal studies for fragile X are indicated. The etiology of these findings is not known.

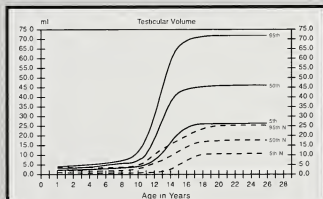
William L. Clarke, MD

Figure 1



Standardized curves for ear length of males with fragile X syndrome (solid line) and normal individuals (broken line).

Figure 2



Standardized curves for testicular volume of males with fragile X syndrome (solid line) and normal individuals (broken line).

## Psychiatric Disorders Associated With Fragile X in the Young Female

Females heterozygous for fragile X chromosomal variations are usually less affected than males, with only 35% exhibiting mental retardation. Studies by others have demonstrated specific short-term memory deficits, characteristic Wechsler IQ test subtest profiles, and frontal lobe deficits, suggesting verbal reasoning strengths and visual-spatial deficits in fragile X girls. Freund et al evaluated the prevalence of psychiatric and behavioral disturbances among a group of 17 fragile X females. This group was matched to 17 non-fragile X females who were similar in age, IQ, and socioeconomic status. All fragile X girls had cytogenetically confirmed fragile X syndrome. Cytogenetic testing was available on 12 of the 17 controls. The age range of the fragile X girls was from 4 to 27 years; that of the control group from 7 to 27 years. IQs averaged 78.2 in the fragile X group and 80.5 in the control group. Socioeconomic status ranged from lower to upper middle categories in both groups.

The Diagnostic Interview for Children and Adolescents-Parent version was administered to parents to ascertain psychiatric diagnoses. These diagnoses were based on *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition, Revised (DSM-III-R)

criteria, and included additional modifications for depression in the developmentally disabled. Adaptive behavior was assessed utilizing the Vineland Adaptive Behavior Scales, Survey Form. This assessed behaviors in 3 domains including communications, daily living skills, and socialization. The Revised Behavior Problem Checklist was given to parents and teachers to assess problem behaviors, including conduct, socialized aggression, attention problems, anxiety-withdrawal, psychotic behavior, and motor-tension excess. Cognitive assessment was performed with either the Stanford-Binet Intelligence Scale, Fourth Edition, or the Wechsler Intelligence Scale for Children-Revised.

Fragile X females have significantly more avoidant disorder of childhood and adolescence (ADCA) or avoidant personality disorder (65%), more mood disorders (47%), and more stereotypy-habit disorders (35%) than the control group. The majority of the fragile X girls (5 of 8) with a current or past mood disorder also met the criteria for ADCA. Stereotypy included repetitive smelling of objects, hand biting, excessive nail biting, hand clapping, or head banging. The frequency of stereotypy with ADCA and/or mood disorder was 83%. Female fragile X subjects also demonstrated

significantly lower socialization scores, especially in the area of interpersonal skills, eg, a lack of friends, as well as difficulty initiating, maintaining, and ending social conversations appropriately. Both parents and teachers report higher scores on the anxiety-withdrawal scale, behaviors which would include being shy, easily embarrassed, fearful, depressed, and sad.

DNA insert size was determined for 13 of the fragile X females and a significant correlation was shown between the size of the insert and IQ. In addition, the anxiety-withdrawal scores correlated positively with base-pair insert size when controlling for IQ, ie, increasing insert size correlated with increasing severity of anxiety and withdrawal behaviors.

Freund LS, Reiss AL, Abrams MT. *Pediatrics* 1993;91(2):321-329.

**Editor's comment:** Findings from this paper suggest that there

may be a significant prevalence of psychiatric disability in fragile X females and that the increase in size of the fragile X DNA insert may be associated with lower IQ and increased severity of anxiety-withdrawal symptoms. These associations are intriguing even though the pathogenetic mechanisms remain obscure. The authors have pointed out the significant limitations of their study, which include a small sample size, a wide age range, the lack of DNA testing on control subjects, and broken blindness to group membership. Despite these limitations, this study provides important information to the clinician dealing with these patients and their parents as it suggests that significant psychiatric disorders may occur. Whether or not such disorders will be amenable to treatment is not known. The recognition that certain behaviors may occur in fragile X females points out the need for prospective evaluations.

William L. Clarke, MD

## Girls With Fragile X Syndrome: Physical and Neurocognitive Status and Outcome

Male patients with fragile X syndrome have been the primary research focus as the entity is X-linked; and males, therefore, are more severely affected than females. Hagerman et al broadened the study of the fragile X syndrome to females. Thirty-two fragile X positive girls, 1 to 18 years of age, were compared with their sisters (n=19) who were fragile X negative. Some of the latter may have carried the gene.

Phenotypic features of affected individuals are listed in Table 1. A characteristic facial appearance is presented in Figure 1. A physical index score was obtained by adding up the number of phenotypic abnormalities listed in Table 1. Three of 32 girls had no phenotypic characteristics, 2 girls had 6 of the phenotypic abnormalities, and the remainder had between 1 and 5 phenotypic abnormalities.

Intellectual learning and behavior difficulties were frequent and included hyperactivity, shyness, hand flapping, poor eye contact, tactile defensiveness, impulsivity, and distractibility. Intelligence evaluations revealed an IQ <70 in 25% and <84 in 53% of the girls. No correlation between the extent of X fragility and IQ levels was found. However, the percent fragility statistically correlated (although not strongly) with the physical index score. IQ did not decline with age, which has been reported in boys with fragile X syndrome.

The authors recommended cytogenetic testing of all female siblings of fragile X males.

Hagerman RJ, Jackson C, Amiri K, et al. *Pediatrics* 1992;89:395-400.

**Editor's comment:** Fragile X syndrome does occur in girls. While patients with this syndrome frequently have both developmental and phenotypic characteristics, either may occur alone. Evolution of our knowledge about this syndrome is fascinating. It remains to be seen if imprinting is involved.

Robert M. Blizzard, MD

Table 1  
Physical Features Associated With Fragile X Syndrome

| Phenotypic Feature  | (n = 32)<br>X Positive % | (n = 19 sibs)<br>X Negative % |
|---|--------------------------|-------------------------------|
| Long ears<br>(measurement from<br>top to bottom of pinna)           | 12.9                     | 5.6                           |
| Prominent ears<br>(subjectively estimated<br>as visually prominent) | 56.3                     | 11.8                          |
| Long narrow face<br>(subjectively determined)                       | 46.4                     | 7.7                           |
| High arched palate<br>(subjectively determined)                     | 19.4                     | 5.9                           |
| Hyperextensible meta-<br>carpal phalangeal joints                   | 62.1                     | 38.9                          |
| Double-jointed thumbs   | 33.3                     | 22.2                          |
| Hand calluses<br>(from hand biting)                                 | 3.3                      | 0                             |
| Simian crease   | 21.4                     | 6.3                           |
| Flat feet   | 36.7                     | 23.5                          |
| Murmur or systolic click  | 13.3                     | 0                             |

Figure 1



Two sisters who are fragile X positive. Note prominent ears in both and long narrow face of sister on the left.

# Growth Hormone II: Basic and Clinical Aspects

*Conference Summary: Tarpon Springs, Florida,  
December 3-6, 1992  
Chairmen: B.B. Bercu, MD, and R.F. Walker, MD*

## Allen W. Root, MD

Recent developments in the regulation, physiology, and metabolic effects of growth hormone (GH) were discussed at this conference. Reisine et al and Coy et al described the family of 5 somatostatin (SRIH) membrane receptors identified by their differential binding of various analogues of SRIH and distinguishing modes of regulation and mechanism(s) of action. SRIH receptor-4 is expressed only in the anterior pituitary. Kraicer and Sims reviewed the evidence indicating that GH-releasing hormone (GHRH) acts through a G-protein to increase adenyl cyclase activity and intracellular levels of cyclic AMP, leading to activation of protein kinase A, increased intracellular  $\text{Ca}^{++}$  and exocytic release of GH. SRIH inhibits influx of  $\text{Ca}^{++}$  and release of GH. Melmed described the paracrine inhibitory effect of pituitary insulin-like growth factor 1 (IGF-1) on GH gene transcription and GH secretion. IGF-1 (synthesized in the pituitary folliculostellate cell in response to GH, cortisol, and triiodothyronine and acting through type 1 IGF receptors in the somatotroph membrane) can inhibit the stimulatory effects of GHRH, cyclic AMP, and triiodothyronine on GH gene expression.

Blalock reported that the synthesis and secretion of lymphocyte-derived GH are regulated by GHRH and IGF-1, but not by SRIH or GH itself. Lymphocyte GH stimulates lymphocyte production of IGF-1 and lymphocyte proliferation. Kelley detailed the many actions of GH and IGF-I on the immune system including an increase in macrophage and neutrophil production of superoxide anion — necessary for antimicrobial activity. In human neutrophils, the action of GH is mediated through the prolactin receptor (binding to this receptor is aided by  $\text{Zn}^{++}$ ). Mulligan et al administered GH to 6 men with the acquired immunodeficiency syndrome (HIV+) for 7 days and observed nitrogen retention, decreased protein and increased lipid oxidation, and other anabolic effects. Further study will be required before the role of GH in this and other wasting diseases is determined.

The effects of GH in the aged human and experimental animal were a focus of interest. Rudman reported on extended studies in elderly hyposomatomedinemic (IGF-I <0.35 U/mL) men; after 12 months of GH administration lean body mass had increased to 106% and body fat had decreased to 84% of baseline values. However, among 61 GH-treated subjects, 10 developed carpal tunnel syndrome, 4 gynecomastia, and 3 hyperglycemia. The likelihood of an adverse event occurring

during GH administration was greater if IGF-1 concentrations exceeded 1 U/mL during therapy. Although serum concentrations of IGF-binding protein (IGFBP-3), osteocalcin, and the propeptide of type 1 collagen increased, Marcus et al noted no effect of GH administered for 12 months to healthy elderly women (not specifically selected for low IGF-1 levels) on bone mineral density, lean body mass, or fat composition (by hydrostatic weighing, although fat estimated by skin-fold thickness declined, an effect attributed to fluid retention inasmuch as it occurred within 7 days of initiation of treatment). Thus, any beneficial effects of GH administration to the elderly have yet to be demonstrated conclusively.

The development of several small peptides (6 to 7 amino acids) that stimulate the secretion of GH was discussed by Bowers et al. These peptides are effective when administered parenterally or orally. They stimulate release of GH by a cyclic AMP-independent mechanism, and thus act in a manner different than that of GHRH. Consequently, the GH-releasing peptides (GHRP) act synergistically with GHRH. Bowers hypothesized that the endogenous GHRP ligand (that has yet to be identified but may be related to the endogenous opiate family of peptides) may primarily act to amplify the effect of endogenous GHRH on GH secretion. Chihara et al reported that in short children, a 6-amino-acid GHRP-6, administered as an intravenous bolus injection stimulated GH release in a manner quantitatively similar to that of GHRH and greater than that following insulin hypoglycemia or levodopa, but was more reproducible than was GHRH. Walker reported that the combined administration of GHRP-6 and GHRH to aging female rats for 60 days lead to significantly lower pituitary, adrenal, and kidney weights, and a decreased incidence of pituitary adenomas compared to saline-treated aged control animals. Plasma concentrations of cholesterol were reduced in the GHRP-6/GHRH-treated animals. (A non-peptidyl, small molecule that also stimulates GH release when given orally was described by Schoen et al - Merck.)

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## Growth Hormone Secretion in Turner's Syndrome

Twenty-four growth hormone (GH) profiles in 26 girls with Turner syndrome were compared with those of 26 normally growing short children (18 males, 8 females) and 24 slowly growing short children (17 males, 7 females). All patients studied were prepubertal and less than 12 years of age (study 1). A randomly selected subgroup of 13 Turner girls was restudied during treatment with ethinyl estradiol 0.05 µg/kg/d. Separate samples were obtained, and GH was measured by immunoradiometric assay (IRMA).

A second trial (study 2) was done with 45 girls with Turner syndrome, aged 6.7 to 18.9 years, submitted to continuous blood sampling. A different IRMA kit was used for GH measurements. These patients were divided into 4 subgroups:

1. age less than 12 years, no treatment;
2. age more than 12 years, no treatment and no spontaneous breast development;
3. age more than 12 years, spontaneous breast development; and
4. age more than 12 years, treated with ethinyl estradiol 0.1 µg/kg/d.

Time series analysis of the results was done by Fourier transformation. In addition, the mean GH level of each profile was used for estimation of the differences between groups and for correlation with clinical situations.

In study 1, the mean 24-hour serum concentrations of the Turner girls and of the normally growing short children were both significantly higher than those of the slowly growing short children ( $P < 0.0001$ ). In the Fourier analysis, the dominant periodicity of GH secretion was similar in the 3 groups of children, but the oscillatory activity was lower in the slowly growing children, resulting in a reduced spectral power. Estrogen treatment significantly increased the pulse amplitude but did not change the periodicity.

In study 2, the estrogen-treated Turner girls had a higher mean GH than the others, but the difference was not significant. Fourier analysis did not show significant differences between the 3 subgroups of patients over 12 years of age. There was no relationship between mean 24-hour GH levels and age. Linear regression analysis did not show a relationship between the height (standard deviation scores for Turner references and for bone age) and the mean 24-hour level of GH.

Thus, the authors point out that the regulation of GH pulse amplitude and frequency is normal in girls with Turner syndrome. This clearly shows that short stature in Turner syndrome is not related to insufficient or abnormal GH secretion. The findings agree with clinical therapeutic studies, which suggest that girls with Turner syndrome are relatively resistant to GH treatment and need high doses of GH for improving their growth rate.

Wit JM, Massarano AA, Kamp GA, et al. *Acta Endocrinol* 1992;127:7-12.

**Editor's comment:** The first trials of GH treatment in Turner syndrome were largely related to the reported finding by some groups of reduced release of GH, mainly after the age of 10 to 12 years. Further clinical experience clearly showed that the results of treatment with GH in these patients were in no way related to the results of any measurement of GH secretion. This sophisticated study gives clear confirmation that short stature in Turner syndrome, at least up to adolescence, does not result from abnormal or insufficient secretion of GH. This study may be of importance for future understanding of the short- and long-term effects of GH treatment in Turner syndrome, a very peculiar model of short stature with low biologic GH sensitivity but acceptable responses to supraphysiologic GH doses.

Jean-Claude Job, MD

## Predictive Factors for the Effect of Gonadotrophin Releasing Hormone Analogue Therapy on the Height of Girls With Idiopathic Central Precocious Puberty

Brauner et al studied 14 girls with idiopathic central precocious puberty (CPP) treated with the gonadotropin-releasing hormone (GnRH) analogue Decapeptyl (a long acting preparation of D-Trp<sup>6</sup> GnRH analogue) beginning at a mean age of  $7.1 \pm 0.4$  years. The mean age at the onset of puberty was  $5.7 \pm 0.4$  years. Growth hormone secretion was evaluated by arginine-insulin infusions and was within normal limits in all patients. Bone age was evaluated by the atlas of Greulich and Pyle; target height was calculated by the method of Tanner; and final height was predicted according to Bayley-Pinneau tables. The mean target height was  $161.8 \pm 1.4$  cm, but the mean predicted height prior to the onset of therapy was  $152 \pm 1.8$  cm. GnRH therapy (3.5 mg IM every 25 days) was given for a mean duration of  $3.1 \pm 0.3$  years and stopped either at the request of the patient or when the bone age was more than 12 years. The mean follow-up after cessation of therapy was  $1.4 \pm 0.2$  years.

Estrogen activity was fully suppressed during therapy, as determined by both basal and GnRH-stimulated plasma luteinizing hormone and follicle-stimulating hormone. The mean bone age increased from  $10.6 \pm 0.2$  years to  $12 \pm 0.1$  years over a mean of  $3.1 \pm 0.3$  years. The mean predicted final height increased from  $152 \pm 1.8$  cm at the onset of therapy to  $160.4 \pm 1.3$  cm at the end of therapy and to  $162.2 \pm 1.2$  cm at a mean of  $1.4 \pm 0.2$  years

after the cessation of therapy. Thus, the mean total gain in predicted height was  $10.2 \pm 1.1$  cm. The authors demonstrated that individual gains correlated positively with bone age advance over chronologic age ( $r = 0.66$ ,  $P < 0.02$ ) and with the difference between target height and pretherapy predicted height ( $r = 0.76$ ,  $P < 0.001$ ) and negatively with the height predicted before therapy ( $r = -0.76$ ,  $P < 0.001$ ). Height gains were not correlated with either chronologic or bone age at the onset of therapy or with the duration of therapy.

Brauner R, Malandry F, Rappaport R. *Eur J Pediatr* 1992;151:728-730.

**Editor's comment:** At first glance, these data may be reassuring to the pediatric endocrinologist treating girls with idiopathic CPP with long-acting GnRH analogues. The data suggest that predicted height can be increased as much as 20.5 cm (1 patient); however, final height has not yet been achieved in this group. Thus, although it would appear that growth has continued at normal rates following cessation of therapy, the final data will not be available until growth has ceased in all of these girls. The authors state that the therapeutic effect of GnRH therapy on improving predicted height is best in those with the most accelerated bone ages at the onset of therapy, the lowest initial predicted heights, and the largest

difference between the target height and the initial predicted height. These would appear to be the girls with the most severe disease (final height most affected by their sexual precocity). However, there are some caveats that should be acknowledged. Bone age was never greater than 12 years in any of these patients until discontinuation of GnRH therapy, and bone age of 12 years was the criterion for discontinuation of therapy. Even though the mean advance of bone age and the mean bone age increase are reported, it is important to view the raw data, including the actual range of initial bone ages, to interpret the findings. Clearly a short girl with a bone age of 11 years 6 months would have had little

height benefit during this study, since therapy would have stopped at a bone age of 12 years. In addition, it would be important for the authors to calculate, using stepwise regression analysis, the contribution of each of these variables to the variance in the improvement in predicted height. Thus, the paper provides tantalizing information, but the data are insufficient to answer the question of what factors predict the effect of GnRH analogue therapy on the height of girls with idiopathic CPP. I invite comments through the Letter to the Editors column regarding my deductions.

William L. Clarke, MD

## Autoimmune Addison's Disease: Enzymes as Autoantigens

Two adrenocortical steroid biosynthetic enzymes (17 $\alpha$ -hydroxylase and 21-hydroxylase) have been recognized as autoantigens in patients with autoimmune adrenocortical insufficiency (Addison's disease). Krohn et al<sup>1</sup> identified antibodies against 17 $\alpha$ -hydroxylase (P450c17 $\alpha$ ) in the sera of children with the autosomal recessive type-1 polyendocrine autoimmune syndrome (PAS.I), which consists of at least 2 of 3 entities (hypoparathyroidism, chronic mucocutaneous candidiasis, and Addison's disease). Hypogonadism, vitiligo, alopecia, and pernicious anemia also are often present. Precipitating antibodies against human adrenal homogenates were found by immunodiffusion in 21 of 35 subjects with PAS.I. Further analysis of sera by western blotting revealed antibodies against several adrenal proteins with molecular weights ranging from 19 to 55 kd. Antibodies against the 55 kd protein were present only in the sera of patients with precipitating antibodies detected by immunodiffusion. The 55 kd autoantigen proved to be the P450c17 $\alpha$  protein, as the cDNA of this protein was 98.8% homologous with the gene for P450c17 $\alpha$ . Vector expression of this cDNA resulted in a protein recognized only by serum immunoglobulins from PAS.I subjects. The sera of 2 adult patients with only idiopathic Addison's disease did not contain antibodies against P450c17 $\alpha$ .

Winqvist et al<sup>2</sup> found antibodies against 21-hydroxylase (P450c21) in the sera of 12 of 16 adults with isolated Addison's disease, all of whose sera contained immunoglobulins reacting most strongly with the outer zona glomerulosa of the adrenal cortex by immunofluorescence. Immunoblotting techniques revealed that the autoantigen identified in the sera of these 12 patients comigrated with the P450c21 protein, but not with the proteins of the side-chain cleavage enzyme (P450scc), 11 $\beta$ -hydroxylase (P450c11 $\beta$ ), or P450c17 $\alpha$ . Preabsorption of P450c21 protein with a rabbit antiserum to this protein abolished the reactivity of the patients' sera with the adrenal antigen, whereas preabsorption with antisera to P450scc, P450c11 $\beta$ , or P450c17 $\alpha$  proteins did not. Baumann-Antczak et al<sup>3</sup> confirmed these findings.

steroidogenic enzymes may be autoantigenic. In adult subjects with isolated adrenocortical insufficiency, antibodies against P450c21 were predominant. However, in 4 of 16 patients with antiadrenocortical antibodies studied by Winqvist et al the adrenal autoantigen was not identified, again indicating the immunoheterogeneity of this disorder. The enzyme P450c17 $\alpha$  is expressed in the adrenal cortex, ovary, and testis; P450c21 is expressed only in the adrenal cortex. This suggests that antibodies to these enzymes may be of pathogenetic significance in the different patterns of the 2 diseases, therefore, possibly associated with gonadal and adrenal failure.

The reason that different steroidogenic enzymes prove antigenic in differing forms of autoimmune Addison's disease is unknown; this observation requires confirmation and investigation of the genes and the processing of their products in the 2 disorders. If the enzyme antibodies are of pathophysiologic importance in these disorders, rather than secondary manifestations of the primary abnormality, their mechanism(s) of action are unclear. The antibodies might adversely affect enzyme function, but the manner in which the antibodies enter the cell and gain access to the cytoplasmic reticulum or microsomes is not known. If these antibodies fix complement, they also might act through a complement-mediated insult.

Thus, antibodies against 3 adrenocortical steroidogenic enzymes present in the sera of subjects with autoimmune Addison's disease join the list of autoantibodies to enzymes identified in other autoimmune diseases (autoimmune thyroid disease: thyroid peroxidase; diabetes mellitus type 1: glutamic acid decarboxylase; autoimmune gastritis: H<sup>+</sup>, K<sup>+</sup> adenosine triphosphatase; autoimmune hepatitis type II: cytochrome P450db-1).

Allen W. Root, MD

1. Krohn K, Uibo R, Aavik E, et al. *Lancet* 1992;339:770-773.
2. Winqvist O, Karlsson FA, Kämpe O. *Lancet* 1992;339:1559-1562.
3. Baumann-Antczak A, Wedlock N, Bednarek J, et al. *Lancet* 1992;340:429-430. Letter.
4. Enzymes as autoantigens. *Lancet* 1992;339:779-780. Editorial.

**Editor's comment:** These reports demonstrate that immunoglobulins against steroidogenic enzymes may develop in subjects with autoimmune Addison's disease. In patients with PAS.I, the most common antibody was directed against P450c17 $\alpha$ , but Winqvist et al also identified 1 subject with PAS.I who had antibodies against P450scc, suggesting that in this syndrome,

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## The Androgen Receptor Gene in Androgen Insensitivity Syndromes

This study has used restriction fragment length polymorphism (RFLP) analysis of DNA for studying a large group of 52 patients with karyotype 46,XY and androgen insensitivity syndrome, considered as complete in 27 males having a female phenotype, and as partial in 25 males having ambiguous external genitalia. Endocrine investigation of these patients showed concomitantly high plasma testosterone and luteinizing hormone levels. The 52 patients were followed in 20 different clinics. Twenty-one were familial and 31 were isolated cases. Androgen-binding studies were performed from cultures of genital skin fibroblasts. Genomic DNA was prepared from peripheral blood leukocytes. DNA samples were studied by Southern blot analysis after digestion by 4 different restriction enzymes and hybridization with 3 cDNA probes covering the 3 domains of the androgen receptor. The informative relatives of the probands, i.e., their mothers and unaffected brothers, were studied to the extent it was possible.

Androgen-binding studies revealed that androgen receptor-binding capacity was undetectable in the 27 patients with complete androgen insensitivity. In the 25 patients considered on a clinical and hormonal basis as having partial insensitivity, receptor-binding capacity ranged from 120 to 340 fmol/mg DNA (normal mean,  $650 \pm 200$  fmol/mg DNA), with dissociation constants in the normal range of  $0.5 \pm 0.25$  nM. Thus, androgen-binding studies did not sustain the clinical and hormonal evidence of partial androgen insensitivity.

No large DNA deletion was found in any of the 52 patients. This suggests that in these studies of androgen insensitivity syndromes, abnormalities of androgen receptor could be related to point mutations or to microdeletions, rather than to gross alterations of the receptor gene.

Heterozygosity in the mother was found in 3 of 14 families studied with the HindIII polymorphism, and in 12 of 25 families using the exon 1 CAG repeat polymorphism. This suggests that HindIII and exon 1 polymorphism studies would be of considerable help in prenatal diagnosis of androgen insensitivity in male fetuses and in identification of carrier females, at least for half of affected families.

Lobaccaro JM, Belon C, Chaussain JL, et al. Molecular analysis of the androgen receptor gene in 52 patients with complete or partial androgen insensitivity syndrome: a collaborative study. *Horm Res* 1992;37:54-59.

**Editor's comment:** This genetic approach to the androgen insensitivity syndromes is a considerable work since it is based on multicenter clinical trials. It provides a complete and accurate biochemical study of androgen receptors in genital skin cells and studies of genomic DNA in white blood cells. The first and most important fact is that whatever the degree of clinical androgen insensitivity and lack of cellular androgen receptivity, no large genomic deletion has been found in the many patients studied. Thus, the microdeletions and/or point mutations responsible for androgen insensitivity are still to be elucidated. The second fact is that, even in these conditions, RFLP techniques allow for familial studies in both complete and partial androgen insensitivity syndromes, offering a reasonable chance to detect the carrier females after the study of one index case. Therefore, the possibility of prenatal diagnosis is offered. It is an important step in the complicated and difficult field of the genetics of androgen insensitivity syndromes.

Jean-Claude Job, MD

## Growth Hormone Deficiency in Down's Syndrome Children

In this study, the capacity to secrete growth hormone (GH) was investigated in 20 children with Down syndrome (DS) to determine if GH deficiency plays a role in growth retardation in DS. The subjects (13 boys, 7 girls) were aged between 15 months and 13.9 years, had a height standard deviation score (SDS) ranging from -1.19 to -5.48, a weight SDS of -0.21 to -4.58, and a head circumference SDS from -0.4 to -6.6 below the mean for normal children of the same age and sex. All but 1 severely mentally retarded child attended infant stimulation programs. All but 2 subjects had moderate to severe expressive language impairment and were institutionalized from early infancy. GH was evaluated in all 20 patients by levodopa (125 mg up to 15 kg, and 240 mg between 15 to 30 kg) and clonidine (0.15 mg m<sup>2</sup>) stimulation tests. GH secretory patterns were assessed in 4 patients via integrated 24-hour GH concentration (IC-GH) using a constant withdrawal pump with continuous blood collection every 30 minutes. Normal IC-GH values were considered to be above 3.2 ng/mL. Peak serum GH after levodopa and clonidine stimulation was found to be below 10 ng/mL for both tests in 7 of the 20 children studied. Twelve children showed a disparity between levodopa and clonidine testing. Peak serum GH after levodopa administration was found to be below 10 ng/mL in 5 children; and peak serum GH following administration of clonidine was found to be below 10 ng/mL in 7 children. Four children had reportedly abnormal 12- or 24-hour IC-GH, with mean values below 1.5 ng/mL. These 4 subjects had previously shown GH levels above 10 ng/mL in at least 1 of the

stimulation tests. Additional endocrine testing revealed no thyroid or prolactin abnormalities in any of these patients; serum luteinizing hormone, follicle-stimulating hormone, and testosterone levels were appropriate for age; and insulin-like growth factor 1 (IGF-1) levels were normal in all DS patients.

The authors conclude that the growth retardation observed in these DS children was associated with a reduced serum GH response to levodopa and clonidine stimulation tests, disparity in responses to the stimulatory tests, and low 24-hour IC-GH.

Castells S, Torrado C, Bastian W, et al. *J Intell Disabil Res* 1992;36:29-43.

**Editor's comment:** This study invites us to think that reduced GH secretion plays a role in growth retardation in DS. The authors speculate that DS children have fewer neurons and neuronal connections in the CNS, which accounts for the abnormalities in GH secretion found. However, as the authors pointed out, the discriminator of 10 ng/mL for peak serum GH responses to provocative stimuli is rather arbitrary. Decreased levels after stimulation tests (false-negatives) and disparity among test responses are well known to occur even in normal short children. In these studies, the peak response was between 7 and 10 ng/mL for levodopa in 2 patients, for clonidine in 3 patients, and for both levodopa and clonidine in another patient. The authors did not have their own control values for GH levels. In many laboratories using the same GH kit (Quintapace; Kallestad Inc., Austin, Texas) used in this study,



values between 7 to 10 ng/mL could be considered adequate and would rule out GH deficiency. Moreover, some IC-GH measurements were made with the Hybritech Tandem kit, which records lower GH levels than many other assays. Finally, all of these DS patients had normal IGF-1 levels for age. Therefore, the patients did not meet biochemical criteria for classic GH deficiency to account for growth failure. Unfortunately, only 4 of the patients who had appropriate responses to at least 1 pharmacologic stimuli had IC-GH measured, all 4 being abnormal. There were no such measurements made in the other 16 patients described in this report. Thus, no conclusion should be derived from these very few cases that the etiology of growth failure in DS patients results from inadequate spontaneous GH secretion.

A major pitfall of the study is the fact that obesity was not considered in the equation. Most of the DS patients were obese, as evidenced by the weight and height data; the majority of them were overweight for height. Since obesity is known to cause hyporesponsiveness of GH secretion to provocative stimuli and to reduce IC-GH, it is very possible that these obese DS patients could have responded normally after priming with pyridostigmine,

as has been demonstrated in obese normal children.<sup>1</sup> Elsewhere these authors reported data that showed benefits of GH therapy in DS patients.<sup>2</sup> (This information was reviewed previously in GGH.<sup>3</sup>) Caution must be exercised in obtaining incomplete data and extrapolating results of GH testing to establish the diagnosis of GH alterations in DS to justify treatment with GH. Other genetic mechanisms that relate directly or indirectly to gene abnormalities of chromosome 21 may be most important in determining height in DS patients.

Fima Lifshitz, MD

1. Cordif F, Dieguez C, Casanueva FF. Effect of central cholinergic neurotransmission enhancement by pyridostigmine on the growth hormone secretion elicited by clonidine, arginine, or hypoglycemia in normal and obese subjects. *JCEM* 1990;7(5):1361-1370.
2. Torrado C, Vastian W, Wisniewski, et al. Treatment of children with Down syndrome and growth retardation with recombinant human growth hormone. *J Pediatr* 1991;119:478-483.
3. Lifshitz F. *Growth, Genetics, and Hormones* 1991;8(1):15.

## Constitutional Delay of Growth and Adolescence: Results of Short-Term and Long-Term Treatment With GH

Bierich et al report final adult heights of children with constitutional delay of growth and adolescence (CDGA) who received exogenous growth hormone (GH) therapy and compared this data to the predicted heights prior to treatment. Thirteen boys and 2 girls were studied. Prior to treatment, nocturnal GH secretion was measured and shown to be approximately half that of a control group. In addition, the mean peak growth hormone levels following IV arginine also were half that of normal controls. All of the children were below the 3rd percentile for height, and their bone ages, according to Greulich and Pyle, were retarded by more than 2 years. Height predictions, by the method of Bayley and Pinneau, were performed at the initiation and termination of GH treatment and target height was determined according to the method of Tanner. Duration of GH therapy ranged from 2.5 to 6 years (mean, 3 years); 7 of the children were in early puberty at the start of treatment.

The mean final height of these 13 children was not different from the mean predicted height prior to GH treatment, but was significantly less than the target height. Although the authors did not include a control group for comparison, a meta-analysis of 5 studies of untreated CDGA children was performed. The mean final height of these children was 168.7 cm, while their predicted height was 170.4 cm and their target height was 172.8 cm. No statistics were provided, but it would appear that these differences were not significant. The authors concluded that children with CDGA become relatively short adults, when compared with their parents, regardless of whether they receive GH therapy. They further suggested that studies are needed in which GH therapy is continued until the epiphyses are closed. Bierich et al remind us that similar increases in height may be produced with oral oxandrolone or testosterone, if sexual development is a particular problem.

Bierich JR, Nolte K, Drews K, et al. *Acta Endocrinol* 1992;127:392-396.

**Editor's comment:** This paper confirms previous findings that children given GH will respond with an increase in growth velocity, but suggests that, at least in the group with CDGA, final height is

not affected. These findings are not unexpected, but are important in that some physicians may interpret the prepubertal growth deceleration in children with CDGA as an indication for GH therapy. This paper would suggest that this is not so. It is unclear why GH therapy was discontinued for the patients in this study, but restoration of height deficit (increased height standard deviation scores), may have been the indicator for discontinuance. It would have been interesting to have continued treatment in these individuals until epiphyseal fusion. It is unclear whether such studies are presently underway. The cost of GH therapy is not comparable to that of oxandrolone or testosterone. If similar results can be attained with these drugs, there is little justification for GH treatment of children with CDGA.

William L. Clarke, MD

**2nd Editor's comment:** Although GH treatment in delayed adolescence was once considered as useful based on good short-term results, this study could be interpreted to suggest that GH has minimal effect on final height. This paper, by a group that previously advocated GH in such situations, is to be considered both an interesting contribution to the management of growth delay as well as an example of fair self-evaluation and honesty in this field.

Jean-Claude Job, MD

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## Insulin-Like Growth Factor 1 Improves Glucose and Lipid Metabolism in Type 2 Diabetes Mellitus

Type 2 (non-insulin dependent) diabetes mellitus (DM) is associated with hyperinsulinemia and a degree of insulin resistance. In order to determine the effect of insulin-like growth factor 1 (IGF-1) in patients with type 2 DM, the investigators administered recombinant human IGF-1 (120 µg/kg body weight per dose) by twice daily SC injection for 5 days to 8 adults (2 females) with type 2 DM. During IGF-1 administration: total and free IGF-1 concentrations increased as anticipated; IGF-2 and basal growth hormone (GH) concentrations fell; fasting glucose, fructosamine, triglyceride, insulin, C-peptide, and proinsulin levels declined; postprandial concentrations of glucose, insulin, C-peptide, and proinsulin, and the insulin:glucose and proinsulin:insulin ratios fell. Basal concentrations of glucagon were not changed by IGF-1 administration. The decline in fasting concentrations of glucose, triglyceride, insulin, and C-peptide during treatment with IGF-1 correlated directly with their respective fasting control levels.

The authors suggested that IGF-1 in type 2 DM:

1. decreased glucose concentrations by interaction of free IGF-1 and/or IGF-1 bound to IGF-binding protein 1 with type 1 IGF and insulin receptors in muscle. (Free IGF-1 levels are increased by lower insulin values and, when combined with IGFBP-1, cross the vascular barrier more easily than does IGF-1 bound to IGFBP-3.);
2. suppressed insulin secretion by a direct effect on the pancreatic beta cell; and
3. improved insulin sensitivity by lowering glucose, insulin, GH, and triglyceride concentrations.

They concluded that IGF-1 may have a therapeutic role in the management of patients with type 2 DM.

Zenobi PD, Jaeggi-Groisman SE, Riesen WF, et al. *J Clin Invest* 1992;90: 2234-2241.

**Editor's comment:** This article complements others that report the beneficial effects of IGF-1 in insulin resistant states, such as type 1 (insulin-dependent) DM.<sup>1</sup> In patients with type 2 DM, IGF-1 probably lowered glucose values by increasing glucose transport into muscle, acting through IGF-1 and/or insulin receptors stimulated by free IGF-1 and IGF-1 bound to IGFBP-1 that crossed vascular barriers and then dissociated from rapidly degraded IGFBP-1. It is likely that IGF-1 may have important therapeutic potential in insulin-resistant states as may an incompletely processed form of pro-IGF-2.<sup>2</sup>

Allen W. Root

1. Schoenle EJ, Zenobi PD, Torresani T, et al. Recombinant human insulin-like growth factor-I (rhIGF-I) reduces hyperglycaemia in patients with extreme insulin resistance. *Diabetologia* 1991;34: 675-679.
2. Zapf J, Futo E, Peter M, Froesch ER. Can "big" insulin-like growth factor-II in serum of tumor patients account for the development of extrapancreatic tumor hypoglycemia? *J Clin Invest* 1992;90:2574-2584.

## Growth Hormone Deficiency During Puberty Reduces Adult Bone Mineral Density

Hyer et al measured bone mineral density (BMD) by dual energy X-ray absorptiometry in 60 adults (aged 23 to 76 years) with growth hormone deficiency (GHD, defined as a peak GH response below 5 mU/L after insulin-induced hypoglycemia). Ten of the 60 patients had GHD documented before the completion of puberty and 5 patients had received human GH (0.25 IU/kg IM 3 times a week for a mean of 6 years) until epiphyseal fusion. All patients received physiologic replacement of thyroxine, corticotropin, or sex steroids as needed. A control group of 17 subjects age-matched to these 10 patients also was studied. The larger group of GHD adults was matched to a normal reference population studied with an identical scanner. BMD was measured at the lumbar spine (L2 - L4), the femoral neck, and at Ward's triangle (a region of the proximal femur consisting predominantly of trabecular bone). The coefficient of variation for BMD measurement is 1% at the lumbar spine and 2% at the femoral neck.

The 10 subjects with GHD identified during puberty had a longer duration of GHD than the other 50 subjects, and those who were treated with GH were taller than those who were not treated. The mean BMD in the 5 untreated subjects was significantly lower than that of the controls and that of the GH-treated subjects. The 50 subjects with adult-onset GHD had mean BMDs of  $89.9 \pm 2.2\%$  (lumbar spine),  $96.1 \pm 1.1\%$  (femoral neck), and  $96.0 \pm 2.7\%$  (Ward's triangle) when compared with the reference population. A significant negative correlation was found between the duration of GHD in all subjects and BMD measured at the lumbar spine or Ward's triangle.

**Editor's comment:** These findings suggest that untreated GHD during puberty results in diminished BMD at adulthood and that there may be some reduction in BMD with GHD acquired during adulthood. However, it should be noted that the standard errors of the mean for BMD determinations in the large group of GHD adults are very close to the coefficients of variation for the BMD measurement at the 3 sites. Thus, although a significant negative correlation was shown between duration of GHD and BMD measured at 2 sites, the clinical significance of these findings is not clear.

We recently reviewed reports of diminished BMD in adult men with a history of constitutional delay of puberty and in adult men with treated GHD (GGH 1992;8(3):13). In the latter study, the adults with GHD were all diagnosed and treated prior to epiphyseal fusion. In the present study, Hyer et al reported findings in a large cohort of adults who acquired GHD as adults and show somewhat different findings. Their findings, however, lend further support to the hypotheses that the diminished BMD associated with pubertal delay is secondary to a relative GH insufficiency during early adolescence. In a recent report in the *Journal of Pediatrics* (1993;122:37-45), Saggese measured BMD in 26 children aged 6.5 to 10.7 years with isolated GHD and showed diminished BMD at the distal radius (corrected for chronologic stature and bone ages) that was significantly increased by 12 months of GH therapy. These findings suggest a need for a larger prospective study to describe the relationship between BMD and GH secretion.

Hyer SL, Rodin DA, Tobias JH, et al. *Arch Dis Child* 1992;67:1472-1474.

William L. Clarke, MD

## Congenital Idiopathic Growth Hormone Deficiency Associated With Prenatal and Early Postnatal Growth Failure

The authors identified 52 infants with presumed congenital growth hormone deficiency (CGHD) in whom therapy with GH was initiated before 2 years of age. Seven infants had septo-optic dysplasia, and the remainder had idiopathic CGHD (although imaging studies of the CNS are not reported). Delivery was normal in 67%, assisted in 10%, and by cesarean section in 23%. Two thirds of these infants were males. Mean birth length (48.3  $\pm$  2.8 cm) and mean birth weight (3.14  $\pm$  0.61 kg) were below published normal data ( $P < 0.05$ ); the mean birth length was more than 2 standard deviations (SD) below the mean normal birth length. Infants with idiopathic CGHD were relatively obese at birth. Serum GH concentrations (determined in random or stimulated specimens) were less than 5 ng/mL in 85% of infants and between 5 to 10 ng/mL in the remaining 15%. GH deficiency was isolated in 42% of infants. Postnatally, growth velocity was slow in infants with CGHD. Seventy percent of subjects measured at 6 months, and 91% of these measured again at 12 months, had reported lengths falling below 2 SD from the norm. The investigators concluded that GH deficiency may impair in utero and postnatal growth, and

that GH is an important factor for human fetal and infantile growth.

Gluckman PD, Gunn AJ, Wray A, et al. *J Pediatr* 1992;121:920-923.

**Editor's comment:** There has been uncertainty about the role of GH in fetal and early postnatal growth. However, neonates with GH insensitivity (Laron syndrome) and with isolated GH deficiency due to abnormalities of the GH gene are short at birth and have poor postnatal growth. These observations, and the current data from a large group of infants with CGHD, indicate that GH is a growth factor for the human fetus and infant, although the mechanism(s) through which it exerts these growth-promoting effects (idiopathic growth factor 1, idiopathic growth factor 2, or other growth factor) is unknown. It is also of interest that 15% of infants with CGHD had serum GH concentrations between 5 to 10 ng/mL at diagnosis; however, the nonuniformity of collection and assay of GH specimens makes interpretation of this observation less certain.

Allen W. Root

## Final Height After Growth Hormone Therapy in Peripubertal Boys With a Subnormal Integrated Concentration of Growth Hormone

The aim of this study was to evaluate the effect of growth hormone (GH) treatment on final height in boys treated at a peripubertal age with idiopathic short stature and possibly reduced GH secretion, as measured by continuous 24-hour blood sampling.

The cohort included 28 males with idiopathic short stature, aged 10 to 16 years (mean, 12.65  $\pm$  1.4 years), and a mean target height of  $-2.0 \pm 0.7$  standard deviation score (SDS). The parents of these boys were short also, with a mean height of  $-2.0$  SDS. At the time of study, the boys' mean height was  $-3.2 \pm 0.9$  SDS for age. Their mean growth velocity was  $-2.6 \pm 1.1$  SDS, ie, a mean of 3.4 cm/y, well below a norm of 4.5 cm/y. All patients were born at term, with normal birth length and weight. They were free of chronic disease or dysmorphic syndromes. Their mean bone age, evaluated according to Greulich and Pyle, was more than 2 SD below chronological age. They had a GH response of greater than 10 ng/mL (mean, 17 ng/mL) after stimulation by insulin, arginine, or clonidine. In contrast, the 24-hour integrated plasma GH level, measured by continuous pump blood withdrawal and sampling every 30 minutes, was definitely subnormal, below 3.2 ng/mL (mean, 2.25  $\pm$  0.6 ng/mL). Plasma insulin-like growth factor 1 (IGF-1) was in the low normal range.

The patients and their families were randomly offered GH therapy. Eleven (group B, treated) accepted and received GH 0.75 IU/kg/wk until final height was achieved. GH was administered in 3 weekly doses for the first 2 years and then in daily doses. Seventeen patients did not accept, and served as controls (group A, untreated).

Patients of both groups were followed every 3 months until completion of growth, which was defined by a height gain of less than 0.5 cm for 6 months and complete epiphyseal fusion on X-ray films of the hand. The clinical and hormonal characteristics of groups A and B were very similar, without any significant differences. The Bayley-Pinneau mean predicted heights were 161.8  $\pm$  5.9 cm for group A and 162.1  $\pm$  7.6 cm for group B.

Growth velocity (GV) in the treated group was significantly

greater than in the untreated group during the first 2 years of study. In contrast, during years 3, 4, and 5, GV was slightly less in treated patients than in untreated controls. Plasma IGF-1 rose in both groups. In the untreated group, IGF-1 rose from 9.5  $\pm$  11.0 to 29.4  $\pm$  15.0 nmol/L, demonstrating a rise related to puberty. In the treated group, IGF-1 increased sharply during the first 2 years, reaching mean values of 36.6  $\pm$  15.3 nmol/L and 35.4  $\pm$  8.3 nmol/L, which were significantly higher at this time than control values, but dropped to comparable levels as those of untreated boys after the end of the second year of treatment.

There was no difference between groups A and B in mean age at the onset of puberty, 13.1  $\pm$  1.8 years and 13.3  $\pm$  1.7 years, respectively. Patients receiving GH had a slightly better prepubertal height gain than controls: 8.7 cm  $\pm$  4.0 vs 5.6  $\pm$  2.0 cm (NS); however, the total height gain of treated patients during puberty was not improved: 19.2  $\pm$  4.0 cm vs 19.0 cm  $\pm$  2.2 cm in controls, and the duration puberty was slightly shorter than in controls: 3.2  $\pm$  0.7 years vs 4.0  $\pm$  0.8 years (NS).

Although mean Bayley-Pinneau predicted heights at the onset of the study was the same ( $-1.8$  SDS) in the 2 groups, the final height of the treated group was  $-1.5 \pm 0.6$  SDS vs  $-2.7 \pm 0.7$  SDS in the untreated group. This difference was significant at  $P < 0.04$ .

The authors point out that: (1) the main effect of GH was observed during the first 2 years of treatment, while most patients were prepubertal; (2) very little height gain, if any, was obtained by continuing GH therapy after the onset of puberty; (3) the patients in this study received a GH dosage in the range of classic replacement therapy; (4) GH therapy, administered for several years to peripubertal short boys with so-called neurosecretory dysfunction did not improve their final stature beyond their target height and predicted height; and (5) the mean final height of untreated boys was significantly below their target height and predicted height.

Among the authors' conclusions is that short patients with subnormal integrated concentration of plasma GH, whose GH

response to stimulation tests falls within the normal range, may improve their growth velocity when treated with substitutive doses of GH for 1 to 2 years before the onset of puberty. However, the authors also cited the high cost of the treatment, the potential disappointment if expectations are not met, and the possibility of adverse psychologic effects as factors to be considered in contrast to the incremental low height gains achieved in this specific group.

Zadik Z, Mira U, Landau H. *Horm Res* 1992;37:150-155.

**Editor's comment:** This paper deserves particular attention since it compares the final height of controls with short patients treated from late prepubertal years up to the end of pubertal development. Some degree of GH deficiency could be inferred since they had a slow prepubertal GV and endocrine investigations had shown what has been described as neurosecretory dysfunction. The natural history of the untreated subjects showed that their puberty started at the usual age, with a height below -3 SD; that they had a normal or low-normal gain of height during puberty; and that they reached

a mean final height of -2.7 SD, clearly less than their genetic target height of -1.9 SD and prepubertal predicted height of -1.8 SD. On the other hand, the patients who received GH at usual replacement doses probably gained more than 3 cm in height as compared with the untreated boys, before onset of puberty. The treated boys started puberty with a height of -2.5 SD and reached a final height of -1.5 SD, slightly better than their target height and predicted height.

A long-term study, this work affords pertinent data about a subgroup of constitutionally short boys with a poor prepubertal growth rate and so-called neurosecretory dysfunction. It suggests that GH treatment could be useful before puberty but in a very limited range, and would not be effective after the onset of sexual development. Thus, any decision to initiate GH treatment in such cases has to take into consideration the expected gain—or non-loss—of final height, the burden of treatment, including the cost, the potential present gap between the expectations of constitutionally short children and their families, and the probable long-term outcome of therapy.

Jean-Claude Job, MD

## Wilms' Tumor and Insulin-Like Growth Factor 2

Wilms' tumor is a pediatric malignancy thought to arise when multipotential kidney blastemal cells fail to differentiate after birth and instead continue to proliferate. The occurrence of both sporadic and hereditary forms of Wilms' tumor and the early age of onset of bilateral kidney tumors suggest that Wilms' tumors result when a predisposing germ-line mutation is accompanied by a second mutation or loss of heterozygosity at the disease locus. A potential tumor suppressor gene, *wt1*, was cloned in 1990 by analyzing deletions at chromosomal locus 11p13. These are associated with Wilms' tumors. The *wt1* gene encodes a zinc finger DNA binding protein, called WT1, and this has been found to behave as a transcriptional repressor. The biologic significance of DNA binding and transcriptional regulation by WT1 is underscored by the observation that small deletions and point mutations in the WT1 Zn<sup>2+</sup>-fingers that abolish this DNA binding have been detected in a number of Wilms' tumors, especially in tumors associated with the Denys-Drash syndrome.

The fetal mitogen insulin-like growth factor 2 (IGF-2) is overexpressed in Wilms' tumor. In addition, the overgrowth disorder Beckwith-Wiedemann syndrome, which is characterized by loss of the maternal copy of the IGF-2 gene, also is prone to Wilms' tumors. For these reasons, Drummond et al have examined the interaction between the suppressor protein WT1 and the IGF-2 gene. They found that WT1 binds to multiple sites in the promoter

region of the IGF-2 gene, and that it acts as a potent repressor of IGF-2 transcription *in vivo*. Thus, a molecular basis for the overexpression of IGF-2 in Wilms' tumor has been identified, and these experiments suggest that the *wt1* gene negatively regulates blastemal cell proliferation by limiting the production of a fetal growth factor in the developing vertebrate kidney.

Drummond JA, Madden SL, Rohwer-Nutter P, et al. Repression of the insulin-like growth factor II gene by the Wilms' tumor suppressor WT1. *Science* 1992;257:674-678.

**Editor's comment:** This paper provides further insight into the question of specific molecular mechanisms of growth control, ie, what prevents all cells in all tissues of the body from proliferating indefinitely? In addition, there are many indications that both Wilms' tumor and Beckwith-Wiedemann syndrome are imprinted disorders, ie, they will develop when inherited from a parent of the same sex but will not when inherited from a parent of the opposite sex. The IGF-2 gene has been shown to be imprinted in mice such that only the paternally inherited gene is expressed. Thus, these experiments provide further insight into mechanisms of genomic imprinting as well as overgrowth in cancer.

Judith G. Hall, MD

## New Genes May Shed Light on Cell Growth Control

Two independent lines of work in molecular biology have now begun to converge: research on the cancer-causing oncogenes and research on the signaling pathways that carry messages telling cells to start—or stop—dividing. Cell biologists have found that the pathways that transmit growth signals into the cell contain the proteins made by several known oncogenes. However, the question has been what are the intermediaries between the growth factor receptors and the oncogenes that, when activated, signal the cell to divide and to keep dividing until the oncogene signal is turned off?

One of the most common oncogene proteins involved in this type of signaling is *ras*, which acts as a relay point to the nucleus for all

the growth factor receptors examined so far. When the *ras* protein is locked in a permanent "on" position by a mutation, various types of cancer may result. Researchers have begun to understand how *ras* works (Reddy BV, Khanna SN, Jena P. *Science* 1992;258:1640). They have found that *ras* is turned on only when it has bound to the nucleotide GTP (guanosine triphosphate). It is turned off again via reaction with another protein, called GAP (GTPase-activating protein), that stimulates the breakdown of GTP into GDP (guanosine diphosphate), which in turn inactivates *ras* and remains tightly bound to it.

It would be disastrous, however, if this inactivated form of *ras*



could not be reversibly reactivated. Thus, the need for exchange proteins has been postulated — proteins that would remove the GDP and free up the ras protein so that it could bind new, activating GTP molecules. Such "ras exchangers" have been known for some time in yeast and in the fruit fly, *Drosophila*. Recently, several groups have identified and cloned genes for exchange proteins in mammals. These have been shown to be highly specific, stimulating the release of GDP from ras, but not from 2 other members of the ras superfamily that have different functions and presumably their own exchangers. There is some evidence, however, that the ras exchangers also may serve as a link to exchangers for other oncogene proteins, such as rho.

Marx J. *Science* 1992;257:484-485.

**Editor's comment:** Researchers are gradually beginning to unravel the nature and function of the genes that are essential to

normal cell growth but that, when improperly regulated, lead to devastating disorders such as cancer and neurofibromatosis. The discovery of intermediate proteins in the signaling pathway between growth factor receptors, on the cell membrane, and growth effector proteins such as ras, provides a mechanism for the delicately balanced regulation of cell division and quiescence. In addition, the fact that these intermediary exchanger proteins may serve as links to yet other signaling pathways allows us to begin to see how the incredibly intricate cascade and feedback pathways of the cell work on molecular level. And, as ras scientist Frank McCormick of Onyx Pharmaceuticals observes in Marx's article, this new information "has all sorts of therapeutic possibilities." Drugs that inhibit ras activation might be used, for example, to treat diseases such as cancer and neurofibromatosis, in which growth stimulatory pathways are excessively active.

Judith G. Hall, MD

## Relationship Between Urinary and Serum Growth Hormone and Pubertal Status

This study of correlations involved 31 prepubertal and 29 pubertal subjects. Three different groups were studied: (1) 21 patients, aged 6.9 to 18.2 years (7 prepubertal, 14 pubertal) who had received cranial irradiation of 18 to 24 Gy for acute lymphoblastic leukemia; (2) 18 subjects aged 3.8 to 18.9 years, among whom 10 were normal siblings of the irradiated patients and 8 were normal subjects with genetic short stature (10 prepubertal and 8 pubertal); and (3) 12 boys investigated once or twice for constitutional delay of growth and puberty (CDGP) for a total of 21 studies, among which 14 had 4- to 6-mL testes and 7 had testes with a volume of 8 to 12 mL.

Growth hormone (GH) secretion was evaluated as a 24-hour profile, with sampling every 20 minutes in groups 1 and 2, and as an overnight 12-hour profile in group 3. Urine was collected concurrently with blood sampling. Serum GH was assayed by the immunoradiometric assay (IRMA) technique. Urine concentration of GH was measured by a 2-step IRMA on dialyzed urine, with a sensitivity of 0.8 pg/mL and interassay coefficients of variation of 6.6% to 8.4%. The results were expressed as nanograms of GH per gram of creatinine. Renal function was checked and considered normal in all study subjects, although some of those with a history of leukemia had at times received short courses of 1 or 2 aminoglycosides.

The results in prepubertal children ( $n=17$ ) showed a close correlation between mean serum GH (integrated concentration) and urinary GH:  $r=0.88$  in group 1,  $0.84$  in group 2, and  $0.82$  in group 1+2 with  $P<0.001$ . There also were significant correlations in prepubertal children between nanograms of urinary GH per grams of creatinine and both the maximal peak ( $r=0.86$ ) and the mean pulse amplitude ( $r=0.71$ ) of the serum GH profile.

In the pubertal children of groups 1 and 2, considered together ( $n=22$ ) or separately, there was no such relationship:  $r=0.26$  (NS) for the mean GH;  $r=0.29$  (NS) for the peak; and  $r=0.34$  (NS) for the mean amplitude of GH peaks on the profile.

In the early pubertal boys (stage 2) investigated for CDGP, the correlation was highly significant between urinary GH and mean serum GH ( $r=0.74$ ,  $P<0.001$ ), but less significant with the mean amplitude of pulses ( $r=0.4$ ,  $P<0.05$ ) and not significant with the peak serum GH value.

The authors point out that although GH excreted in urine represents less than 0.002% of cumulative serum GH, the correlations found are very close in prepubertal children and rather good

at early puberty. This is in contrast with the lack of correlation in late pubertal subjects. They conclude that measuring urinary GH may be a test for screening GH secretion in children, but it is inappropriate from mid to late puberty. They also stress that the impact of physiologic and pathologic changes of renal function upon filtration and excretion of GH by kidneys needs further investigation before considering urinary GH measurements as a reliable tool.

Crowne EC, Wallace WHB, Shalet SM, et al. *Arch Dis Child* 1992;67:91-95.

**Editor's comment:** There is some contrast between the good correlations between urinary and serum GH found by the authors up to early puberty and their rather negative conclusions. Their discussion is extensive, including many previous studies on urinary GH, done with more or less similar methodologies, and possibly this is the main reason for concluding in this sense. Whatever the reasons for this contrast, my opinion is that after more than 5 years of extensive work and a great number of clinical studies, measurement of GH in urine has never been proven to be a reliable means for appreciating somatotrophic secretion in clinical situations or for longitudinal studies in physiology of growth.

Jean-Claude Job, MD

**2nd Editor's comment:** The answer to the question, "Can measurement of urinary GH be used to diagnose growth hormone deficiency?" remains elusive after 30 years of investigation. In 1963, Geller and Loh (*J Clin Endocrinol Metab* 1963;23:1107) first attempted to do this, as have many others. Confirmation that GH deficiency can be diagnosed by the reported techniques has been elusive. Interpretation of the data in the abstract above is similarly guarded. Fortunately, Dr. Margaret MacGillivray, who has had a long-term interest in this question, will be writing an article for GGH after reviewing the literature and conferring with the investigators currently working in this field. We look forward to her review and summary. Hopefully, Dr. MacGillivray will give us a broad perspective and a definitive answer to our question.

Robert M. Blizzard, MD



## MEETINGS CALENDAR

**September 6-8, 1993** Frontiers of Paed Neuroendocrinol, London, Eng. Info: Dr MO Savage. Tel: 44-71-601-8487; Fax: 44-71-601-8505.

**September 12-15, 1993** Ann Mtg of the Eur Soc for Paed Research (ESPR), Edinburgh, Scot. Sci Info: Prof N McIntosh. Tel: 44-31-667-2617; Fax: 44-31-668-2605. Genl Info: ESPR '93, Edinburgh Conf Ctr. Tel: 44-31-449-5111; Fax: 44-31-451-3199.

**September 23-25, 1993** Natl Mtg of the Italian Soc of Paed Endo and Diabetol, Bari, Italy. Info: Dr L Cavallo. Fax: 39-80-536-4450.

**September 30-October 2, 1993** Intl Symp on Developmental Endocrinol, Geneva, Switzerland. Info: Profs PC Sizonenko/M Aubert. Tel: 41-22-3824-592; Fax: 41-22-3824-588.

**October 28-31, 1993** Somatotrophic Axis & the Reproductive Process in Health and Disease, Baltimore, MD. Info: Dr BK Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

**November 7-11, 1993** Wkshp on the Superfamily of Receptors for Growth Hormone, Prolactin, Erythropoietin & Cytokines, Haifa, Israel. Info: Dr Z Hochberg. Tel: 972-3-635-5038; Fax: 972-3-635-1103.\*

**November 7-11, 1993** Molecular Basis of Endo Diseases, Barcelona, Spain. Info: Dr C Pavia. Tel: 34-3-2804-000; Fax: 34-3-2033-959.\*

**November 12-17, 1993** 45th Postgrad Assembly of the Amer Endo Soc, San Francisco, CA. Info: CHuck. Tel: 301-571-1802; Fax: 301-571-1869.

**December 9-12, 1993** GHRH, GH, IGF-1: Basic and Clinical Advances, San Diego, CA. Info: Dr BK Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

**February 6-10, 1994** 3rd Intl Symp on Insulin-Like Growth Factors, Sydney, Australia. Sci Info: Dr R Baxter. Tel: 61-2-516-6111; Fax: 61-2-516-1273. Genl Info: E Loveridge. Tel: 61-2-956-8333; Fax: 61-2-956-5154.

**June, 1994** 7th Intl Congress of Auxology, Budapest, Hungary.\*

**June 1-4, 1994** 1st Intl Mtg of the Growth Hormone Research Soc, Aarhus, Denmark. Info: Dr JS Christiansen/JOL Jorgensen. Tel: 45-86-1255-55/Ext 2084; Fax: 45-86-1378-25.\*

**June 15-18, 1994** 76th Ann Mtg of the Amer Endo Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1802; Fax: 301-571-1869.

**June 22-25, 1994** 33rd Ann Mtg of the ESPE, Maastricht, The Netherlands. Info: Congrex Holland. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

**June 30-July 3, 1994** 2nd Intl Conf on Prader-Willi Syndrome, Cambridge, Eng. Info: Dr BM Laurence.\*

**October 29-November 3, 1994** 46th Postgrad Assembly of the Amer Endo Soc, Toronto, Canada. Info: C Huck. Tel: 301-571-1802; Fax: 301-571-1869.

**November 6-11, 1994** 15th World Cong of the IDF, Kobe, Japan. Sci Info: Prof S Baba. Tel: 81-78-303-0055; Fax: 81-78-302-7303.

\*Confirmations not received upon publication.

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# GROWTH

## Genetics & Hormones

Vol. 9 No. 3

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### Clinical Significance of Urinary Growth Hormone Measurements

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In the past decade, renewed interest in the measurement of urinary growth hormone (GH) output has occurred. Reports of suboptimal spontaneous GH production in short children whose GH stimulation

tests were normal prompted this interest.<sup>1,2</sup> These children were classified as having GH deficiency because of defective neuroregulation of GH secretion. Assessment of spontaneous GH production requires either serial blood sampling at 20-minute intervals for 12 to 24 hours or constant withdrawal of blood via an indwelling catheter.<sup>1-3</sup> The former approach gives information about GH pulse frequency and amplitude, as well as mean and pool GH concentrations. The latter also yields an integrated GH level but provides no information about GH pulses. Both procedures are uncomfortable, labor intensive, and inappropriate for small children. Recently, the diagnostic validity of these tests has been challenged because mean or pooled GH concentrations of healthy children matched for age and pubertal stage overlap with concentrations observed in short children. Since timed urine collections integrate GH output, it was reasoned that measurement of urinary GH excretion would reflect endogenous GH production and provide a safe alternative method to screen short children for GH deficiency.

#### ASSESSMENT METHODOLOGY

Since 1985, the assays that have been used to re-evaluate the usefulness of urinary GH measurements include either improved radioimmunoassay (RIA) techniques; newly developed, ultrasensitive enzyme

**Table 1: Urinary GH Output in Normal Subjects**

| Study                 | Assay                       | Sensitivity of Assay | Age (y) | Growth Hormone/Creatinine (ng/g) |
|-----------------------|-----------------------------|----------------------|---------|----------------------------------|
| Albini <sup>16</sup>  | RIA after 50x concentration | 0.15 ng/mL           | 7-15    | Mean 35.3 ± 2.6                  |
| Granada <sup>19</sup> | RIA after concentration     | 4 pg/mL              | 5-12    | Mean 33 ± 22                     |
|                       |                             |                      | 11-16   | Mean 48 ± 27                     |
|                       |                             |                      | Adult   | Mean 9 ± 4.4                     |
| Hashida <sup>23</sup> | ELISA                       | 1.2 pg/mL            | <2      | Range 78 to 113                  |
|                       |                             |                      | 2-5     | Range 19 to 51                   |
|                       |                             |                      | 6-15    | Range 7.8 to 25                  |
|                       |                             |                      | 28-35   | Range 1.1 to 5.2                 |
| Tanaka <sup>21</sup>  | ELISA                       | 3 pg/mL              | 2-16    | Mean 13 ± 11.2                   |
| Porquet <sup>10</sup> | IRMA                        | 0.4 pg/mL            | 3-10    | Mean 10.7 ± 0.2                  |

RIA = radioimmunoassay

ELISA = enzyme-linked immunosorbent assay

IRMA = immunoradiometric assay

#### In This Issue

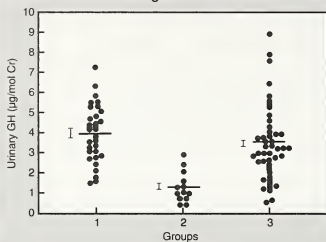
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Figure 1



Urinary GH output ( $\mu\text{g/mol creatinine}$ ) in healthy subjects (1); GH-deficient subjects (2); and children with idiopathic growth failure (3).<sup>16</sup>

$$\frac{\mu\text{g}}{113} \times 1000 = \text{ng/g creatinine}^{16}$$

linked immunosorbent assays (ELISA); or immunoradiometric assays (IRMA).<sup>4-10</sup> This article summarizes the information obtained from this research, and evaluates the utilization of urinary GH measurements for clinical and investigative purposes.

The authenticity of urinary GH has been confirmed by high-performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis, sephadex gel filtration, and double-monoclonal IRMA.<sup>5,10-12</sup> The major form of GH in urine is a 22 kd peptide; the presence of a 20 kd form has been identified only in extensively concentrated urine.<sup>11</sup> Unfortunately, less than 0.001% of the GH secreted by the pituitary is excreted intact in urine. The remainder is degraded by the liver, kidneys, and peripheral tissues. Renal function has a profound effect on urinary GH levels. In healthy individuals, the fraction of GH in urine is the end product of glomerular filtration, subsequent reabsorption, and catabolism within tubular epithelial cells.

Prior to 1970, attempts to quantitate GH output in urine failed because of insensitive assays. In addition, the presence of interfering substances caused widely discrepant results and overestimations of GH excretion. In 1972, Hanssen improved the specificity and sensitivity of double-antibody GH RIA by first dialyzing and then concentrating the urine fifty-fold.<sup>4</sup> These improvements minimized the problem of interfering substances, but the sensitivity was still limited to 0.15 to 0.3 ng/mL (150 to 300 pg/mL). Since 1985, significant gains have been made in developing ultrasensitive ELISA or IRMA assays that measure urine GH levels as low as 0.4 to 4 pg/mL. By using antibody-coated polystyrene beads to concentrate GH in urine, it is now possible

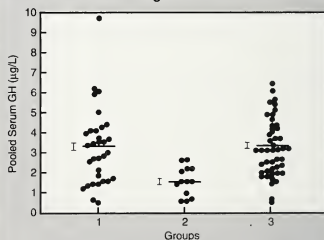
to omit prior dialysis and concentration steps for most samples. The recent development of a sensitive double-monoclonal IRMA has further increased the specificity of urine GH measurements.<sup>10</sup>

Disagreement exists as to the best method of standardizing urinary GH output in children of varying ages and sizes. The methods used include assessment of timed urine collections (ng/12 h or ng/24 h) based on chronologic age; weight (kg); surface area ( $\text{M}^2$ ), and creatinine excretion.<sup>5,14-16</sup> Since the amount of GH present in 24-hour urine collections from healthy subjects is extremely small, standardization on the basis of age, weight, or surface area results in considerable overlap of values between groups. The data are normalized most frequently on the basis of creatinine excretion. However, this approach may result in significant error because creatinine is dependent on lean body mass, which changes with age and clinical state.<sup>17</sup> For example, the reduced lean body mass of hypopituitary children or elderly individuals is reflected in lower excretion of urinary creatinine, which falsely elevates estimates of urinary GH based on creatinine.<sup>16-18</sup> Conversely, puberty is associated with an increase in lean body mass and creatinine excretion, which leads to blunting of the pubertal rise in urinary GH. Due to these uncertainties, some investigators have reported the absolute outputs of urinary GH in nanograms per time interval without standardizing for weight or creatinine.<sup>15-19</sup>

## URINARY AND PLASMA GH CONCENTRATIONS

Urinary GH excretion correlates positively with plasma GH concentrations in most studies (mean or pooled GH levels and peak GH concentrations after growth

Figure 2

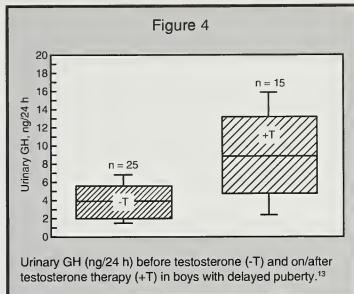
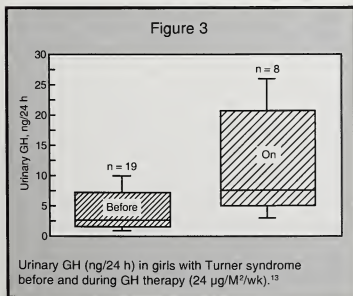


Pooled serum GH concentrations in healthy controls (1); GH-deficient subjects (2); and children with idiopathic growth failure (3).<sup>16</sup>

hormone-releasing hormone [GHRH] or stimulation tests). Also, the GH content of first morning urine specimens may correlate positively with 12- or 24-hour urine collections.<sup>14,16,20-22</sup> A positive correlation was seen in prepubertal and early pubertal children when plasma and urinary GH levels were computed throughout puberty, as reported by Crowne et al.<sup>22</sup> However, this correlation was not seen in subjects with advanced puberty. Although most investigators report a close relationship between urinary GH output and plasma GH levels, measurement of urinary GH excretion cannot be used to calculate pituitary GH production because the amount of intact hormone in urine is such a tiny fraction of the quantity produced. Therefore, small differences in excretion of urinary GH may produce large differences in calculation of the secretion of pituitary GH. Furthermore, widespread metabolism of GH in the body, coupled with renal processing of GH, undermines the validity of estimated GH production.

Girard<sup>13</sup> recently reported that urinary GH output did not correlate with the dose of GH given to hypopituitary children, supporting evidence that the hormone undergoes extensive changes prior to appearance in urine.

In healthy children and adolescents, the mean output of urinary GH varies between 7.8 and 48 ng/g of creatinine (Table 1, page 1). Higher estimates of urine GH excretion were reported in studies utilizing RIA techniques than in those using ELISA or IRMA methods. The lack of agreement may be due to differences in the antibodies used or to matrix factors in the RIA method, since higher concentrations of urine were required. Alternatively, it is possible that omission of the concentration step prior to assaying by the ELISA or IRMA methods may have resulted in an inability to accurately measure the minute quantities of GH in dilute urines.<sup>7,9,15,16,19,21,23</sup>



## DISCUSSION

Many investigators have concluded that measurement of urinary GH output is not a valid screening test for GH deficiency in children who are failing to grow but who have normal GH responses to standard stimulation tests.<sup>16,21</sup> Studies demonstrate that the overlap of urinary GH values between normal children and short subjects is similar to that observed for pooled serum GH levels in these populations (Figures 1 and 2). Consequently, neither urinary GH measurements nor serial serum GH studies appear to give reliable diagnostic information about the status of short "GH sufficient" children.<sup>16,24,25</sup>

Urinary GH output will identify children with severe GH deficiency, as well as subjects who have GH excess.<sup>5,7</sup> In the former group, standard GH stimulation tests are still needed for confirmation of GH deficiency. In acromegalic subjects, urinary GH output and plasma GH levels appear to yield similar diagnostic information at baseline and following therapeutic intervention. Therefore, urinary GH measurements may be of little additional clinical value in acromegaly.

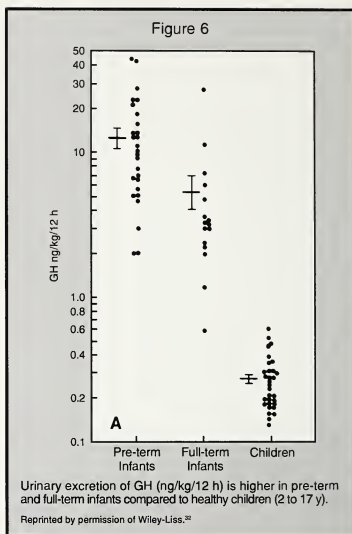
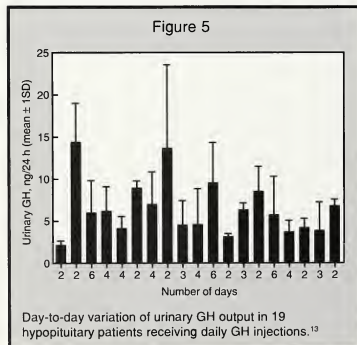
Urinary GH measurements also have been informative in studying physiology. Estimates of urinary GH output have been used to monitor the effects of treatment with GH or testosterone.<sup>13</sup> Not surprisingly, hypopituitary children treated with GH have increased outputs of GH compared to the low values observed in the baseline periods. Also of note, girls with Turner syndrome who received higher than standard doses of GH exhibit greater outputs of urinary GH than treated hypopituitary children (Figure 3). Following testosterone treatment (50 to 100 mg/mo), boys with delayed adolescence excrete at least twice as much GH in urine as compared to pretreatment values (Figure 4). The latter observation confirms



that androgen-stimulated GH secretion is reflected in urine. The output of urinary GH in testosterone-treated boys has been shown to be similar to the amount observed in girls with Turner syndrome who received higher doses of GH. Urinary GH measurements have also been used to monitor the effects of dose and frequency of GH injections in GH-deficient children. However, considerable day-to-day variability has been observed in hypopituitary subjects receiving daily GH therapy (Figure 5).<sup>13,26</sup>

Urinary GH measurements have increased our knowledge about the relationship between the output of insulin-like growth factor 1 (IGF-1) and GH in urine. These levels are positively correlated. Hypopituitary children treated with GH show a prompt rise in both urinary IGF-1 and GH excretion. This observation confirms that renal excretion of IGF-1 is GH-dependent.<sup>27</sup>

Measurement of urinary GH output has provided qualitative information about the relative output of GH from infancy through adulthood (Figure 6 and Figure 7). The data suggest that more GH is excreted in urine during infancy than in childhood or adulthood.<sup>23,28,29</sup> A similar pattern of IGF-1 excretion also is observed in these age groups. During early childhood, the positive relationship evidenced between GH and IGF-1 in urine differs from the relationship seen in plasma, since plasma IGF-1 levels are low during this period of rapid somatic growth. Opinions differ as to the interpretation of urinary GH data in pubertal children. When the data are standardized for weight or creatinine, the output of urinary GH does not increase in puberty.<sup>5,19</sup> A pubertal rise in urinary GH output is seen only when the data are not standardized (ie, expressed as nanograms per 12 or 24 hours).<sup>15,19</sup>



Abnormalities of glomerular or tubular function interfere with the reliability of GH measurements in urine. Urinary GH correlates negatively with creatinine clearance and positively with  $\beta_2$ -microglobulin and albumin excretion.<sup>30,31</sup> Thus, urinary estimates of GH excretion are inaccurate in children with renal insufficiency or diabetic nephropathy.

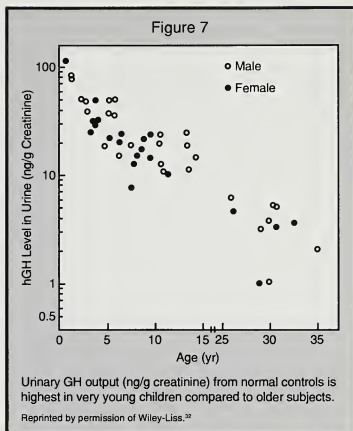
## CONCLUSIONS

Measurement of urinary GH excretion is not a valid screening test for GH deficiency in short children who have normal GH stimulation tests. This method will identify the child with severe GH deficiency and the child with GH excess, but confirmation by serum GH measurements is still needed. Furthermore, assessment of urinary GH provides no information about GH pulsatility and cannot be used in patients with abnormal renal function. Since urinary testing is noninvasive, repetitive measurements of urinary GH can be used to gather information about relative outputs of GH over time in small children and to assess changes in urinary GH excretion before, during, and after therapeutic interventions aimed at increasing or decreasing GH production. Also, measurements of urinary GH and IGF-1 suggest

that a closer relationship exists for these peptides in urine than in plasma for all ages. To date, however, there is insufficient evidence in the literature to prompt this author to recommend measurement of GH in urine except as a possible research technique. Even in the latter instance, caution in interpretation of the data is mandatory, because only trends in production — rather than production rates — can be evaluated.

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## Contiguous Gene Syndromes

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In 1986, Schmickel<sup>1</sup> proposed the term contiguous gene syndromes for a group of disorders characterized by microdeletions or microduplications of chromosomal segments associated with clusters of single gene disorders. These disorders were recognized clinically prior to their cytogenetic localization, distinguishing them from the classic group of deletions or duplications recognized cytogenetically prior to, or concomitantly with, their clinical delineation (eg, Wolf-Hirschhorn syndrome, cri du chat syndrome, 18p deletion, and 18q deletion). Regardless, these classic chromosomal deletion or duplication syndromes may yet be revealed as contiguous gene

syndromes once the size and extent of the critical region on the chromosome (as determined by molecular analysis) has been correlated with the clinical phenotype.

Schmickel proposed 2 types of contiguous gene syndromes — those with and those without visible cytogenetic abnormalities.<sup>1</sup> Both types of cytogenetic abnormalities have been described for the same disorder, so that in fact, there is probably a phenotypic spectrum for each specific disorder relating to the size and location of the deletion. Schmickel described 7 autosomal and 1 X-linked contiguous gene syndromes. Since his paper, most of the contiguous gene syndromes described have involved microdeletions. This may be due to the fact that the phenotypic effects resulting from microdeletions are more obvious clinically than those associated with microduplications. As outlined by Ledbetter and Cavenee<sup>2</sup> and by Schinzel,<sup>3</sup> microdeletion and microduplication syndromes have several features in common (Table 1, page 6).

The identification and delineation of contiguous gene syndromes has been evolving. The ability to study these conditions is dependent on 3 components:

- (1) The identification and clinical evaluation of patients with suspected contiguous gene syndromes. Most patients with these syndromes display varying degrees of mental retardation; thus, one might suspect a contiguous gene syndrome in a mentally retarded individual with one — or multiple — mendelian traits that are not usually associated with mental retardation. Chromosome analyses should be performed to search for cytogenetic abnormalities that may lead to further delineation of the disorder.
- (2) Access to high-quality cytogenetics. The newly recognized contiguous gene syndromes were initially detected by routine metaphase chromosome analyses, which identified obvious structural chromosome abnormalities (eg, translocations, ring chromosomes). High-resolution analyses of over 500 bands have then been used to characterize the critical chromosome regions of a specific syndrome.<sup>2</sup> More recently, the isolation of molecular probes from these critical regions coupled with the use of fluorescent *in situ* hybridization (FISH) techniques, has facilitated the detection of deletions along chromosomes that previously appeared normal via high-resolution analyses, in disorders such as Prader-Willi/Angelman syndrome, Miller-Dieker syndrome, and DiGeorge syndrome (DGS).<sup>4-7</sup> FISH is an important adjunct for studying disorders mapped to telomeric regions of chromosomes that have been associated with subtle or cryptic translocations inherited from one parent<sup>6,8</sup> (eg, Miller-Dieker syndrome, Wolf-Hirschhorn syndrome, and cri du chat syndrome). Cytogenetic studies of both parents using FISH may be beneficial for determining the risk for recurrence in subsequent offspring and as a means of prenatal diagnosis in families where inheritance of such a disorder has already occurred. Based on the higher sensitivity and specificity of FISH, this technology may displace high-resolution chromosome analyses in the diagnosis of many of the contiguous gene syndromes.
- (3) The mapping of critical chromosome regions can be further enhanced by employing standard molecular techniques, including Southern blot analysis and polymerase chain reaction.<sup>2</sup>

## CLASSIC MICRODELETION SYNDROMES

**Langer-Giedion syndrome (LGS)** was first described in 1969.<sup>9</sup> The clinical features of LGS include sparse scalp hair, bulbous or pear-shaped nose, cone-shaped phalangeal epiphyses, and multiple cartilaginous exostoses. The majority of

patients are mentally retarded, although there is quite a range in the degree of retardation such that some patients have been reported to have normal intelligence.<sup>10</sup> Prior to the distinction of LGS a similar disorder, **trichorhinophalangeal (TRP) syndrome**, was described by Giedion in 1966.<sup>11</sup> Patients with TRP are clinically similar to those with LGS (also known as TRP2) except for the absence of multiple cartilaginous exostoses and mental retardation. Beginning in 1980, several patients with LGS were described with *de novo* deletions of 8q24.1. Subsequent studies have identified deletions of 8q24.1 in about 50% of patients.<sup>12</sup> It has been proposed that the critical regions coding for TRP1 and multiple exostoses (ME; a condition that is also an isolated autosomal dominant disorder) are located in close proximity along this region of 8q.<sup>13</sup> Although it now appears that both TRP1 and LGS (or TRP2) are mapped to this region, linkage studies of families with ME have not demonstrated linkage to chromosome 8 in all families.<sup>13</sup> Based upon these findings, ME may be a heterogeneous condition localized in the critical region of 8q24.

The association of **aniridia and Wilms tumor** was first described in 1964.<sup>14</sup> Subsequently in 1978, a deletion of chromosome band 11p13 was identified in patients with a complex of abnormalities,

Table 1  
**Features of Microdeletion/  
Microduplication Syndromes**

1. The syndromes were recognized prior to knowing the cytogenetic etiology, although occasionally chromosomal abnormalities have been reported.
2. Usually these syndromes are sporadic, but occasionally they are familial or dominant.
3. Cytogenetic abnormalities are usually detectable by high-resolution chromosome analysis. Studies using fluorescent *in situ* hybridization (FISH) may become more practical.
4. Not all patients have visible cytogenetic abnormalities, although the frequency may increase with the use of FISH. However, some patients may have submicroscopic molecular deletions.
5. Specific features of the syndrome may occur as single mendelian traits.
6. Multiple, unrelated loci that are physically contiguous in the critical region are thought to be involved. The patient's phenotype frequently correlates with the deletion or duplication of these contiguous genes.

Adapted from Ledbetter and Cavenee<sup>2</sup> and Schinzel.<sup>3</sup>



**Figure 1: Critical Chromosome Regions**

The critical chromosome regions for the syndromes presented herein are designated on the ideogram. The critical regions for these disorders are designated on the left side of the chromosome for deletions and on the right side for duplications as follows:

| Region |   |
|--------|---|
| AHD    | Arteriohepatic dysplasia (Alagille syndrome) (del 20p11.23p12.2)      |
| ATMR   | Hemoglobin H/ $\alpha$ -thalassemia-mental retardation (del 16p13.3)  |
| BWS    | Beckwith-Wiedemann syndrome (dup 11p15)                               |
| CDCR   | Chorioideremia, deafness, clefting, retardation (del Xq21)            |
| CES    | Cat-eye syndrome (dup 22q11)  |
| CMT1A  | Charcot-Marie-Tooth disease, type 1A (dup 17p11.2p12)                 |
| DGS    | DiGeorge/Velocardiofacial syndrome (del 22q11)                        |
| DMD    | Duchenne's muscular dystrophy and contiguous genes (del Xp21)         |
| GCPS   | Greig cephalopolysyndactyly (del 7p13)                                |
| HOLO   | Holoprosencephaly (one form) (del 7q34)                               |
| KAL    | Kallmann syndrome and contiguous genes (del Xp22.3)                   |
| MDS    | Miller-Dieker syndrome (del 17p13)                                    |
| PWS/AS | Prader-Willi/Angelman syndrome (del 15q12)                            |
| RB     | Retinoblastoma (del 13q14.11)   |
| RTS    | Rubinstein-Taybi syndrome (del 16p13.3)                               |
| SMS    | Smith-Magenis syndrome (del 17p11.2)                                  |
| TRP    | Trichorhinophalangeal/Langer-Giedion syndrome (del 8q24.1)            |
| WAGR   | Wilms tumor, aniridia, genital abnormalities, retardation (del 11p13) |

including Wilms tumor, aniridia, genital abnormalities, and mental retardation (WAGR).<sup>15</sup> A number of genes have now been mapped to this region, including catalase and the beta subunit of follicle-stimulating hormone (FSH $\beta$ ), in addition to the Wilms tumor gene (WT1) and aniridia (AN2).<sup>16</sup>

**Drash syndrome**, comprised of the triad of nephropathy, Wilms tumor, and genital abnormalities, may, at least in some cases, also be associated with the WAGR microdeletion syndrome.<sup>17</sup> In addition, the Wilms tumor appears to be associated with acquired homozygosity of WT1 and preferential nonrandom loss of the maternal allele, suggesting an imprinting effect.<sup>18</sup> Lastly, WT1 has also been implicated in abnormalities of renal development, suggesting that the known spectrum of this microdeletion syndrome may be expanded with future research.<sup>19</sup>

In many respects, the association of **retinoblastoma (RB)** and **deletion of chromosome 13q14.11** was one of the first microdeletion syndromes to be described and one of the first genes to be cloned.<sup>20,21</sup> In addition to RB, children with visible deletions may have mental retardation and facial dysmorphism, including broad nasal bridge, upturned nares, elongated philtrum, and thin upper lip.<sup>22</sup> The tumor appears to arise by reduction to homozygosity of the RB locus.<sup>21</sup>

**Prader-Willi syndrome (PWS)**, first described in 1956, consists of neonatal hypotonia, feeding difficulty, and genital hypoplasia. Hyperphagia and obesity develop during the first 1 to 2 years of life.<sup>23</sup> Other characteristics associated with PWS include distinctive facial features, short stature, small hands and feet, and hypopigmentation. In 1981, Ledbetter

et al<sup>24</sup> described a small interstitial deletion of chromosome 15 between bands q11 and q13 found in about half of PWS patients. In 1987, another disorder, **Angelman syndrome (AS)**, was also discovered to have a similar deletion of the same region of chromosome 15.<sup>25</sup> Angelman syndrome is characterized by microcephaly, macrosomia with prominent tongue, hypotonia, ataxic gait, excessive laughter, seizures, and severe mental retardation; some patients also have hypopigmentation.<sup>26</sup> PWS and AS have now been shown to be the prototypes of genomic imprinting in humans.<sup>27</sup> The majority of PWS individuals have deletions of paternal origin. Thirty percent of PWS patients with no deletion have been found to have maternal disomy of chromosome 15, usually heterodisomy, with no paternal chromosome 15 contribution. This may arise by maternal nondisjunction, resulting in a trisomy 15 conceptus with loss of paternal chromosome 15. In addition, a small nuclear ribonucleoprotein (snRNP) was identified as a maternally imprinted candidate gene for PWS.<sup>28</sup> Conversely, AS has been found to be associated with maternally derived deletions of chromosome 15 in over half of the cases. However, only 3% to 5% of cases have been shown to have paternal disomy of chromosome 15, primarily isodisomy.<sup>29</sup> The remaining AS patients have biparental inheritance, with a copy of chromosome 15 from each parent and no cytogenetic or molecular deletion.<sup>29</sup> Several families with multiple affected individuals have now been described with AS but without cytogenetic deletions; in each case the same chromosome 15 has been maternally inherited, suggesting paternally imprinted mutations of the hypothetical



AS gene.<sup>30</sup> Recently, the mouse pink-eyed deletion gene has been mapped to the PWS/AS critical region. This deletion gene may be involved in the hypopigmentation seen in both disorders as well as oculocutaneous albinism type II.<sup>30</sup>

**Miller-Dieker syndrome** is a rare malformation syndrome consisting of type I lissencephaly and dysmorphic facies, resulting from a deletion of chromosome 17p13.3, initially described in 1983.<sup>32</sup> Visible deletions of 17p13.3 have been identified in about 50% of patients.<sup>33</sup> In patients without visible deletions, large molecular deletions have been detected. The use of FISH has enhanced detection of chromosome 17 deletions associated with Miller-Dieker syndrome.<sup>6</sup> In addition, probes isolated from the Miller-Dieker critical region have identified molecular deletions of 17p13.3 in a small percentage of patients with isolated lissencephaly.<sup>34</sup>

**DiGeorge syndrome (DGS)**, first described by DiGeorge in 1965, is characterized by a group of defects resulting from abnormalities in the development of the third and fourth branchial arches, producing thymic hypoplasia, parathyroid hypoplasia, and conotruncal cardiac defects, in addition to facial dysmorphism.<sup>35</sup> The majority of DGS patients initially come to a physician's attention due to congenital heart defects; a small percentage initially present with manifestations of hypocalcemia.<sup>35</sup> Although the pathogenesis of DGS appears to be fairly consistent, (ie, maldevelopment of the branchial arches), the etiology is heterogeneous.<sup>36</sup> Most cases of DGS are sporadic, although familial cases have been known. Prior to the use of molecular techniques, about 15% of cases were shown to have cytogenetic abnormalities, primarily deletions of 22q11<sup>37</sup>; however, other chromosome abnormalities, including deletion of 22q11, also have been described.<sup>37</sup> In addition, DGS has been associated with teratogenic

exposure, including alcohol, isotretinoin, and maternal diabetes.<sup>36</sup> It has also been observed in conjunction with other genetic disorders, including **velocardiofacial syndrome (VCFS)**, **Zellweger syndrome**, and **Kallmann syndrome**.<sup>36</sup> Recent studies have confirmed the presence of microdeletions of chromosome 22q11 in most patients with DGS. Cytogenetically visible deletions are apparent in as many as 30% of DGS cases, and molecular deletions are detectable in 90% of cases.<sup>38,39</sup>

**Velocardiofacial syndrome (VCFS, or Sprintzen syndrome)** is characterized by palatal defects, including cleft palate; hypoplastic alae nasi with a long nose; learning disorders or mental retardation; and congenital heart disease, primarily conotruncal defects. It is inherited in an autosomal dominant fashion<sup>40</sup> with as many as 90% of VCFS patients having visible deletions of 22q11. In about 90% of these patients, the 22q11 deletions have been shown by FISH or molecular analysis.<sup>41,42</sup> Lastly, although some patients with CHARGE association (ventricular septal defect, vertebral anomalies, anal atresia, tracheoesophageal fistula, radial and renal anomalies) also have DGS-type anomalies, it appears that only a small percentage of CHARGE patients have deletions of chromosome 22 demonstrable by cytogenetic or molecular techniques.<sup>41</sup>

## RECENTLY DESCRIBED MICRODELETION AND MICRODUPLICATION SYNDROMES

### Microdeletion Syndromes

As of 1986, one contiguous gene deletion syndrome on the X chromosome had been described. This involved **deletion of Xp21**, which included the genes for Duchenne's muscular dystrophy, chronic granulomatous disease, the McLeod phenotype, retinitis pigmentosa, glycerol kinase deficiency, adrenal hypoplasia, and ornithine transcarbamoylase deficiency.<sup>43</sup> More recently, 2 new contiguous gene deletion syndromes of the X chromosome have been described. The first — **a deletion of Xp22.3** — is characterized by X-linked ichthyosis with steroid sulfatase deficiency, Kallmann syndrome, chondrodysplasia punctata, mental retardation, short stature, ocular albinism, and may be expanded to include Aicardi syndrome and Goltz syndrome.<sup>44</sup> The second is a **deletion of Xq21** that is associated with choroideremia, deafness, cleft lip and palate, and mental retardation.<sup>45</sup>

A number of new contiguous gene/microdeletion syndromes have been described since Schmickel's paper was published in 1986. **Greig cephalopolysyndactyly syndrome** (comprised of craniosynostosis associated with polysyndactyly and occasionally with mental retardation) was found to be

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associated with translocations or deletions of chromosome 7p13, suggesting that this may be a contiguous gene deletion syndrome.<sup>46</sup>

Muencke and colleagues recently have shown association of holoprosencephaly and associated features in patients with different sized deletions of chromosome 7q34.<sup>47</sup> The features appear to vary with the size and extent of the deletion.

The association of  $\alpha$ -thalassemia with mental retardation has been linked to deletions and subtle cryptic translocations involving chromosome 16p13.3.<sup>48</sup> In a group of 8 patients, 4 were found to have unbalanced chromosome translocations and the remainder had deletions of 16p13.3 as detected by high-resolution cytogenetic or molecular analysis, including FISH.<sup>49</sup> However, there is also an X-linked form of this disorder.<sup>50</sup>

**Rubinstein-Taybi syndrome (RTS)** is phenotypically characterized by dysmorphic facial features, including a beaked nose, prominent columella, hypoplastic maxilla, and down-slanted palpebral fissures; broad thumbs and first toes; and varying degrees of mental retardation.<sup>51</sup> After previous association of RTS with chromosomal abnormalities involving 16p13, submicroscopic deletions were identified.<sup>52</sup> In 6 of 24 patients with normal appearing chromosomes, a submicroscopic deletion was detected by FISH.<sup>52</sup> Since at least 1 gene for tuberous sclerosis and adult polycystic kidney disease gene are also located in this region, it will be of interest to determine whether these disorders may also be part of a contiguous gene deletion syndrome with RTS.

**Smith-Magenis syndrome (SMS)**, with deletion of chromosome 17p11.2, was initially described in 1982.<sup>53</sup> The disorder consists of brachycephaly, midface hypoplasia, broad nasal bridge, prominent jaw, short broad hands, speech delayed, varying degrees of mental retardation and behavioral abnormalities, including onychotillomania (compulsive picking at the nails) and polyembolokoilomania (insertion of foreign bodies into orifices).<sup>54</sup> The disorder may be relatively common; more than 100 patients have been described over a relatively short period. Approximately two thirds of patients have evidence of a peripheral neuropathy associated with normal nerve conduction velocities.<sup>54</sup> Although the gene for **Charcot-Marie-Tooth disease type 1A (CMT1A)** is usually associated with a duplication within 17p11.2p12,<sup>55</sup> the CMT1A region does not appear to be deleted in the majority of SMS patients, including those with peripheral neuropathy.<sup>56</sup> Thus, the etiology of peripheral neuropathy in SMS patients is still unclear. In addition, about two thirds of patients with SMS have sleep disorders; 2 patients have been shown to have absence of REM sleep on

sleep study.<sup>54</sup> A number of other patients have demonstrated reduced amounts of REM sleep. Thus, it has been hypothesized that a gene involving REM sleep may be localized to this region. Though in some ways, SMS fits the criteria for a microdeletion syndrome, additional work is needed before it can truly be called a contiguous gene deletion syndrome. While the majority of patients have visible deletions of chromosome 17 revealed by high-resolution cytogenetics, studies of several patients with clinical features of SMS and whose chromosomes appear normal are currently ongoing to detect submicroscopic deletions of this region.

**Arteriohepatic dysplasia (AHD), or Alagille syndrome**, is an autosomal dominant disorder associated with chronic cholestasis and paucity of interlobular bile ducts, dysmorphic facies, posterior embryotoxon, butterfly-like vertebral arch defects, and peripheral pulmonary stenosis or hypoplasia.<sup>57</sup> A recent report by Schnittger et al<sup>58</sup> showed 9 patients with visible deletions of chromosome 20p11.23p12.2.

## Microduplication Syndromes

**Beckwith-Wiedemann syndrome (BWS)** consists of macrosomia, macroglossia, omphalocele, hypoglycemia due to pancreatic islet cell hyperplasia, transverse earlobe creases, hemihypertrophy, and advanced bone age.<sup>59</sup> Individuals with BWS have an increased risk of malignancy, particularly Wilms' tumor, adrenocortical carcinoma, and hepatoblastoma. A small percentage of patients with BWS have been observed to have duplications of chromosome 11p15.<sup>60</sup> However, the majority of BWS patients do not have a visible chromosome 11 abnormality.<sup>59</sup> More recent studies have suggested that parental imprinting may play a significant role in BWS. It appears that the duplication 11p15 in BWS patients is primarily of paternal origin; a few of the patients without cytogenetic duplications have paternal uniparental disomy for markers in the 11p15 region.<sup>61</sup> In addition, there are several cases of maternal inheritance of BWS with an inversion or translocation of chromosome 11 in band p15.5. Thus, in the strict sense, BWS is not a true microduplication syndrome, at least in most cases.

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**Duplications of chromosome 17p11.2p12** have been described.<sup>62</sup> The clinical features of patients with this disorder have been variable, although the majority have hypotonia, decreased reflexes, and club foot. With the finding of duplication of a small region within 17p11.2p12 in association with CMT1A,<sup>55</sup> a number of patients with cytogenetic duplications of this region were studied to determine whether nerve conduction findings consistent with CMT1A would be present. Patients who have duplication for PMP22, the candidate gene for CMT1A,<sup>63</sup> also have findings consistent with CMT1A, while those patients without this PMP22 duplication do not have features of CMT1A.<sup>62</sup> These duplications have been visible without high-resolution cytogenetics. At least one patient with the duplication of 17p11.2p12 and CMT1A also has absence of REM sleep, lending support to the idea that a gene involved with sleep regulation is also located within this chromosomal region.

Another possible contiguous gene duplication syndrome is the **cat-eye syndrome**, which is due to duplication of chromosome 22q and consists of coloboma of the iris and anal atresia.<sup>64</sup> In addition, many of these patients have ear abnormalities and cardiac defects. Analysis of the duplicated region in these patients is currently being studied by McDermid and colleagues.<sup>65</sup>

## FUTURE ISSUES

Advances in molecular genetics and cytogenetics, and the subsequent increase in the number of single gene disorders mapped to specific chromosomal regions, will undoubtedly lead to the delineation of additional contiguous gene syndromes. Several syndromes (ie, de Lange syndrome<sup>66</sup>), are currently under investigation, and others that have been recently mapped may lead to the identification of contiguous gene syndromes located within the same critical region.

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# Results of the Diabetes Control and Complications Trials

Reviewed by William L. Clarke, MD

The Annual Scientific Session of the American Diabetes Association was held in Las Vegas, June 12 through 15, 1993. The highlight of this meeting was the report from the Diabetes Control and Complications Trial (DCCT), presented to the largest gathering (more than 6,000 individuals) ever to be assembled in the 53-year history of the American Diabetes Association. The DCCT is a randomized prospective study of the hypothesis that intensive treatment of insulin-dependent diabetes that reduces hyperglycemia will delay the onset and/or reduce the progression of microvascular complications. More than 1,400 subjects aged 13 to 39 years participated in the trial. There were 2 distinct cohorts: (1) a primary prevention cohort comprised of individuals with diabetes of 1 to 5 years' duration and with no background retinopathy; and (2) a secondary intervention cohort comprised of individuals with diabetes of 1 to 15 years' duration and with retinopathy of minimal to moderate degree. Eighteen percent of the primary cohort were adolescents, while 10% of the secondary cohort were adolescents.

Subjects were randomized in each group to an intensive treatment or conventional treatment group. The goal in the conventional treatment groups was to prevent symptoms of hypoglycemia or hyperglycemia. They were treated with 1 to 2 daily insulin injections, daily self-monitoring, quarterly glycosylated hemoglobin determinations, diet and exercise education, and quarterly physician visits. Glycosylated hemoglobin values were not reported to the physician or patient unless they exceeded 2 standard deviations (SD) above the mean for this treatment group. Self blood glucose monitoring was not used for daily treatment management. The goal for the intensive treatment groups was to have pre-meal blood glucose values between 70 and 120 mg/dL; post-meal glucose levels <180 mg/dL; 3:00 AM blood glucose levels >65 mg/dL; and glycosylated hemoglobin levels at the upper limit of the normal range. These groups were treated with 3 or more daily insulin injections or with continuous subcutaneous insulin infusion pumps. Blood glucose was measured 4 or more times per day, and abnormal values prompted frequent changes in insulin, diet, and exercise. Monthly clinic visits were required.

Ninety-nine percent of individuals from both cohorts completed the entire study. Retinal and renal

data were collected on more than 98% of patients and neurologic evaluations were available for more than 97%. Although the intensive treatment groups did not achieve the goal of maintaining glycosylated hemoglobin values at the upper range of normal, there was always a consistent 1% to 2% difference between their mean levels and those of the conventional treatment groups. Adolescents consistently had glycosylated hemoglobin values approximately 1% above the average for nonadolescents. Capillary blood glucose levels were measured quarterly before and 90 minutes after each meal, and averaged across subjects. Mean blood glucose averaged 155 mg/dL in the intensive treatment groups and 231 mg/dL in the conventionally treated groups.

Retinal evaluations included stereoscopic photographs of the fundi, which were evaluated by blinded readers. Retinal findings were similar in both groups in the primary intervention cohort (background retinopathy at time zero) for the first 3 years; however, by the end of the study, a 76% reduction in retinopathy was seen in the intensive treatment group compared with the conventional treatment group. In the secondary intervention cohort (background retinopathy at time zero), progression of retinopathy in the intensive treatment group was reduced by 34% compared with the conventional treatment group. All subgroups, including adolescents, benefited from intensive insulin therapy.

Nephropathy was evaluated by albumin excretion. Microalbuminuria (albumin excretion rate [AER] between 40 and 300 mg/24 h) was reduced by 38% in the combined intensive treatment group as compared to the conventional treatment group. Clinical albuminuria (AER  $\geq$ 300 mg/24 h) was reduced by 56% and clinical nephropathy (AER  $\geq$ 300 mg/24 h

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and creatinine clearance  $<70$  mL/min) was reduced by 60%. Neuropathy, measured by heart rate variation to deep breathing and postural changes and nerve conduction studies, was reduced by 60% in the intensive treatment group. There were no differences between the 2 groups with regards to the development of hypertension or elevated triglyceride levels, but low-density lipoprotein cholesterol was significantly lower in the intensive treatment groups. No differences were observed in neurobehavioral or quality-of-life measures between the intensive and conventional treatment groups. Intensive therapy of insulin-dependent diabetes did not reduce quality of life or adversely affect neurobehavioral or psychological parameters.

The complications of intensive therapy included: weight gain (an average 10 lbs more than in the conventional treatment groups); catheter infection; and severe hypoglycemia, for which the incidence was 3 times greater in the intensive treatment groups.

The investigators stated that the benefit ratio could be less favorable in patients with recurrent severe hypoglycemia or hypoglycemic unawareness; those with far advanced complications such as renal failure; patients with coronary artery or cerebral vascular

disease; or children less than 13 years of age (who were not included in the study).

The conclusion of this study is that the majority of insulin-dependent diabetic patients should be treated with intensive therapy with the expectation that the long-term outcome will be measurably improved. The study was not designed to determine the level of glucose control at which the prevention of complications could be maximized while minimizing the risk of severe hypoglycemia. However, the data clearly support the concept that any reduction in glucose level has potential benefits. The debate regarding the role of glucose control and the development of microvascular complications of insulin-dependent diabetes mellitus has been concluded.

#### Erratum

In **GROWTH, Genetics, & Hormones** Vol. 9, No. 2 (June 1993) an error on page 13 identifies growth-promoting mechanisms in Dr. Root's editorial comment as *idiopathic*-like growth factor 1 and 2. The correct description should have been *insulin*-like growth factor 1 and 2.

#### Abstracts From the Literature

### Effects of Changes in Nutritional Conditions on Growth and on Timing of Puberty

Longitudinal studies of growth in different countries provide an index of the nutritional and hygienic status of their populations. Secular trends in growth and its relationship with socioeconomic levels are given by J.M. Tanner in a retrospective analysis.<sup>1</sup> Whatever the country, the era, and the general conditions of study, similar data were obtained, with increased height change observed between successive generations within specific ethnic societies and among socioeconomic strata within each ethnic group.

The preschool years, between 18 and 24 months and 3 and 4 years, seem the age period when different trends between contemporary classes, between generations, and between children of the developing and industrialized countries mainly appear. This is the period when the legs are growing faster relative to the trunk, and when larger trends for leg length than for sitting height are found. A current hypothesis includes not only nutrition but morbidity as a factor in the young to account for the social/secular trends. For example, growth patterns relate to the degree of catch-up growth occurring following successive episodes of infection, during which growth has slowed down. Restoration of the genetically endowed growth potential requires a considerable temporary increase of energy and protein intake that is not available for all and, when lacking, decreases the achievement of the genetic potential for height.

This is a different mechanism and timing from that which establishes the largely genetic difference between short and tall adults when all grow up in optimal circumstances. What controls this normal genetic difference – which is quite different from

the social class/secular trend mechanism – is unclear. It may be related to individual genetic differences relating to the various components of the growth hormone – IGF-1 growth axis.

Nutritional conditions are important also for the timing of puberty. This is strongly suggested by follow-up studies of adopted children relocated from poor areas to developed countries. J.P. Bourguignon and colleagues<sup>2</sup> observed that precocious puberty occurred in 8 of 32 children adopted in Belgium. They developed an experimental model to this phenomenon utilizing male rats, studying both hypothalamic maturation of the gonadotropic control, and testicular content of elongated spermatids. When compared to those of a small litter, pups from a large litter showed a reduced growth rate before weaning, and then a similar growth rate after weaning at 21 days, followed by earlier hypothalamic and testicular maturation at age 35 days. Refeeding at different times and reduction of litter size, which changes the feeding levels before weaning, showed that food-restricted pups when refeed resumed a normal growth rate and had an accelerated hypothalamic and testicular maturation advanced for their body size. This was observed only when refeeding had occurred before the age of weaning. This suggests that hypothalamic maturation of the gonadotropic control is sensitive to nutritional conditions during a limited critical period before the onset of puberty.

1. Tanner JM. *Horm Res.* 1992;38(suppl 1):106-115.

2. Bourguignon JP, Gerard A, Alvarez Gonzalez ML, et al. *Horm Res.* 1992;38(suppl 1):97-105.

**Editor's comment:** Though the relationship between nutrition and growth has been often documented and long debated, it is still not completely clear. Many data support the idea that optimal nutrition improves growth in infancy and early childhood, and, consequently, stature growth is greater in adults. The analysis by Tanner, based on several reported studies, leaves no doubt regarding this historical fact, evident from his own studies. He now adds the hypothesis of the associated role of hygienic conditions, and suggests the interrelation of morbidity and dietary events in the preschool age as a main factor for the secular trends in growth and also for the social differences in these trends. He presents a stimulating way to analyze new longitudinal data and better understand the population studies.

However, the time of onset of puberty and the height reached when sexual development starts are variable among individuals. This is important in medicine. The data reported by Bourguignon and colleagues stress the influence of appropriate early

nutrition, but with consequences different from those shown by population studies. Thus, nutrition may affect final height through different mechanisms. An interesting perspective is gained by presenting these 2 completely different studies in the same abstract.

Jean-Claude Job, MD

**2nd Editor's comment:** The Editorial Board of GGH has urged that we present a lead article on secular growth. This we will do in good time, but the recently published article by Dr. Tanner, and reviewed here by Dr. Job, is currently available and is a superb analysis of the quantitative and qualitative environmental factors affecting growth. Those interested in this topic now are urged to read Dr. Tanner's extensive and thorough presentation.

Robert M. Blizzard, MD

## Factors Predictive of Sustained Growth in Children After Renal Transplantation

This paper reports growth data on renal transplant recipients collected from the North American Pediatric Renal Transplant Cooperative Study. Participating centers record data at the time of transplant and at 6-month intervals thereafter. Data include height, weight, serum creatinine, type and amount of immunosuppressants, and graft survival. Height data are reported as Z scores and catch-up growth is defined as a gain of 1 standard deviation (SD) or more. The first 300 of 1,553 patients with a functioning graft for at least 2 years were entered into this study: 64% were males, 24% had never been dialyzed, 55% had received a living-related donor kidney, and 15% had undergone retransplantation.

Baseline mean Z score was  $-2.41 \pm 0.09$  and the mean change in Z score at year 2 was  $0.18 \pm 0.06$  ( $P < 0.01$ ). Data were also analyzed for different age groups. Those <1 year of age at diagnosis had the lowest initial Z score and the greatest improvement (approximately 1 SD). The 2- to 5-year-old group had a change in Z score of 0.5 SD, while those from 6 to 12 years had an increase of  $0.1 \pm 0.7$  and those from 13 to 18 years had a decrease ( $-0.21 \pm 0.08$ ) (Table 1). Catch-up growth occurred in 50% of those <1 year of age at transplantation, in 25% of the 2 to 5 year olds, in 16% of the 6 to 12 year olds, and in 6% of the 13 to 18 year olds. No differences were found in baseline Z scores for males or females, for those with or without prior dialysis, or in recipients of a living-related donor or cadaver kidney. As expected, those with a previous transplant were significantly shorter than those receiving an initial transplant, and children with aplastic or hypoplastic kidneys or with obstructive nephropathy had a greater initial height deficit. A 1.0 mg/dL increase in serum creatinine was associated with a 0.15 decrease in Z score following transplantation, but similar analysis using prednisone dose per kilogram of body weight did not demonstrate a significant relationship. The 112 patients who had a rejection episode in the first month posttransplantation had no significant Z score change at 2 years.

**Editor's comment:** This paper demonstrates improvement in height Z scores posttransplantation in a large group of young children whose end-stage renal disease was caused by a variety of disorders, but not in those above 5 years of age. Despite catch-up growth, height Z scores did not normalize in any group. The younger children, who were also those with the greatest initial baseline height deficit, were those who sustained the greatest height gain increments posttransplantation. This response is similar to that seen in growth hormone-deficient children in response to exogenous growth hormone. Unfortunately, this group of children is the group for whom posttransplantation mortality is the greatest. The authors note that most children older than 6 years of age at transplant did not show catch-up growth. Thus, as pointed out by the authors, the findings suggest that other treatment for growth failure is needed and that a controlled trial of recombinant growth hormone in children with end-stage renal disease posttransplantation may be warranted.

William L. Clarke, MD

Table 1  
Relationship of Age at Transplantation to  
Subsequent Growth

| Age (y) | n   | Baseline<br>Z Score<br>(mean $\pm$ SEM) | Change in<br>Z Score*<br>(mean change $\pm$ SEM) |
|---------|-----|---|--|
| 0-1     | 22  | $3.04 \pm 0.31$                         | $0.92 \pm 0.31$                                  |
| 2-5     | 64  | $2.10 \pm 0.16$                         | $0.54 \pm 0.12$                                  |
| 6-12    | 137 | $2.34 \pm 0.14$                         | $0.11 \pm 0.07$                                  |
| 13-18   | 77  | $2.21 \pm 0.21$                         | $0.21 \pm 0.08$                                  |
| Total   | 300 | $2.41 \pm 0.09$                         | $0.18 \pm 0.06$                                  |

n = number of subjects.

\*At 2 years after baseline.

## Putative X-Linked Adrenoleukodystrophy Gene Shares Unexpected Homology With ABC Transporters

Adrenoleukodystrophy (ALD) is an X-linked disorder that leads to central nervous system demyelination and adrenal insufficiency. It can result in death within a few years, although the phenotype varies widely even within a family. The main biochemical abnormality found in this disorder is the accumulation of saturated very-long-chain fatty acids (VLCFA) due to impaired  $\beta$ -oxidation in peroxisomes.

ALD was the subject of the recent film, "Lorenzo's Oil." In this docudrama, a couple whose 6-year-old son, Lorenzo, has been stricken with the disease circumvent the medical establishment in an attempt to find a cure. The oil referred to in the title consists of erucic acid and oleic acid, which returned the raised plasma concentrations of saturated VLCFAs to normal. Unfortunately, this treatment (touted by the film as a cure for ALD) has since been shown in a 5-year controlled study to be ineffective for many ALD patients whose condition continues to deteriorate. Also of importance is that other patients who have not received the treatment remain stable for many years. Thus, it is likely that Lorenzo's condition remained stable due to chance and not to "cure" from the oil.

A gene that is thought to cause the ALD defect has been cloned recently by Patrick Aubourg's group in Paris. This gene was cloned utilizing a positional cloning approach, in which a gene is mapped to particular chromosomal region using restriction fragment length polymorphisms (RFLPs) and candidate genes in that region are then identified and used as probes for genes containing mutations in the affected families.

While it was originally thought that VLCF-CoA synthetase was the most likely candidate for the ALD gene, the gene identified by Aubourg's group bears no sequence resemblance to this or 3 other enzyme genes involved in peroxisomal  $\beta$ -oxidation. Instead, it is highly homologous with a peroxisomal membrane protein that is involved in peroxisome biogenesis and that belongs to a family of membrane proteins known as the adenosine triphosphate-binding cassette transporters. This family includes the multidrug-resistant gene product, the cystic fibrosis

transmembrane conductance regulator, and genes that map in the human major histocompatibility complex region. The members of this gene family are involved in transport of proteins, amino acids, inorganic ions, and peptides in both prokaryotes and eukaryotes. Thus, the sequence of this newly identified gene suggests that ALD may be caused by defective transport of VLCFA-CoA synthetase into the peroxisomal membrane, rather than by a deficiency in the VLCFA-CoA synthetase enzyme itself.

Mosser J, Douar A-M, Sarde C-O, et al. *Nature*. 1993;361:726-730.

Moser, HW. *Lancet*. 1993;341:544.

**Editor's comment:** It is unfortunate that a popular film, "Lorenzo's Oil," would present as fact a hypothesis that has not withstood the test of a controlled trial, and would seek to further widen the rift that exists today between the general public and the medical and scientific establishment. The fallacies of treatment and flagrant misinformation in this film are duly recorded under the title "Pernicious Treatment," by Fred S. Rosen in *Nature* 1993;361:695, and in a film review by Dr. Hugo Moser in *Lancet* 1993;341:544. Both of these outstanding scientists are appalled at the filmmaker's lack of responsibility in researching the truth and to patients with ALD, to physicians who are treating them, and to the United Leukodystrophy Foundation.

However, the discovery of a gene that may represent the causal factor in ALD is very exciting, and brings with it the hope that in the near future, families afflicted with this tragic illness will have access to an understanding of why the disease happens and new therapies that may be effective in all cases. Prenatal diagnosis should be possible, and gene therapy is certainly a possibility as clinical trials are underway for gene therapy in a number of other disorders involving the central nervous system.

Judith G. Hall, MD

## Pregnancy After Age 50: Application of Oocyte Donation to Women After Natural Menopause

A recent study by Sauer et al has shown that women as old as 59 years retain the ability to bear children if hormone replacement therapy and in vitro fertilization of oocytes from younger donors are provided. The researchers were able to establish pregnancies in 9 out of 14 women, aged 50 to 59 years. Three of these women had delivered at the time of publication; 4 were progressing normally beyond the second trimester; and 2 of the 9 pregnancies had obstetric complications involving preterm labor, preeclampsia, growth retardation, and/or gestational diabetes. Of the 7 pregnant or delivered women, 4 had never previously conceived.

As might be expected, however, there did seem to be a "male factor" in the study, as the partners who donated the sperm were also over age 50. A low overall fertilization rate was noted, with most of the nonfertilized eggs occurring among the 5 couples who were unable to conceive.

The authors conclude that there is little doubt that the uterus remains receptive to embryo implantation and can sustain normal pregnancy well beyond the limits of natural reproduction. They thus conclude that it is the aging of the ovaries and

oocytes, and not the uterus, that is responsible for most adverse fertility events associated with aging. Although there have been no serious complications, the authors do indicate that there are not enough data at this time to presume that the incidence of adverse results will not increase in women over 50. The psychologic consequences of giving birth after age 50 are also discussed in the paper, and the point is made that many children are raised by grandparents in various cultures.

Sauer MV, Paulson RJ, Lobo RA. *Lancet*. 1993;341:321-323.

**Editor's comment:** This study demonstrates that women well beyond natural menopause may still achieve implantation of transferred embryos and carry these pregnancies to term. As the authors note, the average life expectancy and quality of life in our society are increasing, but physiology has limited women in their 50s and beyond. This new technology allows women the same range of choices that men have always enjoyed: the chance to concentrate on a career without worrying as much

about the "biological clock," or the chance to have children with a second partner, should a woman be divorced or widowed.

On the negative side, however, there are costs that must be considered as well. *In vitro* fertilization technology is extremely expensive. In addition, the authors note that extensive medical and psychologic screening should be conducted with women of advanced age who are considering pregnancy; the screening

process is also very expensive. At a time when we are realizing that we must somehow curtail skyrocketing medical costs — even to the point where rationing of health care is being considered — such expensive elective procedures must be carefully evaluated and debated.

Judith G. Hall, MD

## Short-Term Growth Hormone Treatment Does Not Increase Muscle Protein Synthesis in Experienced Weight Lifters

Yarasheski et al studied whether recombinant human growth hormone (GH) administration enhances muscle protein anabolism in experienced weight lifters. The fractional rate of skeletal muscle protein synthesis and the whole body rate of protein breakdown were determined using a constant intravenous infusion of  $C^{13}$  leucine in 7 young adult males who were experienced weight lifters. The studies were performed at the beginning and at the end of 14 days of subcutaneous GH administration at  $40 \mu\text{g/kg/d}$ , which is the dosage used often in treatment of GH-deficient patients ( $0.3 \text{ mg/kg/d}$ ). GH administration increased fasting serum insulin-like growth factor 1 (IGF-1) levels (Figure 1), but did not increase the fractional rate of muscle protein synthesis or reduce the rate of whole body protein breakdown from  $103 \pm 4$  to  $108 \pm 5 \text{ mol/kg/h}$ . The authors state that the findings suggest that short-term GH treatment does not increase the rate of muscle protein synthesis or reduce the rate of whole body protein breakdown, metabolic alterations that would promote muscle protein anabolism in experienced weight lifters attempting to further increase muscle mass.

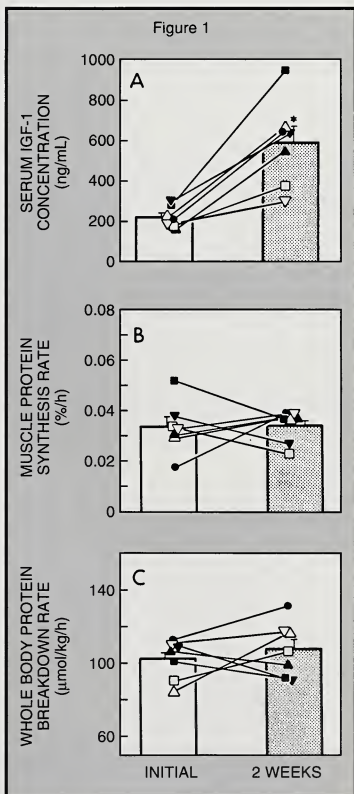
Yarasheski KE, Zachwieja JJ, Angelopoulos TJ, et al. *J Appl Physiol*. 1993;74:3073-3075.

**Editor's comment:** These authors previously reported that recombinant human GH at  $40 \mu\text{g/kg}$  given 5 d/wk to healthy sedentary young men in conjunction with a 12-week muscle-building exercise program produced increments in muscle protein synthesis rate and muscle strength comparable to those achieved by sedentary young men doing an identical muscle-building exercise program but receiving placebo injections. However, the earlier study did not exclude the possibility that GH administration might augment muscle protein synthesis during the early phase of treatment, since muscle protein synthesis was determined only before and after 3 months of GH treatment.

The previous study (Am J Physiol. 1992;25:E261-E262, abstracted previously in GGH. 1992;8[1]14) did not consider the possibility that GH administration might enhance muscle protein synthesis in experienced weight lifters or bodybuilders who had already achieved a large muscle mass using heavy resistance exercise training or that GH administration might further increase muscle mass by supplementing with another potential anabolic stimulus. This study is important because skilled weight lifters and bodybuilders represent the most likely abusers of GH for muscle anabolism.

As is characteristic of these authors, the studies were done in an exquisite manner. The data speak for themselves. GH in such patients is not of value in increasing muscle mass. Pass the word along to the athletes who wish to spend astronomical sums of money in the hope that they will increase their competence.

Robert M. Blizzard, MD





## MEETINGS CALENDAR

**October 5-9, 1993** 43rd Ann Mtg of ASHG, New Orleans, LA. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

**October 28-31, 1993** Somatotrophic Axis & the Reproductive Process in Health & Disease, Baltimore, MD. Info: Dr B Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

**November 7-11, 1993** Wkshp on the Superfamily for Receptors of GH, Prolactin, Erythropoietin & Cytokines, Haifa, Israel. Info: M Zur. Tel: 972-3-635-5038; Fax: 972-3-535-1103.

**November 14-17, 1993** 45th Postgrad Assembly of the Amer Endocrine Soc, San Francisco, CA. Info: C Huck. Tel: 301-571-1803; Fax: 301-571-1869.

**December 9-12, 1993** GHRH, GH, IGF-1: Basic & Clin Advances, San Diego, CA. Info: Dr B Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

**February 6-10, 1994** 3rd Intl Symp on Insulin-Like Growth Factors, Sydney, Austral. Sci Info: Dr R Baxter. Fax: 61-2-516-1273. Genl Info: E Loveridge. Tel: 61-2-956-8333; Fax: 61-2-956-5154.

**March 13-15, 1994** March of Dimes Clin Genet Conf, Kissimmee, FL. Info: C Blagowidow. Tel: 914-997-4524; Fax: 914-428-8203.

**March 15-17, 1994** Amer Coll of Med Genet, 1st Ann Mtg, Kissimmee, FL. Info: E Strass. Tel: 301-571-1826; Fax: 301-530-7079.

**May 2-5, 1994** APA/APS/SPR Ann Mtg, Seattle, WA. Info: D Anagnostelis. Tel: 708-427-1205; Fax: 708-427-1305.

**June 1-4, 1994** 1st Intl Mtg of the GH Research Soc, Aarhus, Denmark. Info: Drs J Christiansen/J Jorgensen. Tel: 45-86-1255-55/Ext 2084; Fax: 45-86-1378-25.

**June 8-14, 1994** 54th Ann Mtg of the ADA, New Orleans, LA. Info: ADA. Tel: 703-549-1500/Ext 330. Fax: 703-836-7439.

**June 15-18, 1994** 76th Ann Mtg of the Amer Endocrine Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1803; Fax: 301-571-1869.

**June 22-25, 1994** 33rd Ann Mtg of the ESPE, Maastricht, The Netherlands. Info: Prof J Van den Brande. Tel: 31-30-32-0521; Fax: 31-30-33-4825.\*

**July 17-24, 1994** 3rd Eur Cong of Endocrinol, Amsterdam, The Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

**August 20-25, 1994** 7th Intl Cong on Obesity, Toronto, Can. Info: CME Office. Tel: 416-978-2719; Fax: 416-971-2200.

**September 24-29, 1994** 9th Intl Cong on Hormonal Steroids, Dallas, TX. Info: Dr E Simpson. Tel: 214-648-3260; Fax: 214-648-8683.

**October 30-November 3, 1994** 46th Postgrad Assembly of the Amer Endocrine Soc, Toronto, Canada. Info: W Johnson. Tel: 301-571-1807; Fax: 301-571-1869.

\*Confirmation not received upon publication.

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# GROWTH

## Genetics & Hormones

Vol. 9 No. 4

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### Relevance of the Genetics of Embryologic Development

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*Professor of Pediatrics*

*Department of Pediatrics*

*University of Texas Southwestern Medical Center  
Dallas, Texas*

*GROWTH, Genetics, & Hormones* is an appropriate title under which to present advances in developmental genetics since many developmentally important genes have been recognized through their hormone-like influence on cell growth. The connection between genes, hormones, and birth defects is well illustrated by the virilization of female fetuses with autosomal recessive 21-hydroxylase deficiency.<sup>1</sup> That genetic factors contribute substantially to human malformations cannot be disputed.

Some 721 single birth defects and 1,040 syndromes are included among the 3,500 mendelian disorders catalogued by McKusick,<sup>2</sup> comprising almost 50% of the total.<sup>3</sup> The genetic contribution to birth defects is further underscored by the discovery that many malformations occurring in the absence of a positive family history (ie, sporadic malformations) actually result from spontaneously arising gene rearrangements and/or are now known to demonstrate parent-of-origin effects from inactivation of either the maternal or paternal allele of a gene through genomic imprinting.<sup>4</sup> Now that most genes found to regulate development of lower animals, such as arthropods and nematodes, are being identified in humans, it becomes possible to explore their potential role in human development and to examine similarities between developmental abnormalities in these species and in humans.

#### DEVELOPMENTAL GENES AS GROWTH FACTORS

The roundworm *Caenorhabditis elegans* has a small genome and a transparent embryo, which allows observation of virtually all developing tissues.<sup>5</sup> Among many interesting mutations identified in this species are those that delay or accelerate the timing of development (heterochrony). For example, a timing mutation called *lin-12* alters genital development. The DNA sequence of the gene involved exhibits homology with mammalian epidermal growth

#### Letter From the Editor

In Dr. Wilson's article, homeobox genes are referred to for one of the first times in *GGH*. These important genes control fundamental aspects of development, such as segmentation of the blast or embryo. These regulatory genes affect the activation of multiple target genes and the differentiation of certain cell types. Most genetic mutations cause damage more directly by producing defects in enzymes or receptors. One of the important aspects of understanding the action of these genes is that multiple cells may be affected, as reported from a defect in a homeobox gene known as *Pit-1*. The protein product of the *Pit-1* gene is abnormal; theoretically, it may not bind to DNA or, if it does bind, it cannot effectively activate transcription. Both the Snell and Jackson dwarf mice have abnormal *Pit-1* genes that lead to multiple hormone defects.

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## Letter From the Editor (continued)

Defects in other homeobox genes occur, such as the *Hox 1.5* gene that produces mice fetuses without thymus and parathyroid glands and with defects of the heart and arteries to produce a DiGeorge-like syndrome. You as a reader of *GGH* and of Dr. Wilson's article may wish to read further about defective homeobox genes and the anatomic and metabolic abnormalities that result. The following references provide further insight into the importance of these organizational genes.

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factors that also provide extracellular signals during mammalian development. Similar sequences occur in the *Notch* neurodevelopmental gene of the fruit fly and in several human genes. These genes may be candidates for human birth defects thought to result from arrested development.<sup>6</sup> The developmental pathways they regulate may be potential targets for therapy to prevent the occurrence of the defects.

## DEVELOPMENTAL GENES AS BLUEPRINTS

The fruit fly *Drosophila melanogaster* has been used extensively to analyze the effects of genes on development. Indeed, *Drosophila* geneticists have defined 5 groups of genes that regulate early pattern formation – the genes that specify the fruit fly basic body plan.<sup>7</sup> Despite obvious differences in the shapes and sizes of mammals and insects, the discovery of human counterparts to many of these fruit fly genes suggests that they are relevant to human development (Figure 1).

Pattern formation in insects begins prior to fertilization, when follicle cells surrounding the female germ cells orient oocyte cytoplasm according to "head" and "top" of the future embryo. In other words, expression of maternal genes regulates the initial stages of embryogenesis (Figure 1, column 1). Mutations in these maternal genes may cause headless embryos, like those produced by the *caudal* mutation, or deficient ventral structures exemplified by the *dorsal* mutation. After fertilization, products from several groups of embryonic genes (which have been named from their spatial or temporal expression pattern, their putative functions, or phenotypes resulting from mutations) orchestrate the next step in development, which is segmentation. First, gap genes (Figure 1, column 2) such as *kruppel* demarcate major embryonic regions. Next, pair-rule and segment polarity genes (Figure 1, columns 3 and 4) specify body segments, with appropriate anteroposterior orientation (polarity).<sup>7</sup> Mutations in these genes disrupt the formation of structures normally derived from the different body segments. For instance, mutations in the gene *wingless* (now called *Wnt-1* because of its homology to the integration site for mouse mammary tumor virus) alters thoracic segments so that wings do not develop. Finally, the expression of homeotic genes gives identity (labia, antennae, limbs) to the segmental units (Figure 1, column 5). The term homeotic refers to the development of normal structures at abnormal locations. For example, normal legs form where antennae normally reside in the *antennopedia* mutations.<sup>8</sup> Characterization of several homeotic genes revealed a highly conserved DNA sequence, which was named the homeobox.<sup>8</sup>

Figure 1  
Gene Families Responsible for  
*Drosophila* Segmentation and Their Mouse  
and Human Homologues



When DNA probes designed to detect the *Drosophila* homeobox sequence were employed to search for similar DNA sequences in other species, it was discovered that organisms as diverse as frogs, mice, and humans had such genes. Indeed, 4 major clusters of homeobox-containing genes have now been found in mice and humans; they have been termed *Hox* and *HOX* genes, respectively (Figure 1, column 5). The nomenclature for these homeobox-containing genes has been confusing since they were named originally in order of discovery. Several were initially assigned to the wrong cluster and different schemes were used for different species and by different investigators. However, consensus has been reached on a naming scheme in which the clusters are called A, B, C, and D and the individual gene loci are designated numerically according to their physical location (3' to 5') within a given gene cluster, hence A1,2,3,...; C4,5,6,..., etc. This scheme assumes that each cluster will have 13 gene loci. The human *HOX A* through *D* clusters map respectively to chromosomes 2, 7, 12, and 17 (Figure 1). Although the functional significance of *HOX* genes in humans remains to be proven, the structure and organization of the *HOX* clusters in humans is remarkably similar to that of other species, such as the mouse and in *Drosophila*, where function has been implied from analysis of segmental expression patterns and mutations.<sup>7,8</sup>

Given the homeobox as a paradigm, investigators sought and found DNA sequences in mammalian genes that corresponded to sequence motifs of other *Drosophila* genes involved in pattern formation. Such sequence similarities in different species are termed homologies, and their genes homologues. One such configuration was detected near the homeobox sequence of the *Drosophila* pair-rule

gene, *paired*; it was called the paired box or *Pax* motif. Eight mouse *Pax* genes (*Pax1* through *Pax8*) have so far been found (Figure 1).<sup>9</sup> In some instances, the DNA sequences of certain oncogenes (ie, mammalian *c-rel*, *GLI*, *Wnt-1*) were found to be homologous to those of insect developmental control genes (ie, insect *dorsal*, *kruppel*, *wingless*). In short, some oncogenes are actually pattern genes in disguise. Of interest to endocrinologists is another sequence motif, the POU box, which is common to pituitary-specific transcription factors. These factors can affect synthesis of pituitary proteins (ie, prolactin, growth hormone) in nonendocrine cells such as HeLa cells.<sup>10</sup> Just as the paired box and homeobox motifs occur together in the fruit fly gene *paired*, a POU box and homeobox occur together in pituitary transcription factors.

As shown in Figure 1, there are now mammalian homologues for each group within the hierarchy of genes that specify the *Drosophila* body plan. Presumably, these function to organize the basic body plan in mammals much like they do in insects, but the precise roles of the gene products are not known. Thus, at the current time, human development is like a repertory theater: the actors are known but not their roles in a given performance.

## FUNCTIONAL TESTS FOR DEVELOPMENTAL GENES

The mouse is an ideal model with which to study human development since its development closely resembles that of humans yet occurs over a time span short enough for analysis. Recent advances in molecular genetics that facilitate manipulation of the mouse genome have provided a means to explore the functions of putative mammalian developmental genes. In short, a gene of interest is altered, introduced genetically into (transgenic) mice, and the effects on development analyzed in progeny that express the mutation. In experiments using transgenic techniques, the DNA of an exogenous gene, the transgene, is introduced into a mouse egg. In this type of work genes can be inactivated, or knocked out, to examine what happens when the normal function of the gene is lost (loss-of-function mutations). Alternatively, genes can be over-expressed by joining them to regions of DNA that promote expression of the gene (promoters), causing them to be expressed at a high level. This produces gain-of-function effects. These technologies have been used to generate a number of mutant mice that have provided insight into mammalian development, with implications for human birth defects. For example, overexpression of the *Hox 1.1* gene produced mice with small jaws reminiscent of the Pierre Robin syndrome in man.<sup>9</sup>



Table 1

| Gene(s)          | Function             | Developmental Process Affected | Human Malformation      |
|------------------|----------------------|--------------------------------|-------------------------|
| <i>Oct-3</i>     | Transcription factor | Zygote cleavage                |                         |
| <i>Wnt-1</i>     | Signal transduction  | Axis formation                 |                         |
| <i>Xhox</i>      | Transcription factor | Axis formation                 |                         |
| <i>FGFs</i>      | Signal transduction  | Gastrulation                   |                         |
| <i>Hox(s)</i>    | Transcription factor | Segment specification          |                         |
| <i>Pax(s)</i>    | Transcription factor | Segmentation                   |                         |
| <i>NF-1</i>      | Signal transduction  | Cell growth                    | Neurofibromatosis       |
| <i>WT-1</i>      | Transcription factor | Differentiation                | Wilms' tumor            |
| <i>SRY</i>       | Transcription factor | Differentiation                | Gonadal dysgenesis      |
| <i>GLI</i>       | Transcription factor | Differentiation                | Grieg syndrome          |
| <i>KALIG-1</i>   | Cell adhesion        | Cell migration                 | Kallmann syndrome       |
| <i>c-kit</i>     | Signal transduction  | Cell migration                 | Piebald trait           |
| <i>COL1A1</i>    | Mechanical integrity | Tissue growth                  | Osteogenesis imperfecta |
| <i>Fibrillin</i> | Mechanical integrity | Tissue growth                  | Marfan syndrome         |

Changes in the cervical vertebrae were suggestive of anterior-to-posterior segment transformations caused by homeotic mutations in the fruit fly. Deficient expression of the *Hox 1.5* gene produced mice with absent thymus, missing parathyroids, and cardiac anomalies. The phenotype was similar to that of the human DiGeorge syndrome.

Mutations have also been characterized in several naturally occurring mouse mutants. The phenotype of the mouse *undulated* mutant with its severe vertebral anomalies has been explained by a mutation in the *PAX-1* gene (Figure 1). The coat color and hematologic defects in the *W* mutation have been mapped to the *c-kit* oncogene (Table 1).<sup>9</sup>

## APPLICATION TO HUMAN BIRTH DEFECTS

Table 1 lists selected genes or gene families that regulate or otherwise participate in developmental processes. Mutations in some genes have been found in human genetic disorders. It must be emphasized that the number of developmentally important gene families and the number of genes within such families is growing rapidly, and the assignment of human malformations to mutations in these genes is only beginning to emerge. The mutations identified to date highlight 3 major avenues for future investigation. The first is correlating normal function, or loss of function, of a gene product with a recognized clinical phenotype. For example, the fragile bones of osteogenesis imperfecta correlate well with loss of mechanical strength normally provided by type 1 collagen in bones. Similarly, the pie-bald trait correlates with loss of influence of the *c-kit* oncogene protein on melanoblast migration. Another example is the loss of the regulatory function of the G protein-related product of the *NF-1* gene in neurofibromatosis.

The second avenue is the use of DNA sequence and/or protein structure to identify human genes exhibiting homology with developmental control genes defined in lower species. The Grieg syndrome, with craniosynostosis and digital anomalies, has been traced to the *GLI* oncogene family that is homologous to the *kruppel* gene of the fruit fly (Figure 1).<sup>11</sup>

The third approach is the use of genetic linkage or chromosomal rearrangements to map putative developmental genes. Chromosomal deletions were important in defining the *WT-1* gene involved in kidney embryogenesis, the *SRY* gene responsible for male sex determination, and the *KALIG-1* gene that influences the migration of neural cells that contribute to olfaction and gonadotropin secretion. Mutations in these genes occur in Wilms' tumor, gonadal dysgenesis, and the X-linked Kallmann syndrome, respectively.<sup>12</sup>

For most developmental disorders, a combination of the gene function-phenotype correlation (candidate gene), gene structure (DNA sequence homology), and gene localization (reverse genetic, or position cloning) approaches will be used to identify genes of potential developmental importance in humans, with mouse genetic manipulation (transgenic and gene-targeted mice) as a key arbiter of their functional significance.

A closing example will illustrate how these developmental approaches offer new perspectives on complex disorders. Situs or laterality defects comprise a large spectrum, including multiple or absent spleen(s), pulmonary isomerism, cardiac defects (ie, transposition, anomalous pulmonary venous return), midline liver, and intestinal malrotation. Pure situs inversus viscerum or syndromes of mixed situs (heterotaxy-Kartagener and Ivemark syndromes) can exhibit familial patterns consistent with

autosomal recessive inheritance. One affected fetus has been described with a translocation breakpoint at chromosome region 12q13.<sup>13</sup> The similar mouse *inversus* (*iv*) mutation has been explained as a change from directed to random situs and linked to the immunoglobulin heavy chain locus on mouse chromosome 12 (human 14q32).<sup>14</sup> The theory of Brown and Wolpert,<sup>15</sup> derived from a variety of developmental systems, provides an approach to this spectrum of human malformation.

As diagrammed in Figure 2, the first steps towards right-left differences involve specification of the anteroposterior (A-P) and dorsoventral (D-V) axes. Mutations that reverse the A-P or D-V axes would produce pure situs inversus without functional consequences. The next step involves development of a midline axis and bilateral symmetry, probably before the primitive streak stage. Monozygotic or conjoined twinning are examples of midline axis disruption, and both are associated with situs defects.

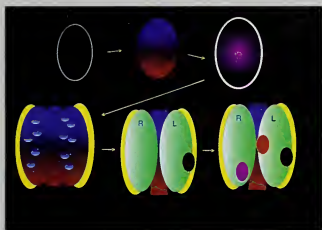
Once bilateral symmetry is established, a handed (asymmetric) molecule<sup>15</sup> is postulated that transports a morphogen or contracts in one direction (rightwards in Figure 2). In the case of morphogen transport, note that high concentrations of morphogen will occur at the midline on the right and at the periphery on the left (Figure 2). If the combination of high morphogen-peripheral molecule signals formation of a spleen, then this is a mechanism for splenic situs. Mutations inactivating the asymmetric molecule or its morphogen gradient would cause random situs determination as observed in the human and mouse heterotaxias. Failure of localized embryonic regions to respond to these signals would lead to isolated laterality defects such as malrotation or dextrocardia. This stepwise transformation of equivalent body halves is reminiscent of segment transformation in *Drosophila* and relevant to the presence of a human homeotic cluster at chromosome band 12q13. No homeobox sequences have been detected on mouse chromosome 12 or human 14q32, but characterization of the mouse *iv* gene is underway using the approaches outlined in this article.<sup>14</sup>

## SUMMARY

The explosion of new knowledge about the developmental genetics of simpler organisms has revolutionized the approach to studying human malformations. The discoveries have unified biology by implicating the same or similar genes in controlling the formation of diverse body plans, by revealing new relationships such as that between tumor growth and embryonic differentiation, and by dramatizing the partnership between clinical medicine and basic science. Although certain human malformations will be irreversibly determined at the

earliest stages of zygote cleavage, they will be detectable by preconceptional, preimplantation, and/or prenatal screening. Other birth defects may be responsive to maternofetal therapy with novel hormones, growth factors, and other treatment strategies. Advancing this multispecialty enterprise of gestational endocrinology should be a highlight of 21st century medicine.

Figure 2  
A Model for Human Situs Determination



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## Letter to the Editor

We read the appraisal by Professor MacGillivray<sup>1</sup> of the clinical significance of urinary growth hormone measurements with great interest and agree with many of her observations and reservations. The fraction of plasma GH excreted in the urine is very small, renal factors may account for 50% of the variability of excretion,<sup>2</sup> and there is a wide range of urinary GH values in normal children. It is surprising that the tiny fraction of GH excreted in the urine is so well correlated to plasma levels over the collection period.

However, what Professor MacGillivray considers to be of clinical significance could be debated. She suggests that urinary GH measurements fail to distinguish between a group of short normal children who have suboptimal spontaneous production of GH from short normal children who have normal GH production on serial blood sampling over a 12- to 24-hour period. The only reason why this distinction might be of relevance is presumably to select the former children for GH treatment, but as far as we are aware, there is no evidence that such children derive greater benefit from additional GH than any other group of short normal children. Therefore, the distinction is not of proven clinical benefit or usefulness.

On the other hand, it is very important to distinguish classical GH deficient children from short

normal children, however subdivided, because GH treatment in the latter group must still be regarded as experimental and has not yet been proven to be of long term benefit. We have shown that urinary GH measurements, using sound age- and sex-related reference ranges for comparison,<sup>2</sup> are useful in defining what Professor MacGillivray calls "severe" GH deficiency and indeed have a better predictive value (89% vs 45%) than stimulation tests.<sup>3</sup> We have regarded the near total overlap of values in short normal children with values for the whole population to be of clinical value rather than the reverse.

However, the case for the clinical use of urinary GH measurements should not be overstated. The jury is still out, but there are grounds for mild optimism. The case against the use of a hypoglycemic stimulus of GH release has been proven.<sup>4</sup>

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# The Importance and Methods of Using Animal Models to Study Human Disease

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Increasingly, animal models are being used to provide insights into the pathogenesis of human endocrinologic disorders as well as bone dysplasias and malformations, and in the laborious process of gene mapping and isolation. The most important mammalian model is the laboratory mouse, although other species also are coming under study. This brief review reports some of the major successes in using mouse models in the study of human disease.

## CANDIDATE GENES AND CHROMOSOME HOMOLOGY

Although there are 23 pairs of human chromosomes and only 20 pairs of mouse chromosomes, and the

overall appearance of the chromosomes is different, segments of chromosome have been identified in mice and humans that appear to have been conserved because they contain 2 or more identical marker loci in both species. Such *syntenic segments* can be used to provide clues to gene mapping. If a human disorder is homologous to a mouse mutant, and the mouse mutant has been mapped and attributed to a syntenic segment, then the gene location on the mouse chromosome will point to a putative location for the gene on the human chromosome. For example, several mouse mutants with pigmentary and inner ear abnormalities show homology to the Waardenburg syndromes.<sup>1</sup> Type 1 Waardenburg syndrome was localized to 2q37 in a syntenic region that in the mouse contains the *spotch* (*Sp*) mutant.<sup>2</sup> Because the *Pax3* gene in the mouse mapped close to the *Sp*, it was investigated and mutations were found in 2 different alleles of *spotch*.<sup>3</sup>

Tassabehji et al<sup>4</sup> and Baldwin et al<sup>5</sup> then demonstrated mutations in the HuP2 gene (which is the homologue of the mouse *Pax3* gene) in human type 1 Waardenburg syndrome.

The study of mouse paired box (*Pax*) genes and their human equivalents has been extremely fruitful.<sup>6</sup> Like homeobox genes, *Pax* genes were originally discovered in *Drosophila*. They code for proteins containing a DNA-binding domain, and as such are transcription factors that turn on other genes to regulate embryologic development. There are at least 6 *Pax* genes in mice and humans; in addition to *Pax3* mutations, 2 other *Pax* genes have been found to be mutated and associated with congenital anomalies. Mice homozygous for mutations of the *Pax1* gene show the undulated (*un*) phenotype with a shortened kinky tail, kyphosis due to vertebral defects, and absence of acromion scapulae. *Pax6* mutated in small eye (*Sey*) in the mouse and in dominant aniridia, mapping to 11p13 in the human.<sup>6</sup>

Another important example of the identification of development genes through comparative gene mapping and candidate genes is that afforded by the study of retinitis pigmentosa. This disorder is heterogeneous, with several autosomal dominant, recessive, and X-linked forms recognized. The mouse mutant retinal degeneration-slow (*rds*) was found to be due to a mutation at the locus for a membrane protein, peripherin. Mutations at this locus were also found in some human families with autosomal dominant retinitis pigmentosa.<sup>7</sup>

## GENETIC MARKERS AND CONTROLLED MATING

Mapping genetic disorders depends on being able to study a large number of genetic markers that segregate in matings producing affected animals. This means not only that the markers themselves must be available but also that the study animals should be heterozygous for these markers.

A detailed map of the mouse genome is rapidly being constructed using variable number tandem repeat (VNTR) probes,<sup>8</sup> microsatellite repeats,<sup>9</sup> and polymorphism among stably inserted murine leukemia proviruses.<sup>10</sup>

As mouse strains are inbred, most animals will be homozygous at marker loci. This disadvantage has been largely overcome by 2 techniques: inter-specific crosses and recombinant inbred strains. The laboratory mouse *Mus musculus* will mate with the species *Mus spretus*. As the 2 species have diverged over several million years, there are different alleles at most marker loci; the offspring will be heterozygous at most marker loci, allowing the mapping of disease loci in the laboratory mouse parent. Recombinant inbred strains are derived by inbreeding the offspring of crosses between 2 laboratory

strains of mice. Different strains will have different alleles at many marker loci.

When 1 of the parental strains has a specific disease susceptibility for a polygenic disorder, recombinant inbred strains can be used in order to identify the loci responsible for the susceptibility to particular chromosome segments.

## COMMON DISORDERS

Common diseases with a polygenic component, such as diabetes mellitus, epilepsy, and hypertension, have long been considered a geneticist's nightmare because of the difficulty in analyzing the interaction of several genetic loci with environmental factors. The ability to set up specific matings in mice, together with the increasing specificity of the mouse genetic map, is allowing investigators to tackle some of these problems.

Examples of these studies include work on the non-obese diabetic (NOD) mouse, which is an animal model of type 1 diabetes,<sup>11</sup> and work on the mutant diabetes (*db*) mouse, which is a possible homologue of type 2 diabetes.<sup>12,13</sup> The epileptic (*EI*) mouse has been studied as a model of human temporal lobe epilepsy. Two susceptibility genes have been identified, one each on chromosomes 2 and 9.<sup>10</sup>

Genetic susceptibility to infection can also be studied more easily in mice. Alleles at a single locus on mouse chromosome 1 (*Lsh*) appear to confer resistance to leishmaniasis, salmonellae, and tuberculosis.<sup>14</sup> It has been suggested that this locus is involved in macrophage activation. There is some evidence of a human homologue to this gene.<sup>15</sup>

Rat strains that are naturally hypertensive and prone to stroke have been used to study the genetics of these traits by using linkage analysis. Susceptibility genes have been identified both on the rat X chromosome and on chromosome 10 in a region homologous to human chromosome 17q. The human angiotensin 1-converting enzyme also maps to 17q, and the rat homologue of this gene maps close to the hypertension gene on rat chromosome 10.<sup>16</sup>

## CONSTRUCTING ANIMAL MODELS

The cloning of specific genes has made feasible the creation of animal models of genetic disorders. The method used is gene targeting in mouse embryonic stem cells, ie, inserting DNA into the cells (transfecting) with constructed plasmids containing part, but not all, of the gene in question, together with selectable markers. This leads to mutation of the gene by homologous recombination in a small proportion of cells. Selected stem cells in which targeting has been successful then can be introduced into a mouse embryo. Some recipient adult mice will be mosaic



for the mutated gene; if they carry mutations in the germ cells, a proportion of their offspring will carry the mutation in every cell. A mouse model of Gaucher disease has been created in this way,<sup>17</sup> and a mouse model of cystic fibrosis has recently been reported.

The same technique is being used to study the effects of genes responsible for embryologic development. For example, LeMouellic et al<sup>18</sup> introduced a null mutation into the mouse *Hox 3.1* gene. They demonstrated in homozygous mice that there was an anterior shift of trunk segments, an example of which was the appearance of an extra pair of ribs attached to the first lumbar vertebra. This approach also was used to prove that the testis-determining factor (Tdf) gene was the only segment of DNA needed to change a female XX mouse into a male.<sup>19</sup>

## DIFFERENCES IN MANIFESTATION OF GENE-SPECIFIC MUTATIONS

Direct molecular homology between mouse and human mutations does not guarantee phenotypic similarity. For example, the *mdx* mouse has no dystrophin in skeletal muscles and the resultant disease is directly homologous with Duchenne muscular dystrophy,<sup>20</sup> yet little detectable muscle weakness or progressive morphologic abnormality (apart from the diaphragm) is observed, although these are major components of Duchenne muscular dystrophy.

Another example is that the hypoxanthine-guanine phosphoribosyl-transferase (*Hprt*)-deficient mouse shows none of the clinical effects seen in humans with Lesch-Nyhan syndrome, which has the same enzyme defect. This presumably is due to alternative metabolic pathways in the mouse.<sup>21</sup> Another variant in the difference of expression by the same gene-specific mutation occurs in respect to mode of inheritance. Various hemolytic anemias due to red cell defects are directly homologous in mice and humans (see Table 1).<sup>22</sup> However, the mode of inheritance of spherocytosis types 1 and 2 in the mouse is autosomal recessive, whereas in humans it is autosomal dominant.

It has been suggested that some pathologic processes are dependent on absolute time rather than relative (biologic) time.<sup>23</sup> If this is true, it may be impossible to produce a convincing mouse model of some human genetic disorders with late onset, such as Huntington disease.

## CONCLUSIONS

Many potential animal models of human disease exist, but these have yet to be associated with their human counterparts because they have not been exhaustively studied.<sup>24,25</sup> Sometimes a mouse mutant can point to the etiology of a human abnormality

that has not previously been contemplated. For example, it has been suggested that some infants have a constellation of abnormalities similar to those caused by the mouse mutant *Disorganization*.<sup>26</sup> An infant was reported with right-sided tibial hypoplasia with a high degree of polydactyly of the toes on the same side. An ectopic digit was situated on the lower abdomen. Mice heterozygous for the *Disorganization* gene have similar defects, and may even have complete limb duplication, demonstrating that apparently teratomatous lesions can be caused by a single gene.

Potential homologues are first recognized at the phenotypic level; further confirmation of true homology can be obtained by comparative gene mapping and, ultimately, by demonstrating homology at the level of the gene sequence in both species. Examples of mouse models identified at 1 of 3 levels – phenotype, genotype, and mapping – are presented in Table 1.

The ability to construct specific matings in the mouse also allows for the genetic analysis of multifactorial "common" disorders, a subject well reviewed by Todd.<sup>27</sup>

In summary, both gene mapping and the new techniques recently developed to study molecular biology have placed us on the threshold of dissecting much of the pathophysiology that we as physicians observe. The same mapping and techniques utilized in animal models permit correlations and conclusions that otherwise would not be possible. These cannot be ignored by physicians dealing with congenital anomalies or errors in metabolism.

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Table 1  
Some Human Disorders and Proposed Mouse Homologues

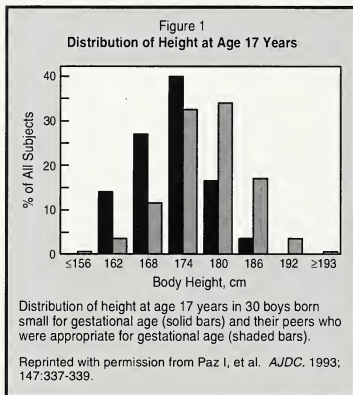
|  |  | <u>Basis of Homology</u> |
|--|--|--------------------------|
| <b><u>Hematological Disorders</u></b>              |  |                          |
| Chediak-Higashi syndrome                           | <i>bg</i> - beige  | Phenotype                |
| Hermansky-Pudlak syndrome                          | <i>coa</i> - cocoa, <i>pe</i> - pearl, <i>sdly</i> - sandy   | Phenotype                |
| Wiskott-Aldrich syndrome                           | <i>sf</i> - scurfy   | Mapping                  |
| Spherocytosis (beta-spectrin def)                  | <i>ja</i> - jaundiced  | Genotype                 |
| Spherocytosis (ankyrin def)                        | <i>nb</i> - normoblastic anaemia   | Genotype                 |
| Spherocytosis (alpha-spectrin def)                 | <i>sph<sup>tm</sup></i> - spherocytosis  | Genotype                 |
| <b><u>Endocrine Disorders</u></b>                  |  |                          |
| Familial idiopathic gonadotropin deficiency        | <i>hpg</i> - hypogonadal   | Phenotype                |
| Type 2 diabetes                                    | <i>db</i> - diabetes   | Phenotype                |
| Type 1 diabetes                                    | <i>nod</i> - nonobese diabetic   | Mapping                  |
| Pituitary dwarfism                                 | <i>mn</i> - miniature  | Mapping                  |
| Testicular feminization                            | <i>Tfm</i> - testicular feminization   | Genotype                 |
| <b><u>Metabolic Disorders</u></b>                  |  |                          |
| X-linked hypophosphatemic rickets                  | <i>Hyp</i> - hypophosphatemia, <i>Gy</i> - gyro  | Mapping                  |
| Krabbe disease                                     | <i>twi</i> - twitcher  | Genotype                 |
| Menkes syndrome                                    | <i>Mo</i> - mottled  | Mapping                  |
| Lesch-Nyhan syndrome                               | <i>Hprt</i> - hypoxanthine guanine phosphoribosyltransferase deficiency  | Genotype                 |
| MPS type VII                                       | <i>gus<sup>mps</sup></i>   | Genotype                 |
| Ornithine transcarbamylase deficiency              | <i>spf</i> - sparse fur  | Genotype                 |
| Carbonic anhydrase deficiency                      | <i>Car-2<sup>l</sup></i> - carbonic anhydrase II null allele   | Genotype                 |
| Pelizaeus-Merzbacher disease                       | <i>jp</i> - jimpy  | Genotype                 |
| Phenylketonuria                                    | <i>Pah</i> - phenylalanine hydroxylase   | Genotype                 |
| <b><u>Skeletal Abnormalities</u></b>               |  |                          |
| Cleidocranial dysostosis                           | <i>ccd</i> - cleidocranial dysplasia   | Phenotype                |
| Osteopetrosis                                      | <i>gl</i> - grey lethal  | Phenotype                |
| Osteogenesis imperfecta                            | <i>fro</i> - fragilitas ossium   | Phenotype                |
| Conradi disease                                    | <i>Bpa</i> - bare patches  | Mapping                  |
| <b><u>Limb Abnormalities</u></b>                   |  |                          |
| Fraser syndrome                                    | <i>my</i> - blebs, <i>bl</i> - blebbed   | Phenotype                |
| Split hand and foot                                | <i>Dac</i> - dactylaplasia   | Phenotype                |
| Weyers oligodactyly                                | <i>ol</i> - oligodactyly   | Phenotype                |
| Greig syndrome                                     | <i>Xt</i> - extra toes   | Mapping                  |
| Cenani-Lenz syndrome                               | <i>ld</i> - limb deformity   | Phenotype                |
| <b><u>Skin/Pigmentary Abnormalities</u></b>        |  |                          |
| X-linked ectodermal dysplasia                      | <i>Ta</i> - tabby  | Mapping                  |
| Incontinentia pigmenti                             | <i>Str</i> - striated  | Mapping                  |
| Goltz syndrome                                     | <i>Td</i> - tattered   | Mapping                  |
| Piebaldism   | <i>W</i> - Dominant white spotting   | Genotype                 |
| Waardenburg syndrome type 1                        | <i>Sp</i> - splotch  | Genotype                 |
| Albinism - tyrosinase negative                     | <i>c</i> - albino  | Genotype                 |
| <b><u>Eye Abnormalities</u></b>                    |  |                          |
| Aniridia   | <i>Sey</i> - small eye   | Genotype                 |
| Retinitis pigmentosa (AD) (peripherin abnormality) | <i>rds</i> - retinal degeneration-slow   | Genotype                 |
| <b><u>Miscellaneous</u></b>                        |  |                          |
| Spondylocostal dysostosis                          | <i>Mv</i> - malformed vertebrae, <i>Rf</i> - rib fusion, <i>rv</i> - rib vertebrae, <i>rh</i> - rachiterata, <i>pu</i> - pudgy | Phenotype                |
| Situs inversus                                     | <i>iv</i> - situs inversus viscerum  | Phenotype                |
| Duchenne muscular dystrophy                        | <i>mdx</i> - X-linked muscular dystrophy   | Genotype                 |

## Are Children Born Small for Gestational Age at Increased Risk of Short Stature?

In Israel all children are evaluated at 17 years of age for military service, and thus the authors were able to determine adult or near-adult heights of 30 boys and 34 girls born from 1970 to 1971 at 39 weeks gestation who were small for gestational age (SGA = birth weight below the 3rd percentile for the study population) and who had no identifiable cause for being SGA. The mean height of the males at 17 years of age was

169.4 cm and that of the females was 160.3 cm. These values were 5.8 cm and 3.4 cm (respectively) less than those of control subjects of appropriate size for gestational age (AGA). The distribution of heights was shifted to the left in comparison to that of the AGA population in both sexes (Figure 1). Mean weights were 2.1 kg and 1.9 kg less than those of AGA boys and girls, respectively, but body mass indices were similar in SGA and AGA subjects. The investigators also calculated the odds ratio that SGA individuals would be less than the 10th percentile for height as an adult (4.13 for males, 3.32 for females).

Paz I, et al. *AJDC*. 1993;147:337-339.



**Editor's comment:** These data indicate that otherwise normal SGA infants are significantly smaller as adults than are AGA infants. Although data on bone age and stage of sexual maturation are not provided, the authors cite other data indicating that neither bone age nor age of pubertal onset is delayed in SGA subjects. Chaussain et al<sup>1</sup> reported that the mean adult height of 21 French SGA boys born at term was 162.8 cm and that of 23 SGA girls 147.6 cm; this is 7.2 cm and 9.9 cm, respectively, less than adult heights predicted in earlier childhood. Interestingly, the adult heights of the French SGA subjects were substantially lower than those of the SGA Israelis. Whether any therapeutic intervention would be useful or should even be considered in regard to the growth of these subjects is a matter of current investigation.

Allen W. Root, MD

1. Chaussain JL, et al. Actual versus predicted final adult height in patients with intrauterine growth retardation (IUGR). *Pediatr Res*. 1993;33:S38. Abstract 210.

## Pseudotumor Cerebri and hGH Administration: A Report by the National Cooperative Growth Hormone Safety Subcommittee

Pseudotumor cerebri (PC), or idiopathic intracranial hypertension, occurs with a frequency of 1:100,000 per year in the general population. The incidence is high in obese women, but reviews of the literature report a sizable proportion of patients ages 5 to 15 years, although infants also may be affected. Permanent visual loss, which is due to damage of the optic disc as a result of papilledema is the major complication. Treatment is aimed at prevention of visual deficits and prolonged symptoms by reducing intracranial pressure. The prognosis is excellent with proper management.

Several diagnostic criteria for PC have been defined, including elevated cerebrospinal fluid (CSF) pressure without abnormal CSF composition. Normal brain appearance with normal or small ventricles is found with imaging studies. Headache, visual disturbances, and papilledema are the most common clinical findings in adults and adolescents. Infants and young children may present with irritability, apathy, or somnolence, rather than headache. Dizziness and ataxia also may occur. Older children and adolescents typically complain of headache, sometimes accompanied by nausea and vomiting. Preadolescents appear more likely than adults or adolescents to manifest symptoms other than

headache and papilledema, including lateral rectus paresis, vertical strabismus, facial paresis, and back and neck pain.

Numerous conditions and risk factors have been linked to the development of PC, including obesity or significant weight gain, steroid withdrawal, and Addison disease. The link to obesity and weight gain may be related to extra ovarian estrone production

**Table 1**  
**Growth Hormone Treatment History in Patients With Pseudotumor Cerebri**  
(n=24)

| Treatment Status  | n= |
|---|----|
| GH therapy discontinued, symptoms improved/resolved                 | 12 |
| GH therapy discontinued, patients rechallenged, symptoms reappeared | 4  |
| GH therapy discontinued   | 7  |
| Disposition unknown   | 1  |

in adipocytes, which is believed to stimulate CSF formation. In children, obesity does not appear to be an important factor. Conditions that may be associated with PC include hypothyroidism and iron deficiency anemia. These associations are not well established and may represent chance occurrences. Drugs also have been implicated, including corticosteroids, tetracycline, minocycline, nalidixic acid, trimethoprim/sulfamethoxazole, indomethacin, isotretinoin, danazol, and oral contraceptives.

Between 1985 and the present, Genentech, Inc. learned of 24 instances (12 female, 12 male) of "diagnosed" or "suspected" PC in patients receiving growth hormone (see Table 1). Twenty cases involved patients receiving Protropin® (somatrem for injection) therapy, 3 cases involved Humatrope® (somatropin, rDNA origin, for injection), and the type of GH was not specified in the 1 remaining case.

In 7 of the 12 patients in which GH therapy was continued, symptoms of PC resolved spontaneously over a period of months. Three patients continued to receive GH and symptoms had not resolved at the most recent examination. In 2 other cases, it was not learned whether GH had been withdrawn and restarted.

Sixteen of the 24 total PC patients were reported through the Protropin® National Cooperative Growth Study. Table 2 provides diagnoses, age, and treatment duration information. It is important to note that most of these 16 patients had 1 or more of the previously mentioned conditions or risk factors associated with PC.

The management of these patients varied but was consistent with guidelines in the literature. The most common modes of therapy are directed at relieving symptoms associated with elevated CSF pressure and at protecting vision. Therapies include acetazolamide, which reduces CSF formation; corticosteroids; periodic lumbar punctures to alleviate intracranial pressure; and, occasionally, lumboperitoneal shunting. In none of the cases reported to Genentech was surgical intervention performed, ie, subtemporal decompression or optic nerve sheath fenestration. Permanent visual loss did not occur in any of the patients.

In summary, PC has been reported rarely in patients treated with GH. Some of the patients had concurrent conditions known to be associated with PC. Therefore, the role of GH therapy is

Table 2  
Reports of Pseudotumor Cerebri\*  
in GH-Treated Patients  
(n=16)

**Sex:** 10 Females, 6 Males

**Age:** Range = 7 to 18 years; Mean = 12.5 years

**Duration of Treatment:** Range = 0.1 to 62 months;  
Mean = 9.0 months

| Diagnosis  | n= |
|--|----|
| Idiopathic GH Deficiency                         | 8  |
| Short Stature due to Chronic Renal Insufficiency | 5  |
| Prader-Willi Syndrome                            | 1  |
| Turner Syndrome                                  | 2  |

\* Data derived from the Protropin® National Cooperative Growth Study.

not known, although a possible association has not been excluded. Traditional management has been successfully employed in treating this condition; in some cases, GH therapy was discontinued.

**Editor's comment:** When this report crossed my desk, it seemed important enough to be abstracted. Therefore, I elected to share this information with those of you who did not read the original report. In my experience with more than 1,000 patients on hGH, I have seen PC occur only once. It regressed quickly with no change in GH dose. For those who wish to read further, a comprehensive paper from the FDA [Malozowski S, Tanner LA, Wysowski D, Flemming GA. Growth hormone, insulin-like growth factor 1, and benign intracranial hypertension. *New Engl J Med.* 1993; 329:665-666] appeared in August. This paper includes all the data available to Genentech as well as data on 3 cases of pseudotumor cerebri that occurred in subjects treated with IGF-1.

Robert M. Blizzard, MD

## Gene Associated With Amyotrophic Lateral Sclerosis (Lou Gehrig's Disease) Codes for Superoxide Dismutase

Amyotrophic lateral sclerosis (ALS) is a late onset, ultimately fatal disease characterized by the degeneration of motor neurons, causing a progressive paralysis that can proceed for many years before causing death. In addition to Lou Gehrig, whose illness gave the disease its popular name, ALS is also well-known for its attack on the brilliant astrophysicist Stephen Hawking, author of *A Brief History of Time*. Most cases of the disorder are sporadic, but approximately 10% are familial.

Rosen et al have identified the SOD1 gene on human chromosome 21 that codes for cytosolic superoxide dismutase (SOD). This gene is the most likely candidate for the ALS defect. The SOD enzyme catalyzes the conversion of superoxide ( $O_2^-$ ), a toxic-free radical, into hydrogen peroxide ( $H_2O_2$ ), which can then be converted to water by glutathione peroxidase or catalase.

Superoxide is a highly reactive molecule involved in numerous physiologic and pathologic processes. It also is highly destructive. For example, free radicals have been implicated in

DNA breakage leading to cancer and aging, and in damage to other cellular structures. Superoxide is released in abundance during the respiratory burst of activated phagocytic leukocytes and plays a role in inflammation. Many bioactive molecules, such as nitric oxide, also react with superoxide, affecting their levels in various tissues. Reperfusion, which injures numerous tissues, also involves superoxide.

How might a defect in SOD1 contribute to the very specific pathology found in ALS? Free radicals have been proposed to cause neuronal injury in several neurological disorders, including Parkinson's disease and ischemic brain injury. Thus, one possibility is that SOD1 activity is reduced in ALS patients, leading to an accumulation of the toxic superoxide radical. Alternatively, the activity of SOD1 might be increased, leading to excessive levels of hydrogen peroxide and, subsequently, of the highly toxic hydroxyl radical. Overexpression of SOD1 in transgenic mice was found to lead to an apparently specific



defect in the motor neurons of the tongue and hindlimbs. This indicated that the SOD1 gene can selectively affect motor neurons.

In a companion piece in the same issue of *Nature*, neurologist James McNamara and biochemist Irwin Fridovich discuss another possibility for the involvement of free radicals in the neuronal injury in ALS. In acute neuronal injury due to ischemia or hypoglycemia, abnormally high concentrations of glutamate accumulate in the extracellular space and excessive activation of neuronal glutamate receptors ensues, thereby literally exciting these glutamate-receptive neurons to death. Epidemiologic evidence hinted at a link between ALS and glutamate when ingestion of excess amounts of a glutamate analogue found in certain nuts was implicated in a form of ALS and Parkinson dementia in Guam. More direct evidence of this excitotoxic mechanism for chronic injury of motor neurons was provided by studies with spinal cord explants, which showed that incubation of these cultures with a glutamate-uptake blocker, causing excess glutamate to accumulate at the extracellular receptors, selectively kills motor neurons. Apparently the glutamate binding by specific receptors requires an influx of calcium, which in turn causes generation of high levels of superoxide. The death of these neurons in culture can be prevented by the addition of SOD1. Thus, a subtle increase of superoxide free radical over the course of a lifetime, caused by decreased SOD1 activity, might lead to the slow death of motor neurons.

Rosen DR, et al. *Nature*. 1993;362:59-62.

McNamara J, Fridovich I. *Nature*. 1993;362:20-21.

**Editor's comment:** *The identification of the genetic defect responsible for this devastating illness sheds light on possible mechanisms for its pathogenesis, as well as potential approaches to therapy for ALS, and possibly even for other similar disorders such as Parkinson's disease. For example, antagonists of the glutamate receptors, which are inhibitors of the enzymes that cause production of superoxide free radical, or treatment with superoxide dismutase itself might be effective as*

*therapies. If, on the other hand, ALS is caused by elevated levels of SOD1, therapies which block its production or activity may be useful. In addition, prenatal diagnosis for this disorder should now become possible, as about 10% of ALS cases are familial with autosomal dominant inheritance. Given the late onset of ALS, carrier testing may also be useful, since a possible carrier may wish to know his or her carrier status before conceiving children.*

Judith G. Hall, MD

## In Future Issues

### Overgrowth Syndromes and Disorders: Definition and Classification

by David Weaver, MD

### The Overgrowth Syndromes: An Update

by Kenneth L. Jones, MD

### Insulin-like Growth Factor 2 and Growth

by Yves Le Bouc, MD

### Neuroendocrinology of Growth Hormone Secretion

by Jesus Argente, MD, PhD

### Serum Polypeptide Hormone Binding Proteins

### Part 1: Growth Hormone Binding Protein Part 2: Insulin-like Growth Factor Binding Proteins

by Allen W. Root, MD

### Treatment of Craniopharyngioma: End Results

by Edward Laws, MD

## Trial of Insulin-like Growth Factor 1 Therapy for Patients With Extreme Insulin Resistance Syndromes

The potential glucose lowering effect of insulin-like growth factor 1 (IGF-1) in individuals with a variety of insulin resistance syndromes was studied. Eleven patients demonstrated extreme insulin resistance syndromes, including 6 with type A insulin resistance syndrome; 2 with congenital generalized lipodystrophy; 2 with leprechaunism; and 1 with an unidentified syndrome that included facial abnormalities, a mild degree of subcutaneous fat atrophy with relatively well-developed musculature, and acanthosis nigricans.

The study had 2 phases: an acute treatment phase and a long-term treatment phase. All subjects, except a 7-month-old baby with leprechaunism, were studied for the acute effects. All were admitted to the hospital and treated with a standard diet; none received any medication other than IGF-1 (recombinant human IGF-1 [FK780], Fujisawa Pharmaceutical, Osaka, Japan). After an overnight fast, patients were given a subcutaneous bolus 0.1 mg/kg. Blood was sampled for blood glucose concentrations at 0, 1, 2, and 3 hours after injection. Two or

more days later, a higher dose of IGF-1 (0.1 to 0.4 mg/kg twice daily) was initiated and given for periods up to 16 months.

Basal concentrations of total circulating IGF-1 were decreased in 6 subjects but normal in the others. Following the acute injection of IGF-1, the circulating IGF-1 concentration decreased very slowly in most and remained elevated for at least 24 hours. In one patient with leprechaunism, the concentration fell rapidly to baseline within 12 hours. The subjects' plasma glucose levels declined 50% to 87% in the low-dose study and 45% to 87% in the high-dose study. Circulating plasma insulin levels fell in response to IGF-1 in a majority of patients. Two patients experienced side effects, including nausea and pallor in one patient and headache in the other.

IGF-1 levels in the long-term study remained at supraphysiologic levels except in 1 of the patients with leprechaunism. Daily plasma glucose profiles demonstrated reductions in both fasting and postprandial levels. In addition, hemoglobin A<sub>1c</sub> and fructosamine concentrations decreased

during IGF-1 treatment. Plasma insulin and C-peptide levels decreased in many of the patients, including those with leprechaunism. Surprisingly, acanthosis nigricans improved slightly in some of the patients. In the 1 patient with high concentrations of plasma testosterone, the degree of hirsutism did not change during therapy. No hypoglycemic episodes were recorded in any patients.

Background retinopathy occurred in 1 patient with congenital generalized lipodystrophy 1.5 months after onset of treatment. Weight gain occurred in some, including those with leprechaunism. One patient with leprechaunism had an increase in subcutaneous fat mass, and the other had an improvement in skin elasticity and in linear growth. Signs of acromegaly did not occur during the limited long-term therapy. Low titers of IGF-1 antibodies were detected in 3 of 8 patients.

These results show that IGF-1 can be used clinically as a hypoglycemic agent in some patients with extreme insulin resistance and in whom insulin is not effective. Seven of the 11 patients (5 with type A syndrome and 2 with leprechaunism) had defective insulin receptor functions. The authors postulated

that IGF-1 may have exerted its blood glucose-lowering effects through its own receptors.

Kuzuya H, et al. *Diabetes*. 1993;42:696-705.

**Editor's comment:** This limited study presents another potentially important clinical use of recombinant IGF-1. Previous therapy, including massive doses of insulin, proved ineffective in controlling hyperglycemia and/or hyperinsulinemia in these patients. The authors point out that IGF receptors are present in high concentrations in muscle but in minimal concentrations in adipose and hepatic cells. Thus, they hypothesize that the glucose-lowering action of IGF-1 may be primarily by its own receptor, inducing glucose transport and stimulating glycogen synthesis. IGF-1 also has been shown to inhibit pancreatic beta-cell function in isolated perfused pancreatic preparations. More sophisticated acute and long-term studies of IGF-1 in these types of disorders are needed. These initial findings, however, are exciting and encouraging.

William L. Clarke, MD

## Failure to Improve Height Prediction in Short Stature Pubertal Adolescents by Inhibiting Puberty With Luteinizing Hormone-Releasing Hormone Analogue

Treatment with long-acting D-Trp6-luteinizing hormone-releasing hormone at a dose of 3.75 mg IM monthly was given for 24 months to 17 endocrinologically normal adolescents of short stature (9 females ages 11.8 ± 1.5 years; 8 males ages 13.2 ± 1.1 years). The patients were referred at pubertal stages II-III according to Tanner, with a height prediction below -2.5 SD according to Bayley and Pinneau tables.

Pubertal progression was suppressed during the 2 years of analogue therapy, but then resumed shortly after the end of treatment. Annual growth rate remained in the prepubertal range during the treatment period and did not increase with the resumption of sexual development. A reduced rate of bone maturation was observed during the 2 years of analogue treatment, but there was no clear-cut increase of the height to bone age relationship at the end of treatment nor after the post-treatment observation period of 12 to 14 months. Thus, after approximately 3 years of study, no significant improvement of predicted adult stature was obtained. There were no side effects, but psychological problems occurred mainly related to the failure to increase height.

The authors conclude that even if methods for predicting adult height are not accurate, the data suggest that use of LHRHa in endocrinologically normal short subjects entering puberty at normal age with a poor height prognosis does not offer enough possible advantages on growth to offset the psychological drawbacks, and this approach cannot be considered as routine treatment in this situation.

Linder D, et al. *Eur J Pediatr*. 1993;152:393-396.

**Editor's comment:** Further studies which are undertaken under rigid protocol are needed to answer the question whether LHRH analogues can be effective in normal short individuals. I suspect that the results of such studies will confirm the data presented here as GH production falls significantly when LHRHa is given. This article is abstracted because it is one of the first to report attempts to increase growth in normal length and intrauterine growth retarded children by using LHRHa. Ten of the subjects had a birth length >-2SDs below the mean height.

Robert M. Blizzard, MD

## Preliminary Study of the Efficacy of Insulin Aerosol Delivered by Oral Inhalation in Diabetic Patients

In this study the efficacy of orally inhaled insulin in normalizing plasma glucose levels during the fasting state in noninsulin-dependent diabetes mellitus (NIDDM) patients was investigated. The subjects were nonobese, nonsmoking NIDDM volunteers aged 35 to 62 years. Body mass index ranged from 19.29 to 27.21, with a mean of 23.94 ± 3.00. The patients had never received insulin therapy. Five were on oral antidiabetic medication, which was discontinued 2 to 4 days before insulin inhalation.

An insulin dose of approximately 1.0 U/kg of body weight was administered as an aerosol by oral inhalation. Aerosol was generated by a raindrop nebulizer from 2 mL of undiluted regular 500 U/mL pork insulin solution. During the aerosolization procedure, the nebulizer was activated 6 times in quick succession. Insulin aerosol generated during these 6 actuations accumulated in the holding chamber. After the sixth firing, aerosol in the holding chamber was inspired as a bolus through

a mouthpiece with a flow rate of 17 L/minute. The small particle size produced by the raindrop nebulizer, the low regulated flow rate, and the holding chamber were employed in combination to minimize impaction and loss of insulin in the mouth. Insulin units available at the mouth were quantified by adding radiolabeled technetium Tc 99m pertechnetate to the insulin solution. Radioactivity deposited on the filter attached to the mouthpiece was counted and found to represent  $27\% \pm 4\%$  of the initial dose of radioactivity that was delivered into the holding chamber. Twenty-seven percent of 24 U of insulin in the chamber (6.48 U) was the amount of insulin available for inhalation at the mouth after 6 actuations. Thus, to deliver a total of 1 U/kg, volunteers inhaled 8 to 13 times from the holding chamber, depending on their body weight. The percentage of deposition in the lungs of each subject was also quantified from a gamma camera scan of the anterior chest after inhalation of a radioaerosol. Quantitative analyses of the lung scans showed that the deposited fraction ranged from 50% to 93% of the inhaled dose. Mean deposition below the larynx was  $79\% \pm 17\%$ . The remainder was deposited in the oropharynx or was exhaled.

After inhalation, plasma glucose and insulin concentrations were monitored while a saline drip was continuously administered intravenously. During a second 12-hour fasting period on another occasion, 3 of the subjects inhaled placebo aerosol generated from 0.9% saline.

The mean plasma insulin level increased from 11.8  $\mu\text{U/mL}$  to 44.8  $\mu\text{U/mL}$ , with an average time  $\pm\text{SD}$  to peak level of  $40 \pm 34$  minutes. The plasma glucose levels decreased from a mean of  $225.5 \pm 46.3$  mg/dL to normal levels of 70 to 115 mg/dL in 5 of the 6 diabetic subjects. In all subjects, maximum percentage decrease from baseline ranged from 43% to 71%, with a mean  $\pm\text{SD}$  of  $55\% \pm 10\%$ . Average time  $\pm\text{SD}$  to the lowest glucose level was  $153 \pm 27$  minutes. In contrast, the mean reduction in glucose levels after placebo inhalation in 3 subjects was  $13\% \pm 9\%$ .

No pulmonary adverse effects or hypoglycemic symptoms were noted. These preliminary results suggested the feasibility of controlling plasma glucose in diabetic subjects during the fasting state by oral inhalation of insulin aerosol. It was pointed out that the time required for the peak insulin level was variable between subjects ( $\text{CV} = 85\%$ ). Nevertheless, this variability did not appear to significantly affect time to maximum decrease in plasma glucose level between subjects, which was more predictable (ranging from 120 to 200 minutes) with a CV of 18%.

Laube BL, et al. *JAMA*. 1993;269:2106-2109.

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**Editor's comment:** This is an exciting and very well done study that proves the short-term efficacy of inhaled insulin in reducing plasma glucose levels in NIDDM patients. The authors of this study were able to overcome the difficulties of delivering insulin to the lungs that plagued previous researchers in this field (Wigley et al. *Diabetes*. 1971;20:552; Elliot et al. *Aust Paediatr J*. 1987;23:293) as well as the adverse signs or symptoms that occur with nasal inhalation of insulin (Moses et al. *Diabetes*. 1983;32:1040; Salzman et al. *N Engl J Med*. 1985;312:1078).

Inhaled insulin offers a safe and painless mode of insulin delivery that possibly could completely eliminate insulin injections in the treatment of NIDDM. In addition to avoiding shots by using aerosolized insulin, it may be possible to administer more frequent medication to diabetics and, therefore, allow them more flexibility in daily life while attaining better control of the disease. Of course, more studies, including those utilizing insulin-dependent patients, are necessary to derive more information about the kinetics, action, and metabolism of inhaled insulin. Also, more information is needed to ascertain if there is a difference in allergic reactions or other complications between inhalation and injection modes of therapy, as well as long-term safety and efficacy of this new treatment modality.

Clinical proof of practicality of inhaled treatment may open a new horizon in endocrinology. The authors have raised the possibility that other peptide hormones currently administered by injection, such as growth hormone and calcitonin, might also be effectively delivered as an aerosol through the lungs.

Fima Lifshitz, MD

**2nd Editor's comment:** Laube and colleagues have presented some intriguing new information concerning an alternative method of insulin delivery. Clearly their work is an advance over earlier studies of inhaled insulin, but should be regarded as very preliminary. No information is given regarding insulin kinetics (onset of action, duration, etc.) nor the actual patient time involved in administering the 8 to 13 inhalations necessary to deliver 1 U/kg. Reductions in blood glucose ranged from 43% to 71%, demonstrating tremendous variability in the biologic activity of a dose that is standardized when it leaves the inhaler. Such variability increases the potential for both severe hypoglycemia and recurrent hyperglycemia. In addition, frequent administration of short-acting insulin would not obviate the need for long-acting insulin to prevent fasting ketosis and the hyperglycemia associated with the dawn phenomena. Finally, experience in treating children and adolescents who have asthma with inhaled corticosteroids and beta-adrenergic agents suggests that the accuracy of inhalation treatment relies heavily on technical skills that are at best variable. Thus, it is unlikely that such therapy would be a practical tool for achieving near-normal glucose levels.

The need for more convenient, less painful methods of insulin delivery is clear. The *Diabetes Control and Complications Trial* (GGH. 1993;9:3) results suggest that intensive therapy can be associated with a reduction in the microvascular complications of diabetes. The majority of subjects requiring intensive therapy in that study injected insulin 3 or more times daily. Any method that might facilitate frequent insulin administration could be of potential benefit to persons attempting to achieve near-normal blood glucose levels. It would appear, however, that inhaled insulin as described in the present paper might lead to such a variable effect as to be of little value at the present time.

William L. Clarke, MD

## Prader-Willi Syndrome: Consensus Diagnostic Criteria

Because of the difficulty in identifying patients with Prader-Willi syndrome (PWS), the authors developed a list of major and minor diagnostic criteria and supportive findings (see Table 1 and Figure 1) and a scoring system designed to aid in this effort. Each major criterion is assigned 1 point and each minor criterion a 1/2 point; supportive criteria are assigned no value. To establish the diagnosis of PWS in a child <3 years of age, a point score of 5 (at least 4 from the major group) is required, and for a subject >3 years of age a point score of 8 (at least 5 from the major group) is required.

Holm VA, et al. *Pediatrics*. 1993;91:398-402.

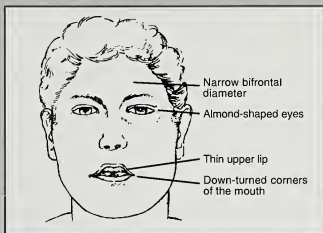
**Editor's comment:** This reviewer has found that one of the most difficult aspects of making the diagnosis of PWS has been to consider this disorder in the differential at the very beginning. Recognition of this disorder is particularly hard in early infancy

Table 1  
Diagnostic Criteria for Prader-Willi Syndrome

- I. Major Criteria (1 point each)
  - A. Hypotonia in the neonatal period
  - B. Failure to thrive in infancy and early childhood
  - C. Rapid weight gain after 1 year of age
  - D. Characteristic facial features (see Figure 1)
  - E. Hypogonadism
    1. Small phallus, cryptorchidism (male)
    2. Delayed gonadarche
  - F. Developmental delay
  - G. Hyperphagia; aggressive food-seeking behavior
  - H. Deletion of 15q11-13 or evidence of maternal disomy
- II. Minor Criteria (1/2 point each)
  - A. Decreased in utero activity
  - B. Behavioral abnormalities: temper tantrums, violent outbursts, obsessive-compulsive, rigid, argumentative, oppositional, stubborn, lying (5 or more required)
  - C. Sleep disturbances/apnea
  - D. Short stature (relative to bone age)
  - E. Hypopigmentation
  - F. Small hands/feet
  - G. Narrow hands with straight ulnar border
  - H. Esotropia, myopia
  - I. Viscous saliva
  - J. Articulation difficulty
  - K. Skin picking
- III. Supportive Findings (no points)
  - A. High pain threshold
  - B. Decreased vomiting
  - C. Temperature instability
  - D. Scoliosis/kyphosis (in the second decade)
  - E. Early adrenarche
  - F. Osteopenia
  - G. Skilled at jigsaw puzzles
  - H. Normal neuromuscular studies

Adapted with permission from Holm VA, et al. *Pediatrics*. 1993;91:398-402.

Figure 1  
Facial Features in Prader-Willi Syndrome



Reprinted with permission from Holm VA, et al. *Pediatrics*. 1993;91:398-402.

when a myriad of causes may result in hypotonia and failure to thrive. In the male infant with small phallus or cryptorchidism, this diagnosis comes more readily to mind, but in the hypotonic female infant the diagnosis is more difficult, even with standards for clitoral size, which are primarily useful for identifying the enlarged, rather than the small, clitoris. As the hypotonia improves, hyperphagia develops and the older infant or young child becomes obese. Therefore developmental delay becomes apparent and the diagnosis becomes evident as well.

The diagnostic criteria set forth by Holm et al are indeed useful, particularly for general pediatricians, who initially see these patients. In the experience of this editor, these criteria and scoring system have been employed to rule out PWS in obese, developmentally delayed children who are often referred with possible PWS. However, rigid adherence to these criteria is to be avoided and clinical judgment trusted when a given point score is not realized at any one time during the course of this disorder. Further observation will often clarify the situation.

The criteria also point out some important aspects of PWS, including the increased occurrence of premature adrenarche and osteopenia, which is accompanied by a propensity for fracture with minimal trauma. Mention is made of growth hormone therapy for short stature associated with PWS, but that is currently a subject of investigation and significant controversy.

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## MEETINGS CALENDAR

**March 13-15, 1994** "Genes in Development and Cancer" March of Dimes Clin Genet Conf, Kissimmee, FL. Info: C Blagowidow. Tel: 914-997-4552; Fax: 914-997-4560.

**March 15-17, 1994** Amer Coll of Med Genet, 1st Ann Mtg, Kissimmee, FL. Info: E Strass. Tel: 301-571-1826; Fax: 301-530-7079.\*

**April 10-14, 1994** Intl Mtg on Sex Hormones & Antihormones in Endo-Dependent Pathol: Basic and Clin Aspects, Milan, Italy. Info: Drs M Motta/M Serio. Tel: 39-2-2940-6576; Fax: 39-2-2940-4927.

**April 20-23, 1994** 1st Postgrad Clin Endo Course of the Euro Fed of Endo Soc, Gerona, Spain. Info: Prof FF Casanueva. Fax: 34-81-572-121.

**April 28 - May 1, 1994** 3rd Ann Mtg & Clin Cong of the Amer Assn of Clin Endo, New Orleans, LA. Info: L Kepner/A Jones. Tel: 904-384-9490; Fax: 904-384-8124.\*

**May 2-5, 1994** APA/APS/SPR Ann Mtg, Seattle, WA. Info: D Anagnostis. Tel: 708-427-1205; Fax: 708-427-1305.

**May 25-27, 1994** 4th Intl Mtg of Endo, Rome, Italy. Info: Sero Symposia. Tel: 39-6-442-91-229; Fax: 39-6-442-91-324.\*

**June 1-4, 1994** 1st Intl Mtg of the GH Research Soc, Aarhus, Denmark. Info: Drs J Christiansen/J Jorgensen. Tel: 45-86-1255-55/ext 2084; Fax: 45-86-1378-25.

**June 8-14, 1994** 54th Ann Mtg of the ADA, New Orleans, LA. Info: ADA. Tel: 703-549-1500/ ext 330; Fax: 703-836-7439.

**June 17-24, 1994** 3rd Eur Cong of Endo, Amsterdam, Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9474.\*

**June 23-24, 1994** 8th Intl Study Group on Diabetes Treatment with Implantable Insulin Delivery Devices Mtg, Nice, France. Info: Prof J Selam. Tel: 33-1-4234-8376; Fax: 33-1-4354-1564.

**June 15-18, 1994** 76th Ann Mtg of the Amer Endo Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.\*

**June 22-25, 1994** 33rd Ann Mtg of the ESPE, Maastricht, Netherlands. Info: Prof J Van den Brande. Tel: 31-30-32-0521; Fax: 31-30-33-4825.

**June 30-July 3, 1994** 2nd Intl Cong on Prader-Willi Syndrome, Cambridge, England. Info: Dr B Laurance.\*

**July 17-24, 1994** 3rd Eur Cong of Endo, Amsterdam, Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

**August 20-25, 1994** 7th Intl Cong on Obesity, Toronto, Can. Info: Univ of Toronto, CME. Tel: 1-416-978-2719; Fax: 1-416-971-2200.

**October 30-November 3, 1994** 46th Postgrad Assembly of the Amer Endo Soc, Toronto, Can. Info: W Johnson. Tel: 301-571-1807; Fax: 301-571-1869.

**November 2-5, 1994** 20th Ann Mtg of the ISGD/IDF Mtg, Tokyo, Japan. Info: Prof T Kitagawa. Tel: 81-3-293-1711-212.\*

**November 6-11, 1994** 15th World Cong of the IDF, Kobe, Japan. Sci Info: Prof S Baba. Tel: 81-78-303-0055; Fax: 81-78-302-7303.

**June 14-17, 1995** 77th Mtg of the Amer Endo Soc, Washington, DC. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.

**June 25-28, 1995** 34th Ann Mtg of the ESPE, Edinburgh, Scot. Info: Prof Dr W Sippell. Tel: 49-431-597-1626; Fax: 49-431-597-1675.\*

**1996** 78th Mtg of the Amer Endo Soc, San Francisco, CA. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.

**Spring 1996** 34th Ann Mtg of the ESPE, Montpelier, France. Info: Prof C Sultan.\*

**June 5-7 1996** 22nd Ann Mtg of the ISGD, Pittsburgh, PA. Info: Prof AL Drash. Tel: 412-692-5851; Fax: 412-692-5960.\*

**July 20-25, 1997** 16th Cong of the IDF, Helsinki, Finland. Info: Prof M-R Taskinen. Fax: 358-0-411-244.

**July 1997** 23rd Ann Mtg of the ISGD in conjunction with the IDF Mtg, Helsinki, Finland. Info: Prof H Akerblom. Tel: 358-0-471-2701.

**June 22-26, 1997** 5th Intl Mtg of the LWPES/ESPE, Stockholm, Sweden. Info: Prof Dr W Sippell. Tel: 310-825-6244; Fax: 310-206-5843.\*

\*Confirmation not received prior to publication.

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# GROWTH

## Genetics & Hormones

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## Overgrowth Syndromes and Disorders: Definition, Classification, and Discussion

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A recent review of the literature made it apparent that a confusing group of disorders with excessive growth and/or development exists. These have been labeled *overgrowth syndromes*. The confusion exists partly because there is no accepted definitive definition or classification of these disorders. The purpose of this article is to present definitions of, and a classification system for, the known types of overgrowth syndromes and disorders.

### PROBLEMS WITH DEFINITION AND CLASSIFICATION

Problems with definition and classification of overgrowth conditions are illustrated best by a few examples. One classic overgrowth syndrome is the Beckwith-Wiedemann syndrome. Most children with this condition have excessive prenatal and postnatal growth involving both height and weight, and have macroglossia, visceromegaly, and advanced skeletal maturation. The condition is a classic overgrowth syndrome since there is excessive growth in most growth parameters. Interestingly, however, the head size and presumably the brain size in children with Beckwith-Wiedemann syndrome are normal.

The Marshall-Smith syndrome is another example of the confusion associated with overgrowth conditions. This condition is characterized by a prominent calvarium, forehead, and eyes, a low nasal bridge, an upturned nose, micrognathia, widened middle and distal phalanges, best appreciated on radiographs, markedly advanced skeletal maturation, respiratory distress, frequent pneumonias, failure to thrive, and often death during infancy.<sup>1</sup> Even though the Marshall-Smith syndrome is associated usually

### Letter From the Editor

Human growth hormone (hGH) has been used in humans for 35 years (1958-1993). The benefits to growth hormone deficient (GHD) children is well known to all of us. For the first 27 years (1958-1985), we were impressed with the safety of the hormone when it was used in physiologic amounts. In 1985, 2 cases of Creutzfeldt-Jakob disease were described in patients who received hGH many years previously. Subsequently, due to various conjectural reports, concern developed whether hGH produces leukemia, tumor recurrence in the CNS, immunologic deficiency, atherosclerosis, other cardiovascular disease, and/or an increased incidence of diabetes mellitus.

In response to these suspicions, the European Society of Paediatric Endocrinology (ESPE) established a committee of renowned pediatric endocrinologists to review all available data regarding these suspicions and to publish in the official journal of ESPE, *Hormone Research*, a statement concerning the safety and/or toxicity of hGH therapy. This has been accomplished and an official statement from ESPE was published in *Horm Res* 1993;39:92-110. The Lawson Wilkins Pediatric Endocrine Society approved the report.

Continued on page 5

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with a normal birth weight and failure to thrive, the condition has been classified in the past as an overgrowth syndrome.<sup>2</sup> This occurred since normally a dramatic increase in the overall bone age is found with the carpal bone maturation being even more advanced.

The Patterson-David syndrome also illustrates the problem in classifying overgrowth conditions. This relatively unknown syndrome has been confused with leprechaunism (Donohue syndrome).<sup>3,4</sup> Patients with the Patterson-David syndrome present with redundant, loose folds of skin of the hands, large ears, hands, and feet, phallic enlargement in males, generalized bronzed hyperpigmentation, hirsutism, severe mental retardation, and characteristic skeletal changes. In this syndrome the birth weight and length are usually at the 97th percentile; the postnatal length is in the high normal range but the weight typically drops below the 3rd percentile (which probably accounts for the confusion with Donohue's syndrome). Since birth weight and length are increased and since there is redundancy of the skin, large ears, large penis, and large hands and feet, one can make a case that this condition truly is an overgrowth syndrome, despite the postnatal weight deficiency.

A final example includes the localized overgrowth disorders such as isolated macrodactyly. Should these disorders be considered under the umbrella of "overgrowth syndromes"? Perhaps not, but they clearly do represent overgrowth in localized tissues or organs and clearly constitute overgrowth disorders.

## DEFINITION AND CLASSIFICATION

Keeping in mind the above examples, I propose that an overgrowth syndrome or disorder simply be defined as a condition in which there is either localized or generalized excessive growth and/or development for the age and sex of the individual. Under this definition, most overgrowth syndromes or disorders can be classified into 1 of the following 3 general categories:

1. Generalized overgrowth syndromes
2. Regional overgrowth disorders
3. Parameter-specific overgrowth disorders

Generalized overgrowth syndromes which include the classic overgrowth conditions are those in which all or most parameters of growth and physical development are in excess of 2 standard deviations (SD) above the mean for the person's age and sex. The relatively few conditions that fall into this category are listed in Table 1. The regional overgrowth disorders include those in which excessive growth is confined to one or a few regions of the body. An example is benign familial macrocephaly, an

autosomal dominant condition associated with a large dolichocephalic head and normal intelligence. Some of the conditions classified as regional overgrowth disorders are listed in Table 2. Finally, there are parameter-specific overgrowth disorders in which a single or, at most, several growth parameters are in excess of normal. Familial idiopathic obesity and Prader-Willi syndrome are examples that belong to this category. Others are listed in Table 3.

Table 1  
**Generalized Overgrowth Syndromes\***

Bannayan-Riley-Ruvalcaba syndrome  
(Bannayan-Zonana syndrome, Ruvalcaba-Myhre syndrome, or Riley-Smith syndrome)<sup>†</sup>  
Beckwith-Wiedemann syndrome<sup>†</sup>  
Diabetic embryopathy  
(infants of diabetic mothers)<sup>†</sup>  
Elejalde syndrome<sup>†</sup>  
Familial rapid maturation  
Familial tall stature  
Fragile X syndrome  
Gigantism/acromegaly  
Hyperthyroidism, congenital  
Hyperthyroidism, infancy and childhood  
Klinefelter syndrome  
Marfan syndrome  
Nevo syndrome<sup>†</sup>  
Perlman syndrome<sup>†</sup>  
Precocious puberty  
(precocious adolescence)  
Precocious gonadotropin-induced  
adolescence  
Congenital adrenal hyperplasia,  
untreated  
Hormone-secreting tumors  
Interstitial cell tumor with androgen  
production in males  
Granulosa cell tumor with inappropriate  
estrogen production in females  
Simpson-Golabi-Behmel syndrome  
(Golabi-Rosen syndrome)<sup>†</sup>  
Sotos syndrome (cerebral gigantism)<sup>†</sup>  
Teebi-type overgrowth syndrome<sup>†</sup>  
Trisomy 8 mosaicism (Warkany syndrome)<sup>†</sup>  
Weaver syndrome<sup>†</sup>

\* List is not all-inclusive.

<sup>†</sup> Excessive growth and/or weight is usually present at birth in this condition.

The above classification scheme is a modification of the one used by Beighton<sup>5</sup> who divided overgrowth conditions into generalized overgrowth, obesity, localized overgrowth, and digital overgrowth syndromes. Cohen<sup>6</sup> also has categorized overgrowth conditions according to whether the condition is a normal variant of growth, such as familial tall stature, or whether the overgrowth is of prenatal onset as in Sotos syndrome, or whether the overgrowth is of postnatal onset as that occurring with early and excessive production of sex hormones.

## GENERAL CHARACTERISTICS

Because of the marked diversity of features associated with the various overgrowth syndromes and disorders, no general statements can be made about common characteristics in these conditions. The exception is that they are associated with excessive growth or development of one type or another. However, the conditions in the generalized overgrowth category that have excessive growth at birth (denoted by † in Table 1) share a few common characteristics. These characteristics include the following.<sup>6</sup>

1. Weight is generally increased as much as length.
2. The condition is usually associated with various other anomalies.
3. Mental deficiency often is present.
4. Neoplasias occur at a higher than expected frequency.

## INCIDENCE AND NUMBER OF OVERGROWTH SYNDROMES AND DISORDERS

The incidence of each overgrowth syndrome or disorder varies tremendously, being as common as 1 in 1,000 to 1,500 as occurs with the fragile X syndrome, to less than 1 in 1,000,000 births in Elejalde syndrome and others. Elejalde syndrome is a striking prenatal overgrowth syndrome that has been reported in only 3 siblings.<sup>7</sup>

If one accepts the classification system presented above, the number of currently recognized overgrowth conditions is dramatically large. A number of growth parameters and the corresponding number of recognized conditions associated with each of these features is presented in Table 4. These data were generated from 2 syndrome data bases, Pictures of Standard Syndromes and Undiagnosed Malformations or POSSUM and the London Dysmorphology Data Base or LDDB, and clearly indicate the number and complexity of syndromes that can be classified as overgrowth syndromes or disorders.

Table 2  
Conditions Classified as  
Regional Overgrowth Disorders\*

Cutis marmorata telangiectatica congenita  
Familial macrocephaly  
Hemifacial microsomia-macrodactyly syndrome  
Hemihyperplasia (hemihypertrophy)  
Klippel-Trenaunay-Weber syndrome  
Macrodactyly  
Maffucci syndrome  
Neurofibromatosis  
Ollier syndrome  
Patterson-David syndrome  
Proteus syndrome

\* List is not all-inclusive.

## ETIOLOGY AND PATHOPHYSIOLOGY

A whole gamut of genetic etiologies is associated with overgrowth syndromes and disorders. For instance, an autosomal dominant gene mutation (FBN1) is the cause of Marfan syndrome. The Perlman syndrome is produced by an autosomal recessive mode of inheritance. The Simpson-Golabi-Beckwith syndrome is inherited in an X-linked recessive fashion, and familial tall stature is polygenetic in etiology. Genomic imprinting is the usual cause of Prader-Willi syndrome, since

Table 3  
Conditions Classified as  
Parameter-Specific Overgrowth Disorders\*

Berardinelli lipodystrophy or Seip-Berardinelli syndrome (tall stature and advanced skeletal maturation)  
Börjeson-Forssman-Lehmann syndrome (obesity)  
Cohen syndrome (obesity)  
Congenital contractural arachnodactyly or Beals syndrome (tall stature)  
Familial idiopathic obesity  
Marshall-Smith syndrome (accelerated skeletal maturation)  
Michelin tire baby syndrome (subcutaneous lipomatous nevus)  
Prader-Willi syndrome (obesity)  
Teebi macrosomia-microphthalmia-cleft palate syndrome (obesity)

\* List is not all-inclusive.



the disorder usually is associated with either an interstitial deletion (q11 to q13) of the paternally derived chromosome 15 or maternal uniparental disomy of chromosome 15. In many other overgrowth syndromes and disorders, eg, Proteus syndrome, all reported cases have been sporadic and the etiologies of the conditions are unknown.

Because of the diverse genetic mechanisms causing overgrowth syndromes, and because of the varied manifestations of excessive growth found in individuals with overgrowth conditions, there must be a multitude of mechanisms producing excessive growth. It is indeed intriguing to consider the vast knowledge about human growth and the regulation of cell division and growth that we will have when we come to understand all of the mechanisms producing overgrowth and its associated syndromes and disorders.

Most overgrowth conditions result from either hyperplasia, hypertrophy, an increase in the interstitium, or some combination of these 3 factors.<sup>6</sup> With the exception of certain hormone disorders such as untreated congenital adrenal hyperplasia, acromegaly, and diabetic embryopathy, the causes for these changes are unknown. Perhaps abnormal states of insulin-like growth factors (IGFs), their cell-surface receptors, insulin-like growth factor-binding proteins, epidermal growth factors, human placental lactogen (chorionic somatomammotropin), and the regulators of these factors cause many of these disorders.<sup>8,9</sup> In addition, perhaps partial or complete disruption of the normal function of proto-oncogenes or tumor-suppressor genes results in regional overgrowth disorders in some cases, although none is recognized to do so at the present time.

An abnormal accumulation of body fluid, as seen in hydrops fetalis or anasarca, can cause an increase in the size and weight of a fetus or individual. However, these categories of disorders have not been included in the classification scheme presented here since the accumulation of fluid does not truly represent excessive growth in the normal sense of the word.

## EVALUATION, COUNSELING, AND FOLLOW-UP

The evaluation of a child with an unrecognized overgrowth condition should be individualized, and based on the type of overgrowth condition present and the presence of other abnormalities. Such an evaluation might normally include: (1) a careful patient history, family history and physical examination; (2) appropriate physical measurements; (3) complete skeletal survey, including bone age; (4) chromosomal analysis that might include specialized testing for specific conditions, eg, fragile X syndrome; (5) urine analysis for metabolic disorders; and (6) endocrine studies, including serum IGF-1 and thyroid function

Table 4  
Overgrowth Parameters and the Number  
of Syndromes and Disorders Listed in  
2 Syndrome Data Bases

| FEATURE   | Number of Disorders |       |
|---|---------------------|-------|
|   | POSSUM*             | LDDB† |
| Macrocephaly  | 166                 | 137   |
| Macroductyly  | 8                   | 10    |
| Tall stature  | 49                  | 44    |
| Asymmetry of the body<br>with hemihypertrophy/<br>hemiatrophy | 36                  | 16    |
| Truncal and<br>generalized obesity                            | 49                  | 80    |
| Excessive birth weight  | 20                  | 23    |
| Advanced osseous<br>maturation                                | 36                  | 43    |
| Hepatomegaly  | 91                  | 99    |
| Long and/or large ears  | 133                 | 77    |
| Large phallus   | 22                  | 13    |
| Macrotestes   | 13                  | 8     |
| Large hands   | 47                  | 23    |

\* Pictures of Standard Syndromes and Undiagnosed Malformations, Version 3.0, 1991

† London Dysmorphology Data Base, 1991

studies. Other studies would be dictated by the patient's history and examination. In many situations it is appropriate to evaluate the parents and siblings of the affected child. It is also necessary to run serial glucose levels on all neonates with generalized overgrowth to detect hypoglycemia. Children with either the Beckwith-Wiedemann syndrome, hemihyperplasia, or the Simpson-Golabi-Beckwith syndrome need to be evaluated on a regular basis for intra-abdominal tumors. Finally, appropriate genetic counseling and long-term follow-up care should be provided to both the family and the child with an overgrowth condition.

Extensive summaries of overgrowth conditions, in addition to specific information about these conditions, are found in articles and chapters by Beighton,<sup>5</sup> Cohen,<sup>6</sup> and Gorlin and associates.<sup>2</sup> The reader will find these references of benefit in evaluating the child with overgrowth.

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## Letter From the Editor (continued)

The report of ESPE is remarkably thorough and the committee members (E. Martin Ritzén, Paul Czernichow, Michael Preece, Michael Ranke, and Jan Marten Wit) are to be highly commended. Separate consideration in the document is given to each physiologic/pathologic entity under suspicion. Many readers of GGH will wish to obtain a copy and review all or part of this report now or at a future date. Its length prevents its publication here; however, the summary that is listed as a foreword in the manuscript, is reprinted here.

### *Safety of Human Growth Hormone Therapy*

Since the introduction of recombinant human growth hormone (GH), this previously scarce drug has become available in technically unlimited amounts. The indications for its use to promote growth has been widened, and many clinical studies are presently under way to test its usefulness also in other conditions than short stature secondary to a proven GH deficiency. Several reports have pointed at possible side effects of GH therapy. Therefore, the ESPE has called on an ad hoc committee to prepare a document describing the present knowledge on proven or suspected adverse effects of GH therapy concerning infectious agents (notably Creutzfeldt-Jakob disease), connection (if any) with malignancies, with disorders of the immune response, carbohydrate and lipid or water/electrolyte metabolism. Mini reviews on these fields are attached to this document as appendices.

Human GH extracted from cadaver pituitaries have been shown sometimes to be contaminated with the infectious agent causing Creutzfeldt-Jakob disease. This has been found to be the case for many different batches of human GH, prepared in many laboratories using different extraction and purification procedures. Due to the very long incubation period of up to 25 years, no single procedure of purification can be proven to be safe until that period has passed. It must therefore be stated clearly that it is no longer acceptable to use any preparation of GH extracted from human pituitaries. On the other hand, there is no reason to believe that recombinant human GH in any way influences the development of Creutzfeldt-Jakob disease or any other infectious disease.

Brain tumors may be the original cause of GH deficiency, and irradiation of the brain as a therapy for tumors may result in GH deficiency. Therefore, many children who have previously had a malignancy are now under treatment with GH. As of today, comparisons of groups of children with such a medical history, with and without subsequent

GH treatment, have failed to show any difference in relapse rate. Leukemia has been reported to be over-represented in Japanese individuals who at some time previously were treated with GH. In worldwide surveys, the slight increase of leukemia observed after GH treatment is only seen when including patients which have other specific risk factors. When such patients are excluded from analyses, children treated with GH do not differ from the general population.

GH decreases the sensitivity to insulin. Therefore, an individual who is in a pre-diabetic state with markedly impaired maximal insulin release might develop clinically obvious insulin deficiency when GH treatment is begun. There is no evidence that GH impairs insulin secretion. Thus, GH may reveal diabetes mellitus but does not cause it.

GH causes immediate and long-term changes in lipid metabolism. Both GH deficiency and excess (as in acromegaly) may predispose to early atherosclerosis. The full effects of normal substitution doses of GH is presently not fully known, but also in this respect it should be of advantage to the growth hormone deficient individual. The consequences of long-term high-dose treatment need further studies.

GH influences the metabolism of water and sodium through the renin-angiotensin system, causing an increase in total body water. This effect is transient and probably dose-related. It is less pronounced in children than adults but may be of significance in patients with impaired lymph drainage, such as Turner's syndrome. If GH is used for patients suffering from heart disease, blood pressure and fluid retention must be monitored during the early phase of treatment.

Most lymphoid cells possess receptors for GH and IGF-I, and GH administration leads to subtle and variable changes in some laboratory parameters of immune function. No clinical symptoms associated with immune dysfunction have been reported in children receiving GH therapy.

In conclusion, human recombinant GH seems to be a remarkably safe drug when used in conventional substitution doses. However, since it is also used in a number of patients with other causes of short stature and at higher than physiological doses, it is important that methods of long-term (decades!) surveillance are developed. In some countries the whole population of GH-treated individuals may be monitored, in other countries representative samples may be followed. Setting up such an organization should be the responsibility of the national health authorities.

Robert M. Blizzard, MD  
Jean-Claude Job, MD

# The Etiology and Diagnosis of Overgrowth Syndromes

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Although intrauterine growth retardation is well recognized to be a frequent feature in a number of multiple malformation syndromes, much less attention is paid to overgrowth either as an isolated defect in an otherwise normal individual or as one feature of a multiple malformation syndrome. Since the initial delineation in 1963 of the Beckwith-Wiedemann syndrome (BWS), a number of specific overgrowth syndromes have been described. For most, endocrine and other metabolic studies have not been helpful in explaining the mechanisms of overgrowth. Only recently have some of the newer molecular techniques begun to shed light on etiology. The purpose here is to set forth the principal diagnostic features of the most common overgrowth

syndromes and to present, when available, data regarding etiology and/or developmental pathogenesis. Not covered in this review are 2 rare disorders seen in single families: Elejalde syndrome<sup>1</sup> and Nevo syndrome.<sup>2</sup> In addition, a number of conditions with asymmetric overgrowth, including neurofibromatosis, Klippel-Trenaunay-Weber syndrome, Proteus syndrome<sup>3</sup> and isolated congenital hemihypertrophy,<sup>4</sup> have not been included.

## BECKWITH-WIEDEMANN SYNDROME

Initially delineated independently by Beckwith<sup>5</sup> and by Wiedemann,<sup>6</sup> more than 400 cases have been reported. The principal features are set forth in Table 1 and are discussed below.

**Growth:** Mean birth length for males is greater than the 95th percentile for gestational age. Thereafter length parallels the normal curve at or above the 95th percentile including through adolescence. For females, mean birth length is at the 75th percentile and increases to the 95th percentile by 18 months of age. After 9 years, mean weight remains between the 75th and 95th percentile. Advanced bone age, most pronounced during the first 4 years, only rarely persists until maturity. Spontaneous pubertal development occurs within normal limits for chronologic age and around the 50th percentile for bone age.<sup>7,8</sup>

**Other:** Cardiovascular abnormalities, including structural defects and/or cardiomegaly, occur in approximately one-third of patients. Malignant tumors, the majority of which are Wilms' tumor, adrenal carcinoma, and/or hepatoblastoma, occur in about 7% of cases.<sup>7</sup> An increased risk of malignancy seems to be associated in those children who have hemihypertrophy.<sup>3</sup>

**Etiology:** The gene for BWS is located at 11p15.5.<sup>9</sup> Based on a number of clinical observations, it now seems clear that the characteristic phenotype in this condition occurs as a result of a variety of different genetic mechanisms, all of which result in a dosage imbalance of the gene.<sup>10,11</sup> Currently, it appears that the maternal copy of the BWS gene normally is imprinted or inactivated. Therefore, there is normally only 1 active copy of the gene functioning at any given time (ie, the paternal copy). Evidence in support of this is set forth schematically in Figure 1 and includes the following: chromosomal abnormalities that cause duplication of the BWS locus at 11p15.5 produce the BWS phenotype when they are paternally derived and, thus, associated with 2 active copies of the gene. Chromosomal inversions and translocations involving the BWS

Table 1  
Beckwith-Wiedemann Syndrome

| Parameter                 | Abnormalities   |
|---------------------------|---|
| Growth                    | Prenatal and postnatal overgrowth<br>Accelerated osseous maturation   |
| Craniofacial              | Macroglossia<br>Capillary nevus flammeus<br>Prominent eyes with relative infraorbital hypoplasia<br>Prominent occiput<br>Mandibular prognathism<br>Ear lobe creases and/or posterior helical pits   |
| Hyperplasia and Dysplasia | Pancreatic hyperplasia<br>Adrenocortical cytomegaly<br>Large kidneys with renal medullary dysplasia<br>Interstitial cell hypoplasia of gonads<br>Pituitary amphophil hyperplasia  |
| Other                     | Omphalocele or other umbilical defect<br>GI malrotation<br>Diaphragmatic eventration<br>Cardiovascular defects<br>Cryptorchidism<br>Intra-abdominal tumors<br>Hemihypertrophy<br>Polyhydramnios<br>Neonatal polycythemia<br>Hypoglycemia in early infancy |

locus produce the phenotype if they are inherited from the mother. Presumably, disruption of the locus causes activation of a gene that is normally imprinted and thus inactive. Also, the BWS phenotype has been seen in conjunction with paternal disomy, a situation in which both BWS loci are inherited from the father, giving 2 active copies of the gene. Recently Weksberg and colleagues<sup>12</sup> have documented relaxation of imprinting at 11p15.5 in cytogenetically normal, sporadic cases of BWS. These individuals have both maternal and paternal copies of the alleles; however, the maternal copy is inadequately methylated and thus activated.

Another curious finding in BWS that is compatible with the imprinting hypothesis is the observation of an excess of female monozygotic twins discordant for the BWS phenotype. Presumably, this relates to discordant X inactivation in the twins or discordant inactivation at the BWS locus itself. This hypothesis suggests that the process of X inactivation relates to the process of imprinting at autosomal loci. Monozygotic twins with different inactive X chromosomes would also be imprinted differently at the BWS locus, causing discordant phenotypes in a presumed single gene disorder.

A number of endocrine studies have been performed in children with this disorder.<sup>13</sup> Because of the localization of the insulin-like growth factor 2

(IGF-2) gene to 11p, abnormalities in the insulin-like growth factors would seem to represent the most likely cause of the overgrowth. It is of particular interest that the overgrowth in this disorder is in organs known to be rich in autogenous IGF-2, and that the tumors that occasionally occur in patients with this syndrome demonstrate high levels messenger RNA for IGF-2. Despite this, elevated levels of serum somatomedins have not been found in individuals with this disorder. The hypoglycemia is usually noted in the first 24 hours, but may be delayed to the third day of life, and is due to nesidioblastosis with B-cell hyperplasia and hyperinsulinism.

Although 3 cases of hypothyroidism have been documented, thyroid abnormalities do not explain the overgrowth. Studies of growth hormone, adrenal, and gonadal hormone levels are normal.

## SOTOS SYNDROME

The principal features of this disorder<sup>14</sup> are set forth in Table 2.

**Growth:** Birth length is frequently greater than the 97th percentile while birth weight is usually within the normal range. After rapid linear growth throughout the first year of life, the height stabilizes at or just above the 97th percentile. Although only limited data are available, adult height is usually within the upper normal range.<sup>15,16</sup>

The difference between bone and chronologic ages gradually increases from  $0.6 \pm 0.5$  years during the first year to  $1.8 \pm 0.5$  years during the fourth and fifth years. Thereafter, the difference stabilizes at 2 to 2.8 years.

A characteristic metacarpophalangeal profile has been established which is based on evaluation of 16 affected patients.<sup>17</sup> Although all had hand bones longer than the mean for normal individuals, the distal phalanges were short relative to the metacarpals and especially to the proximal phalanges.<sup>16</sup>

Table 2  
Sotos Syndrome

| Parameter                | Abnormalities   |
|--------------------------|---|
| Growth                   | Prenatal onset of excessive size<br>Advanced osseous maturation<br>Large hands and feet   |
| Performance              | Mental deficiency<br>Poor coordination  |
| Craniofacial             | Macrocrania<br>Prominent forehead (dolichocephaly)<br>Frontoparietal balding<br>Down-slanting palpebral fissures<br>Ocular hypertelorism<br>Rosy coloring to cheeks and nasal tip<br>Prognathism with pointed chin<br>High, narrow palate with prominent lateral palatine ridges<br>Premature eruption of teeth |
| Occasional Abnormalities | EEG abnormalities<br>Kyphoscoliosis<br>Congenital heart defects<br>Neoplasm (5%)<br>Hemihypertrophy<br>Abnormal glucose tolerance test (14%)<br>Thin, brittle nails   |

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**Performance and CNS Defects:** Mental deficiency (average IQ, 72) was reported in 85% of individuals affected.<sup>15</sup> Delayed onset of walking and talking almost always is present and clumsiness is common.

Magnetic resonance imaging (MRI) studies of the brain revealed abnormalities of the corpus callosum with complete or partial agenesis or hypoplasia, agenesis of the septum pellucidum and/or septum interpositum, wide or persistent cavum septi pellucidi, hypoplasia of the cerebellar vermi, and large cisterna magna.<sup>18</sup> In addition, in contrast to other disorders associated with macrocrania, individuals with Sotos syndrome have normal sized brains but increased extracerebral and intracerebral fluid spaces.

**Etiology:** The etiology is unknown. Although the majority of cases occur sporadically in otherwise normal families, at least 5 families have been reported in which both parent and offspring are affected, raising the possibility of autosomal dominant inheritance in certain instances.<sup>19</sup>

No consistent endocrine or metabolic abnormalities have been detected, including IGF-1 determinations.<sup>16</sup>

## WEAVER SYNDROME

Initially described in 1974,<sup>20</sup> more than 20 individuals have been reported with this disorder.

**Growth:** Although of prenatal onset, overgrowth is not always present by 1 year of age when most reported individuals are at or above the 97th percentile for length and weight. Of the 2 adults reported, 1 male and 1 female, each had a height and weight greater than the 97th percentile. An accelerated bone age is the rule. The carpal bones were more advanced than the phalanges and metacarpals in most cases.<sup>21,22</sup>

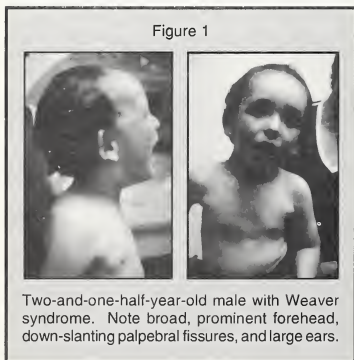
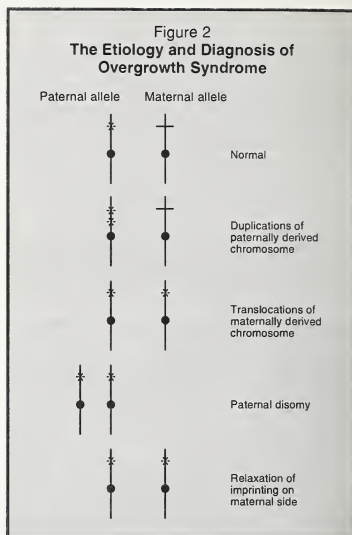


Figure 1

Two-and-one-half-year-old male with Weaver syndrome. Note broad, prominent forehead, down-slanting palpebral fissures, and large ears.



**Performance and CNS Defects:** Although a few affected individuals have performed within the normal range, the majority are mentally deficient with IQ scores ranging from 45 to 70. Hypertonia is the rule. One of the affected adults even lost the ability to walk based on progressive spasticity. A hoarse, low-pitched cry is common in infancy. Computed tomography (CT) scans were obtained in 6 cases. Two studies were normal; 2 revealed a cyst in the septum pellucidum. One revealed nonspecific cerebral atrophy and 1 showed enlarged vessels and hypervascularization in the areas of the middle and left posterior cerebral arteries.

**Craniofacial Characteristics:** Prenatal onset of macrocephaly is common but not invariable, as is the case in the Sotos syndrome.<sup>23</sup> A round face with ocular hypertelorism, down-slanting palpebral fissures, a long philtrum, large ears, and micrognathia are common (Figure 1).

**Limbs:** Features that distinguish Weaver syndrome from other overgrowth syndromes include camptodactyly, widened distal long bones, and clinodactyly of the toes.<sup>23</sup> Other common features include broad thumbs; thin and deep-set nails; prominent fingertip pads, limited elbow and knee extension, and foot deformities.

**Etiology:** The etiology is unknown. Although most cases are sporadic, 2 instances of mildly affected mothers giving birth to severely affected sons raise the possibility of either autosomal dominant inheritance with sex-limited expression or X-linked recessive inheritance (Figure 2).

Endocrine studies, performed in at least 12 affected individuals, were normal with the exception of a 6 7/12-year-old boy with hypothyroidism.<sup>24</sup>

### BANNAYAN-RILEY-RUVALCABA SYNDROME

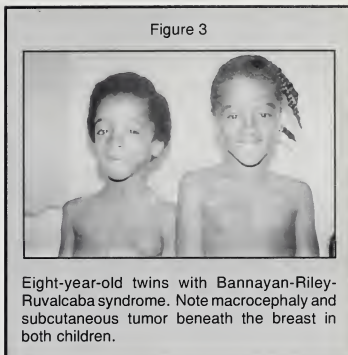
The principal features of this disorder are set forth in Table 3.

**Growth:** Length and weight which are usually greater than the 97th percentile at birth tend to normalize between 3 and 8 years of age, resulting in normal adult stature. Bone age tends to correlate with chronologic age. Macrocephaly, present in the newborn period, remains as a constant feature in adulthood.<sup>25</sup>

**Performance:** Although frank mental deficiency is present in 40-50% of patients, the remainder are of normal intelligence. Electromyographic evidence of a myopathic process has been documented in a number of affected individuals. In some lipid storage myopathy with an increased number of lipid droplets, predominantly in type 1 fibers, was seen.<sup>26</sup>

**Neoplasm:** Mesodermal hamartomas, the majority of which are easily resectable subcutaneous lipomas or hemangiomas, occur in approximately 75% of affected individuals (Figure 3). In addition, ileal and colonic hamartomatous polyps have led to intussusception and rectal bleeding.

**Etiology:** Autosomal dominant inheritance has been documented.



### SIMPSON-GOLABI-BEHMEL SYNDROME

The principal features of this X-linked recessively determined disorder include the following:<sup>27</sup>

**Growth:** A striking prenatal onset of overgrowth occurs with the birth weight as high as 5.9 kg. In 7 out of 8 affected adults, height was greater than the 97th percentile and ranged from 188 cm to 210 cm. The bone age usually is not advanced. Enlargement of the head, which is present at birth, continues in childhood. Ocular hypertelorism, a short broad nose, a large mouth, and macroglossia are common features. Cleft lip, cleft palate, a midline groove of the lower lip, and preauricular pits and tags are seen less frequently. Unilateral coloboma of the optic disc has been noted in one case.

**Performance and CNS Defects:** Mental deficiency is variable with the IQ being normal or somewhat delayed. The average IQ is approximately 1 SD below the mean. Hypotonia is common.

**Other:** Segmental defects of the vertebra are common. Broad halluces and thumbs, postaxial polydactyly of the hands, nail hypoplasia (particularly of the index finger), and partial cutaneous syndactyly of the second and third fingers and toes may occur.

Cryptorchidism and supernumerary nipples occur commonly. Cardiac defects, including bundle-branch block, ventricular septal defects, patent ductus arteriosus, gastrointestinal defects which include intestinal malrotation, pyloric ring and Meckel's diverticulum, and large cystic kidneys occur occasionally.

**Etiology:** X-linked recessive inheritance has been documented. Recent linkage analysis indicates that the locus for this gene maps to the Xq21.3 region.<sup>28</sup> Normal levels of growth hormone and insulin were noted in the 2 affected individuals tested. Partial

Table 3  
Bannayan-Riley-Ruvalcaba Syndrome

| Parameter    | Abnormalities  |
|--------------|--|
| Growth       | Prenatal onset of excessive size<br>Normal adult size                          |
| Performance  | Delayed gross motor function<br>Hypotonia<br>Speech delay<br>Mental deficiency |
| Craniofacial | Macrocephaly with normal<br>ventricular size                                   |
| Eyes         | Prominent Schwalbe lines,<br>prominent corneal nerves,<br>pseudopapilledema    |
| Neoplasm     | Mesodermal hamartomas  |
| Genital      | Pigmentary skin lesions of glans<br>and shaft of penis                         |

expression, including overgrowth and many of the characteristic craniofacial features, has been seen in some of the obligate female carriers. Approximately one half of the reported patients have died of unknown causes prior to 6 months of age.

## DISCUSSION AND CONCLUSION

As more experience has been gained with children affected with various overgrowth disorders, it has become clear that marked clinical similarities exist between them. Many of the diagnostic criteria represent relatively nonspecific growth parameters, and differences in facial features which are often subjective make the differentiation between these syndromes difficult.<sup>18</sup> Therefore, it is important to recognize that certain important generalizations can be made from a practical standpoint when following any child who has overgrowth of prenatal onset. Most importantly, affected children should be evaluated on a frequent basis for the development of tumors. Also important, affected children should be evaluated at an early age since many of these disorders are associated with significant problems that can benefit from early intervention. Finally, it is important to recognize that hemihypertrophy occurs in both BWS and Sotos syndrome. Periodic evaluation for and early recognition of scoliosis secondary to hemihypertrophy hopefully will allow for early intervention and prevention of significant deformity.

### In Future Issues

#### Neuroendocrinology of Growth Hormone Secretion

by Jesus Argente, MD, PhD,  
J.A. Chowen, MD

#### Serum Polypeptide Hormone Binding Proteins

##### Part 1: Growth Hormone Binding Protein Part 2: Insulin-like Growth Factor Binding Proteins

by Allen W. Root, MD

#### Insulin-like Growth Factor 2 and Growth

by Yves Le Bouc, MD

#### Osteochondrodysplasias With Mild Clinical Manifestations: A Clinician's Guide

by Richard M. Pauli, MD

#### Noonan's Syndrome: A Review

by Michael A. Patton, MA, MSc, MD

#### Prader-Willi Syndrome: The Unfolding Genetic Story

by Uta Francke, MD

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# The Differentiation of Constitutional Growth Delay From Nutritional Dwarfism (ND)

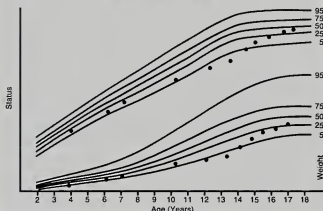
Constitutional growth delay (CGD) is characterized by a retarded linear growth beginning during the first 2 years of life.<sup>1,2</sup> After age 3 years, the growth of CGD patients typically reveals a consistent progression. There is no further fall-off in height-percentile or impairment of growth over time. A growth spurt occurs during adolescence. There are little data regarding body weight progression in CGD, but weight deterioration has only been reported in the first year of life in these patients.<sup>3</sup> On the other hand, in nutritional growth retardation, the longitudinal growth record demonstrates deteriorating weight gain and linear growth.<sup>3</sup> Improvement of the deteriorating growth and weight velocity in this type of patient follows nutritional rehabilitation.<sup>4</sup>

The patient's growth chart shown in Figure 1 denotes a child with short stature and delayed puberty who was diagnosed with CGD at age 13 years. Endocrine testing revealed normal GH release to clonidine stimulation and normal thyroid function tests and prepubertal FSH and LH levels. However, the growth pattern of this patient is not indicative of CGD. Prior to age 10 years, both height and weight progressed normally. After age 10 years, there was a gradual fall-off in weight and in height. The progressive deterioration of height occurred while body weight gain diminished, although body weight for height remained within the normal range ( $\pm 10\%$ ). The deterioration in weight and height gain coincided with dietary efforts imposed by the mother on herself after she stopped smoking and gained weight excessively. The patient adapted to her mother's diet and eating patterns and failed to gain adequate weight and height or to develop sexually. Nutritional rehabilitation restored weight gain and led to catch-up growth. Therefore, the growth pattern of this patient suggests suboptimal weight gain which is consistent with ND rather than CGD.

We take this opportunity to emphasize that weight and height progression should be evaluated together in any patient with short stature. Nutritional growth retardation should be considered when there is a loss of weight percentile, even when weight for height is normal or when weight is above that expected for height. CGD should not be considered when the patient has a steady decrease in weight gain, except in infancy and early childhood, when these patients manifest deteriorating growth with weight for length deficits.<sup>3</sup>

Fima Lifshitz, MD

Figure 1  
Growth Pattern of Nutritional Dwarfing and Recovery



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## Letter From The Editor

The Editorial Board elected to initiate a section in *GROWTH, Genetics, & Hormones (GGH)* called "Clinical Pearls." You each are invited to send us a clinical pearl for possible publication in *GGH*. Please keep it brief, as did Dr. Lifshitz in his writing of the first clinical pearl which is printed on this page. I both hope and believe Dr. Lifshitz' presentation, entitled *The Differentiation of Constitutional Growth Delay from Nutritional Dwarfism* will be helpful in our thinking about growth delay as we see patients in our clinics. You are encouraged not only to contribute pearls yourself but to forward comments regarding Dr. Lifshitz' presentation. When sending comments, please write for inclusion of your comments in our "Letter to the Editor" column. We look forward to hearing from you.

Sincerely,  
Robert M. Blizzard, MD



## Effect of Weight Loss by Obese Children on Long-Term Growth

Epstein et al report on their assessment of height growth in a group of children who were treated in a complex weight loss program 10 years previously. Subjects were enrolled in the original study at 6 to 12 years of age and were initially 20% to 100% overweight for age, sex, and height. The children and at least one parent participated in the weight loss program, which included weekly treatment meetings for 8 to 12 weeks, monthly meetings for 6 to 12 months, and a *traffic light* diet that limited energy intake to 3,780 to 5,040 joules/day. The traffic light diet characterizes foods into color-designated categories, including green foods (primarily low-energy vegetables), yellow foods (basic dietary staples needed for a balanced diet), and red foods (high-energy, low-nutrient-dense foods). Red foods were restricted to 4 servings/wk. When the children were within 10% of ideal body weight, they were placed on a maintenance program and taught to increase their energy intake by 420 J/d per 1 week term until they were no longer losing weight. Subjects were followed prospectively, with anthropometric data collected at 5 and 10 years posttreatment.

One hundred fifty-eight subjects participated in the follow-up studies. Most subjects (80%) were weighed and measured by the principal investigator (PI), while 2% were measured by other physicians since they had moved from the area; 18% self-reported their height and weight. Self-reports were adjusted using regression equations developed by the PI from self-reported estimates of height and weight and measured heights and weights of 1,000 children. Child and midparental height percentiles were constructed based on the National Center for Health Statistics standards.

Mean age at the initiation of weight loss therapy was  $10.4 \pm 1.6$  years; mean height was  $71.6 \pm 26.5$  height percentile; and mean overweight was  $45\% \pm 16.6\%$ . Initially, boys were more overweight than girls. Height percentiles showed a significant decrease from baseline to 5 years, and 5 to 10 years. Weight also changed significantly over time, with boys showing greater

increases in weight than girls. Height percentiles for boys at 0, 5, and 10 years were  $71.3 \pm 25.3$ ,  $64.0 \pm 27.2$ , and  $54.6 \pm 25.9$ , respectively. Height percentiles for boys were significantly greater than midparental height percentiles at baseline and at 5 years but not at 10 years. Height percentiles for girls averaged  $71.8 \pm 25.2$ ,  $60.5 \pm 29.4$ , and  $58.1 \pm 26.2$  at baseline, 5, and 10 years, respectively. Height percentiles were also greater than midparental height at baseline and 5 years but not at 10 years. There were no significant differences or changes in height percentiles for successful versus unsuccessful weight maintainers. Thus, the authors conclude that their weight loss program does not lead to significant long-term reductions in height. They point out that the accelerated height of obese children is often associated with early puberty and earlier growth spurts, but the final height in these children is similar to their midparental height.

Epstein LH, et al. *Am J Dis Child* 1993;147:1076-1080.

**Editor's comment:** This is a very important study. Epstein and colleagues have designed and studied weight loss programs for children in a meticulous fashion for a number of years. Their data have demonstrated some success at weight loss (30% of the children were not obese 10 years after treatment) and now the absence of deleterious effects on final height has been documented. There is concern that children whose energy intake, especially fat, is severely restricted may experience poor growth and delay of puberty. The studies of markedly obese children subjected to moderate calorie restriction and followed prospectively have not been reported previously. The data presented by Epstein et al are reassuring and suggest that greater effort should be made by pediatricians to help children lose excess weight and reduce their risk for obesity-associated disorders of adulthood.

William L. Clarke, MD

## Pharmacologic, Biologic, and Clinical Effects of Recombinant Human Insulin-Like Growth Factor 1 in Growth Hormone Insensitivity Syndromes

The results of coordinated clinical trials of a new recombinant human insulin-like growth factor 1 (rhIGF-1) were presented and discussed in 3 recent papers<sup>1-3</sup> issued from a European symposium.

The first paper,<sup>1</sup> resulting from the cooperation of several groups in Europe and the United States, reviews the data acquired on the pharmacokinetics of this new drug in healthy adult volunteers and in young patients with growth hormone receptor deficiency (GHRD) of the Laron type.

In 3 groups of normal males aged 21 to 40 years, the baseline plasma IGF-1 levels varied among individuals from 100 to 200  $\mu\text{g/L}$ , with intra-individual day-to-day changes of about 10%; the daily rate of endogenous production, estimated from clearance measurements, varied from 27 to 113  $\mu\text{g/kg/d}$ , with a mean of 53  $\mu\text{g/kg/d}$ . Following a single dose of rhIGF-1, 20 or 40  $\mu\text{g/kg}$  given subcutaneously after an overnight fast, absorption was slow, with a  $T_{\text{max}}$  of about 7 hours, and plasma IGF-1 increased in relation to dose, then decreased with a half-

life of about 18 hours. After intravenous injection of 40  $\mu\text{g/kg}$ , the increase of plasma IGF-1 was immediate and reached approximately the same  $C_{\text{max}}$  after subcutaneous injection, and the half-life was near to 22 hours. Daily subcutaneous administration of 20 or 40  $\mu\text{g/kg}$  resulted in a steady trough plasma level slightly higher than the  $C_{\text{max}}$  reached after a single injection, with a  $T_{\text{max}}$  significantly shorter and no changes in other pharmacokinetic parameters. No hypoglycemia occurred at any time. Fasting serum insulin levels significantly decreased after injection of the highest dose.

Two male and 4 female adults with GHRD underwent the same pharmacokinetic studies with a treatment of rhIGF-1 40  $\mu\text{g/kg}$  every 12 hours for 7 days. Baseline plasma IGF-1 levels were 16 to 53  $\mu\text{g/L}$ . Following a single dose of rhIGF-1 40  $\mu\text{g/kg}$ , they reached levels close to 100  $\mu\text{g/L}$ , with a short  $T_{\text{max}}$  of 2 to 4 hours. With twice daily treatment, the mean level of plasma IGF-1 obtained between injections was  $141 \pm 34$   $\mu\text{g/L}$ ; the half-life was 5 to 7 hours which is considerably shorter than

in normal subjects. The differences between healthy volunteers and GHRD patients are consistent with the low plasma level of the major IGF-1 binding protein, IGFBP-3, in GHRD. These results indicate that if substitution therapy with rhIGF-1 is to be employed in GHRD syndromes, the dosages and dosing rates cannot be based directly on pharmacologic data obtained in normal subjects.

The second paper<sup>2</sup> reports follow-up studies of IGF-1, IGF-2, and their 3 specific binding proteins during a 6-month study performed in 28 patients with GHRD aged 3.7 to 22.9 years. They were treated twice daily with rhIGF-1 at doses varying from 40 to 120 µg/kg. The acute effects after a first subcutaneous injection of 40 µg/kg confirmed that the pharmacokinetic pattern of exogenous IGF-1 is determined mainly by the IGFBP-3 concentration in plasma. Results after 7 days and 6 months on treatment showed a dose-related increase of plasma IGF-1 measured just before the morning injection, with considerable variations among individual patients. Plasma GH decreased sharply, on the average by 50% after 3 days, with an inverse relationship between GH and IGF-1 in individual samples; levels remained supranormal at 6 months. IGF-2 decreased steadily after 2 months on rhIGF-1. IGFBP-1 showed no significant changes over time. IGFBP-2 increased during the first 2 weeks and remained constant thereafter. Mean IGFBP-3 levels declined slightly but steadily and significantly during the 6 months of treatment.

The whole of results lead the authors to stress the high correlation between IGFs and IGFBP-3 and to speculate that they combine early in the process of their secretion, probably in the liver. Another speculation from the data leads to a tentative

scheme of regulation of plasma IGF-2 and its related binding protein IGFBP-2 by IGF-1 and by the degree of saturation of the IGFBP-3 binding sites by the amounts of IGF-1 available in plasma or liver.

A very short summary of the clinical results obtained with rhIGF-1 in 31 patients is also presented.<sup>3</sup> The group of prepubertal children given 10 µg/kg twice daily increased their mean height velocity from 3.9 to 7.0 cm/y during 12 months of treatment. The growth rate of those given 120 µg/kg twice daily only increased from 4.6 to 8.6 cm/y. Main adverse events were injection pain (n=16), headache (n=12), and hypoglycemia during the first 3 months of treatment (n=4).

1. Grahnen A, et al. *Acta Paediatr Scand* 1993;391(suppl):9-13.
2. Blum WF, et al. *Acta Paediatr Scand* 1993;391(suppl):15-19.
3. Wilton P. *Acta Paediatr Scand* 1993;391(suppl):20.

**Editor's comment:** These 3 reports, each oriented toward different aspects of the same large study of the effects of recombinant IGF-1 in Laron-type GHRD syndromes, are of great physiologic value for the understanding of the growth-regulating hormonal mechanism as well as of clinical interest for the treatment of a rare but severe cause of dwarfism. Each one deserves full consideration as a good example of the power of a well-organized multicenter collaborative study. However, several points of clinical importance are not presented in these papers, and further reports on the experience gained in the long-term management of GH insensitivity syndromes are needed.

Jean-Claude Job, MD

## A Constitutively Activating Mutation of the Luteinizing Hormone Receptor in Familial Male Precocious Puberty

Shenker et al hypothesized that familial male precocious puberty (FMPP) might be due to a mutant receptor activated in the presence of little or no agonist (luteinizing hormone [LH]). Genomic DNA was isolated from affected males from 8 different families with FMPP, and polymerase chain reaction (PCR) was used to amplify fragments of the LH receptor DNA encoding amino acid residues 441 to 594. This fragment includes transmembrane helices 3 to 6, the second extracellular loop, and the second and third intracellular loops. Sequencing of the PCR product from one patient showed an adenine (A) to guanine (G) transition at nucleotide 1,733 in codon 578. The mutation (GAT to GGT, T = thymine) encodes a substitution of glycine for aspartate and creates a recognition site for the restriction endonuclease *Msp* I. These results were confirmed by sequencing PCR products from 2 other families.

The functional effect of the mutation was tested with wild-type and mutated human LH receptors (LHR) in COS-7 cells by measuring cyclic adenosine monophosphate (cAMP) accumulation. The mutant LHR was associated with a 4½-fold increase in basal cAMP production indicating that it was constitutively active. The mutant receptor responded to increasing concentrations of human chorionic gonadotropin (HCG) with a 50% effective concentration ( $EC_{50}$ ) similar to that of the wild-type receptor.

The authors state that constitutive activation of the LHR-mediated cAMP pathway can explain the dominant mutation and pathophysiology of FMPP since testosterone production by Leydig cells is associated with increased production of intracellular cAMP. The authors suggest that the age of pubertal onset in FMPP may depend on the extent to which the mutant allele is expressed as protein, as well as on the relative expression of other genes necessary for Leydig cell maturation. Since both LH and follicle-stimulating hormone (FSH) are required to activate ovarian hormone production, the mutant LHR could not be expected to cause sexual precocity in females.

Six of the 8 families in the study had origins in the same geographic region, and the surname of 1 affected family appeared in 2 of the other family trees.

Shenker A, et al. *Nature* 1993;365:652-654.

**Editor's comment:** These are exciting findings that not only help explain the pathophysiology of FMPP but also suggest possible mechanisms for other disorders. In the same issue of *Nature* (1993;365:649-651), Parma et al describe similar mutations in the carboxy-terminal portion of the third cytoplasmic loop of the thyrotropin receptor in 3 out of 11 hyperfunctioning thyroid adenomas. These mutations (T to C [cytosine], resulting

in the replacement of Asp 619 by glycine) affect a residue of the thyrotropin receptor. It is homologous to a mutation that leads to the constitutive activation of adenylyl cyclase in the  $\alpha_{1B}$ -adrenergic receptor. In an accompanying editorial entitled "Turned on to Ill Effect" (Nature 1993;365:603-604), Lefkowitz suggests that the mutant receptors as described by Shenker et al and Parma et al displayed properties similar to those of constitutively active mutant adrenal receptors created *in vitro*. Thus, there are probably many other "turned on" disorders with similar etiologies. Lefkowitz cautions, however, that neither group examined the entire sequence of the receptor for other mutations, and other activating mutations could be present in other regions of the receptors.

This new information could potentially lead to new therapeutic interventions for similar pathologic conditions.

William L. Clarke, MD

**2nd Editor's comment:** The investigators have convincingly demonstrated that patients with FMPP have an abnormal LH receptor that appears to activate Leydig cell production of testosterone independent of gonadotropin binding. One wonders if the carrier mothers of boys with FMPP are

hyperandrogenic, as only LH is necessary for theca cell synthesis of androgens. Clinically, these women are not hirsute, and they report no difficulty with conception. Although this disorder was associated with the same mutation in all of the patients in this report, there is genetic heterogeneity as different mutations in the LH receptor have been identified in other patients (Laue L, personal communication).

Abnormalities in G-protein activating receptors leading to decreased function have been observed (eg, for the ACTH receptor in familial glucocorticoid insufficiency),<sup>1,2</sup> this is the first report of a constitutively active receptor. Perhaps other disorders such as Cushing syndrome associated with corticotroph hyperplasia (ie, the CRH receptor) or nodular adrenal hyperplasia (ie, the ACTH receptor) may have a similar pathophysiologic base. In the McCune-Albright syndrome, a mutation in the G-protein itself leads to its independent activation, hyperfunction in multiple tissues, and less selective disease than is observed in a disorder confined to the specific receptor.<sup>3</sup>

Allen W. Root, MD

1. Tsigos C, et al. *J Clin Invest* 1992;92:2458-2461.
2. Clark AJ, et al. *Lancet* 1993;341:461-462.
3. Shenker A, et al. *J Pediatr* 1993;123:509-518.

## Reduction of Bone Density: An Effect of Gonadotropin Releasing Hormone Analogue Treatment in Central Precocious Puberty

Saggese et al determined bone mineral density (BMD) in 13 girls (aged 3.8 to 8.5 years) with central precocious puberty (CPP) before and during a year of therapy with the long-acting gonadotropin releasing hormone (GnRH) agonist D-Trp<sup>6</sup>-GnRH (decapeptyl depot, IPSEN Biotech, Milan). They compared the findings in these girls with data obtained from 2 different control groups: group 1 with 10 prepubertal girls matched to CPP girls according to chronologic age and group 2 with 10 girls matched to CPP girls according to bone age. BMD was determined by single-photon absorptiometry at the distal third of the nondominant radius, and results were expressed as bone mineral content divided by bone width (BMD, g/cm<sup>2</sup>). Each value was the mean of 3 determinations. Bone age was determined by the method of Gruelich and Pyle.

Prior to treatment, BMD was significantly increased in CPP girls compared with age-matched controls ( $0.557 \pm 0.097$  g/cm<sup>2</sup> vs  $0.433 \pm 0.09$  g/cm<sup>2</sup>;  $P < 0.001$ ), but was not different from that of bone age-matched controls. Within 6 months of the onset of GnRH agonist analogue therapy, a significant reduction in BMD was observed. A further significant decrease was observed at 12 months. BMD increased as expected during the 12 months in both control groups. The authors state that their findings are similar to those reported for adult premenopausal women treated with GnRH-analogue for endometriosis. They discussed possible reasons why gonadal function may increase bone mineralization, including (1) an action on the parathyroid hormone-vitamin D endocrine system; (2) an enhancement of growth hormone-somatomedin C axis; (3) a direct effect on bone; or (4) a combination of several factors. However, since the CPP girls in the present study continued to grow at an age-appropriate rate (although reduced from pretreatment rates), impaired growth

hormone secretion could not be the sole cause of the findings. The authors plan to continue these studies as they continue to treat their patients and hope to report on whether bone mineralization recovers once therapy is terminated.

Saggese G, et al. *Eur J Pediatr* 1993;152:717-720.

**Editor's comment:** This paper contributes an important additional piece of information regarding the physiology of bone mineralization during growth and adolescence. It is very important that the authors continue to follow these CPP girls, as well as the control groups, until puberty and final height are achieved in all 3 groups so that the long-term effects of GnRH therapy can be monitored. These findings also suggest the importance of studying BMD in girls with CPP who are receiving recombinant human GH as well as GnRH. Such studies may help identify the relative roles of growth hormone and of sex steroids in bone mineralization.

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## Leprechaunism and the Insulin Receptor Gene

Leprechaunism was first described in 1948 by Donohue<sup>1</sup> as a rare autosomal recessive inborn error of metabolism characterized by severe intrauterine and postnatal growth retardation, elfin facies, decreased subcutaneous and muscular tissue, hirsutism, and prominent genitalia.

Patients with leprechaunism have hyperinsulinism due to severe insulin resistance. The insulin resistance in this syndrome has been associated with an inherited defect in a high-affinity insulin receptor.<sup>2</sup> The central role of insulin is to regulate carbohydrate, lipid, and protein metabolism as well as promote cell growth. Insulin is known to stimulate embryonic reproductive tissue and insulin receptors are expressed very early in embryonic development.

Molecular studies in children with leprechaunism had shown homozygous nonsense or compound heterozygous mutations in the insulin receptor gene,<sup>3</sup> but homozygous deletions had never been reported. In all previously described cases, there was some residual function of the insulin receptor, and it was generally believed that complete loss of insulin receptors was incompatible with fetal life.

Recently, Krook et al<sup>4</sup> and Wertheimer et al<sup>5</sup> reported DNA

studies on the only patients known thus far to have a homozygous, complete deficiency mutation in the insulin receptor gene. Both patients presented with normal organogenesis, survived beyond term, and had the typical features of leprechaunism.

The authors suggested that although the insulin receptor is important for intrauterine growth, neurologic development and organogenesis can occur in the absence of functional insulin receptors.

1. Donohue WL. *J Pediatr* 1948;32:73-9.
2. Elsas LJ, et al. *Am J Hum Genet* 1985;37:73-88.
3. Taylor SI, et al. *Endocr Rev* 1992;13:566-595.
4. Krook A, et al. *Lancet* 1993;342:277-278.
5. Wertheimer E, et al. *Nature Genetics* 1993;5:71-73.

**Editor's comment:** There may be many complex interactions and buffering mechanisms at work during early embryonic development allowing an embryo with a severe genetic deficiency such as this to survive. Some other as yet unknown pathway must compensate for the absence of insulin receptors.

Judith G. Hall, MD

## Mild to Moderate Zinc Deficiency in Short Children: Effect of Zinc Supplementation on Linear Growth Velocity

In zinc-deficient subjects total body clearance of zinc is increased. Two hundred twenty children with short stature underwent evaluation to rule out evidence of systemic or endocrinologic disorder and to measure zinc clearance kinetics (height for age <2 SD). Twenty-one prepubertal children had normal serum zinc concentrations but an increased body zinc clearance rate of  $\geq 20$  mL/kg/h (normal subjects =  $15.1 \pm 0.6$  mL/kg/h). These children were randomly divided into 2 groups, one of which received zinc sulfate 5 mg/kg/d orally for 6 months, the other serving as a control group. After 6 months of therapy, the zinc-treated subjects had significantly increased growth rates (treated: from -3.14 to 2.26 SDS vs controls: from -2.29 to -2.42 SDS) and increased circulating IGF-1 and osteocalcin concentrations in comparison to the control subjects. No cause for zinc deficiency (eg, diabetes mellitus, sickle cell disease, chronic inflammatory bowel disease) was apparent in these 21 children, nor did they have decreased dietary intake of zinc. The authors suggest that measurement of body zinc kinetics may reveal children with mild zinc deficiency. They recommend a trial of zinc therapy in short children with no identifiable abnormality, even if the serum concentration of zinc is normal.

Nakamura T, et al. *J Pediatr* 1993;123:65-69.

**Editor's comment:** Zinc deficiency leads to hyposmia and hypogeusia and thus to decreased caloric intake. It has been most apparent in children and adolescents in Middle and Far Eastern countries where zinc intake is low and intestinal absorption is inhibited by zinc binding agents.<sup>1</sup> The present report suggests that mild zinc deficiency occurs in 10 percent of short

Japanese children. Zinc deficiency in North America has been reported in low income infants and children,<sup>2</sup> but its frequency in otherwise normal short children may not be high.<sup>3</sup> Confirmation of these observations in North American children is necessary before zinc therapy can be recommended routinely.

Allan W. Root, MD

1. Sandstead HH. *Am J Dis Child* 1991;145:853-859.
2. Walravens PA, et al. *Am J Clin Nutr* 1983;38:195-201.
3. Solomons NW, et al. *Pediatr Res* 1976;10:923-927.

**2nd Editor's comment:** Zinc deficiency as a possible cause of growth retardation and/or delay in adolescent sexual development first was considered seriously by Prasad working in Iran and, later, Egypt in the 1960s. Subsequently others have considered, and promoted in some instances, zinc deficiency as an etiologic factor in some children with unexplained short stature. I have remained a skeptic because the data have been unconvincing. This study is a credible attempt to clarify the role of zinc in relation to growth. As Dr. Root states, further studies are important and necessary before zinc supplements are used in the U.S. as therapy for growth failure. Six month studies, even when well controlled, are inadequate to derive conclusions that a particular agent is effective in promoting growth. Within the next year, the Editorial Board will invite 2 experts to write point/counterpoint articles regarding zinc deficiency.

Robert M. Blizzard, MD



## Effects of Calcitriol and Phosphorus Therapy on the Growth of Patients With X-Linked Hypophosphatemia

The growth responses of patients with X-linked hypophosphatemia (XLH) to calcitriol and phosphorus ( $\text{PO}_4$ ) therapy in relation to the patients' anthropometric characteristics and/or their pretreatment and posttreatment biochemistries are presented. Twelve consecutive patients whose therapy with calcium (Ca) and  $\text{PO}_4$  exceeded 1.2 years were studied. The subjects consisted of 4 females and 8 males, whose ages at initiation of treatment ranged from 1.7 to 9.9 years. Diagnosis in each patient was confirmed by the presence of hypophosphatemia, renal phosphate wasting, normocalcemia, and a normal serum parathyroid hormone (PTH) level. Radiologic evidence of rickets was observed in all subjects except 1 patient, and bone biopsy revealed evidence of osteomalacia in all. Retrospective evaluation showed that 6 patients (group 1) presented with a height below the fifth percentile and 6 patients (group 2) presented with a height exceeding the fifteenth percentile. Sexual development and ages of the children in the 2 groups at initiation of treatment were not statistically different, but ages varied from  $4.30 \pm 0.98$  years in group 1 to  $7.00 \pm 1.92$  years in group 2.

Both groups were treated with calcitriol 30 to 65 mg/kg/d administered in a split dose regimen, and K-phos, 20 to 60 mg/kg/d into 4 divided doses during the waking hours. After adjusting the doses over a period of several months, the optimal combination was administered for a term of 1.2 to 8.1 years. The children were followed at 2- to 4-month intervals. At each visit, 24-h urine and creatinine output were determined and serum Ca and  $\text{PO}_4$  levels were measured. Therapy was adjusted to maintain a midmorning serum phosphorus concentration close to 4.0 to 4.5 mg/dL in youths and 4.5 to 5.0 mg/dL in toddlers, while avoiding hypercalcemia and hypercalciuria, which are reflected by a Ca/creatinine (Cr) ratio of  $>0.25$ .

At the initial evaluation, children in group 1 exhibited more severe physical signs; 5 of 6 children had severe bowing of the lower extremities ( $>5$  cm between femoral condyles). This abnormality was present only in 1 of 6 children in group 2 ( $P<0.04$ ). All the children with this finding displayed a marked resolution of the bowing, with reductions of 0 to 2 cm between femoral condyles during the first 2 years of therapy.

Physical manifestations did not correlate with biochemical abnormalities (ie, serum Ca,  $\text{PO}_4$ , Cr levels or Cr clearance). However, the younger children in group 1 tended to have a lower serum  $\text{PO}_4$  concentration and increased urinary  $\text{PO}_4$  excretion.

Both groups required comparable doses of calcitriol ( $51.9 \pm 4.4$  mg/kg/d in group 1 and  $43.8 \pm 6.0$  mg/kg/d in group 2). In contrast, those in group 1 required significantly more  $\text{PO}_4$ ,  $47.3 \pm 5.1$  mg/kg/d vs the group 2 dose of  $31.0 \pm 4.7$  mg/kg/d. In response to therapy, the serum Ca concentration in group 2 increased significantly, but stayed within the normal range. The mean levels were not different from those observed in group 1. The mean urinary Ca excretion in both groups increased during the therapeutic course, but the changes were not significant.

Although serum Cr concentration and Cr clearance in both groups were not significantly different before treatment, urinary Cr clearance declined significantly ( $P<0.03$ ) in children in group 2 and serum Cr increased significantly. Serum  $\text{PO}_4$  concentration increased in the majority of the patients. Treatment also enhanced the urinary  $\text{PO}_4$  excretion in both groups. The levels

attained during therapy were not different between the 2 groups.

The children in group 1 were shorter than those in group 2. The 6 patients in group 1 presented at a mean height percentile of  $1.9 \pm 0.6$  (z score,  $-2.2 \pm 0.14$ ), whereas those in group 2 presented at a significantly ( $P<0.004$ ) greater mean height percentile of  $48.7 \pm 8.0$  (z score,  $-0.2 \pm 0.8$ ). The short stature in group 1 manifested a decline in the mean height percentile ( $53.8 \pm 12.8$ ; z score,  $0.12 \pm 0.38$ ) in infancy to that present  $3.3 \pm 1.1$  years later at initiation of therapy. In contrast, group 2 children sustained significantly less growth failure. During therapy, patients in group 1 maintained a low mean height percentile of  $2.0 \pm 0.9$  (z score,  $-2.3 \pm .24$ ), which was not different from that before therapy, and exhibited a growth velocity of (z score)  $-1.05 \pm 0.52$ . In contrast, children in group 2 significantly ( $P<0.03$ ) increased their mean height percentile to  $64.0 \pm 9.5$  (z score,  $0.44 \pm 0.25$ ) and exhibited ( $P<0.01$ ) a significantly greater growth velocity (z score,  $1.35 \pm 0.51$ ).

The authors concluded that the variable growth responses to therapy were not a consequence of the biochemical responses to therapy; that repair of bowing and, presumably, remission of rickets was not related to the variability of growth increment; and that children who were markedly affected with growth retardation at the time of presentation did not significantly increase their growth rate despite improvement in biochemical, radiologic, and other auxologic measures.

Friedman NE, et al. *J Clin Endocrinol Metab* 1993;76(4):839-844.

**Editor's comment:** This report attempts to elucidate the factors that determine growth response to treatment in XLH, and concludes that clinical and biochemical control are not the main determinants of the growth response achieved with therapy. However, the authors compare XLH patients with different degrees of severity of the disease. Logically, the more severely affected patients will be the most disadvantaged and least responsive to treatment. Although the authors referred to the radiologic evidence of rickets among all patients studied, they failed to quantitate the differences and degrees among the 2 groups described, before and after therapy. It is likely that those patients with more severe disease continued to show bone abnormalities, even after prolonged therapy, which could contribute to the inadequacy of the growth recovery. Similarly, the bone deformities and bowing, and their contribution to the height deficits, were not quantitated before and after therapy. Upper/lower body segments and arm-span assessments, throughout the treatment period, may shed some light on this question. Furthermore, other factors that are important in determining height need to be addressed (eg, height of the patients' parents).

Regardless of the deficits, this paper does point out the need to have a high index of suspicion for XLH so the diagnosis is made early in life and treatment is initiated before bone deformities, rickets, and short stature become evident. Once these signs appear, therapy may not accomplish the desired effects of treatment. An ounce of prevention is worth a pound of cure!

Fima Lifshitz, MD

## Effects of Human Growth Hormone Therapy on Melanocytic Naevi

The growth of melanocytic naevi in normal children and in those with hypopituitarism or Turner syndrome, currently or previously treated with hGH therapy, were studied by using HMB-45 antibody which labels actively growing melanocytes. Color slides of the lesions were evaluated using a computerized image analyzer. The growth rate was calculated over 6 months by the change in diameter expressed as a percentage of the initial diameter. In a separate study, 79 naevi were excised from 58 children and adolescents. Of these, 19 of the patients were not presently using hGH and 39 patients were. (The clinical diagnoses were 21 patients with GH deficiency and 18 patients with Turner syndrome.) After fixation of the tissue, studies were done microscopically using HMB-45 antibody which stains the melanocytes.

The mean growth rate of naevi in controls and patients not treated with hGH was 7.6% to 11.2% over 6 months. In patients on treatment with hGH, the growth rate of naevi doubled. Of the 19 untreated or off hGH, 18 patients did not express melanocyte proliferation. Twenty-two of the 39 patients currently being treated with hGH expressed focal or diffuse intradermal HMB-45 antibody reactivity. In one patient with Turner syndrome, the activity correlated with nontreatment and treatment with hGH. The size but not the number of naevi was increased with hGH.

The authors concluded that differences in sexual maturation, age, and diagnosis could not account for the increased growth of naevi. Secondly, the authors felt that the increased HMB-45 antibody expression was not necessarily associated with neoplastic melanocytes and could have resulted from stimulation of normal melanocytes by endocrine or paracrine factors. Thirdly, an increased frequency of skin tumors in hGH-treated or acromegalic patients has not been reported, and no neoplasms or premalignant transformation was observed in the studies reported here. Long-term follow-up is required to identify delayed or unknown effects of hGH therapy, especially in patients with Turner syndrome who are likely to require high doses to obtain substantial growth effect.

Bourguignon JP, et al. *Lancet* 1993;341:1505-1506.

**Editor's comment:** *The study reported here presents a well-defined effect of hGH on melanocyte activity. The authors are to be congratulated. We all should be more observant of the naevi of patients receiving hGH than has been our practice up to this time.*

Robert M. Blizzard, MD

## Growth Hormone (GH) Receptors, GH Binding Protein and GH: An Autoregulatory System?

There is increasing evidence that between the secretion of growth hormone (GH) and its effects on tissues the intermediary steps play an important physiologic role. The view of some authors is that this could constitute an autoregulatory system. Studies in genetically and prenatally GH-deficient dwarfed rats have shown the persistence of sexual dimorphism and with a very low level of GH secretion that is episodic in males and more continuous in females. In these animals the cellular GH receptors (GHRs) exist at a lower level than in normal rats, and develop with age. Continuous infusion of GH increases the GHR binding sites in both males and females, suggesting that GHR autoregulation by GH is preserved in spite of the congenital and permanent lack of GH. Paradoxically, intermittent daily GH injections in the males had a much smaller effect than continuous infusion on plasma GH binding protein (GHBP) and on GHRs. This experimental finding agrees with the observation that normal female rats in whom sustained plasma levels of GH is the norm have higher levels of GHBP than normal males in whom the pituitary secretion of GH is episodic.

Administration of GHBP together with GH, simultaneously or separately, produced a marked prolongation of both GH and GHBP half-lives, compared with their half-lives when injected alone. The experiments with rat and human GH and GHBP showed that the interaction is species-specific. The studies confirmed that GHBP can act as an efficient trap for GH in the circulation, and suggested that complex formation by GHBP in the extracellular space may also serve to trap the extra amounts of GH entering at the time of pulses in the bloodstream, and to prevent this GH from diffusing back into vessels as the plasma concentration of GH wanes.

As continuous GH exposure is not the optimal pattern for growth stimulation in hypopituitary rats of either sex, it may be considered that upregulation of both GHR and GHBP may render the tissues less sensitive to continuous cellular stimulation by GH.

The authors point out that these data obtained in rats do not mean that the same autoregulatory process exists in humans. While the lack of GHRs or the presence of mutated receptors in humans is obviously associated with clinical resistance to GH, it is not clear whether more subtle variations in receptor number or affinity modulate the effects of GH in other conditions. However, the data suggest that continuous treatment, particularly at suboptimal doses, may result in a falloff in GH response, and that intermittent GH therapy might avoid an excessive increase of circulating GHBP.

An example of an increase in GHR and GHBP associated with reduced growth is given with estradiol treatment in normal male rats, which elevates the baseline of plasma GH. This elevation is not sufficient to explain the phenomenon of reduced growth, as continuous GH infusion in hypophysectomized male rats restores the ability of estradiol to raise GHBP and GHR, suggesting an interaction between estradiol and GH in the liver itself to regulate GHBP output. Finally, the authors speculate on the possible clinical significance of their experimental data. They question whether interactions among GH, GHR, and GHBP, and their modulation by sex steroids, could explain certain changes in responsiveness to GH therapy before or during puberty.

Robinson ICAF, et al. *Acta Paediatr Scand* 1993;391(suppl):22-28.

**Editor's comment:** This interesting report of well-organized animal experiments is potentially important in understanding the effects of chronic GH treatment, particularly the secondary waning of growth velocity, and the changes in dose-response relationships over time or during puberty. However, the role of the autoregulatory system suggested by the authors in rats is

unclear in humans. The messenger role of insulin-like growth factor 1 and its GH-dependent binding protein, possibly the most important step in the whole of the human autoregulatory system, has not been taken into consideration in this experimental work.

Jean-Claude Job, MD

## Failure to Improve Height Prediction in Short-Stature Pubertal Adolescents by Inhibiting Puberty with Luteinizing Hormone-Releasing Hormone Analogue

These investigators administered the long acting analogue of GnRH, D-Trp6-GnRH, 3.75 mg intramuscularly monthly for 24 months, to 17 early (breast or genital stage II or III) adolescent subjects (9 females) with intrinsic short stature but no systemic or endocrinologic disorder. The adult height prediction for these patients was below -2.5 SD (females 149.2  $\pm$  3.4 cm; males 160.5  $\pm$  6.1 cm). During administration of D-Trp6-GnRH pubertal progression ceased, testicular and uterine size decreased, and plasma testosterone and estradiol concentrations declined. Unfortunately, pretreatment growth velocity data are not presented, but during GnRH analogue administration growth rates were within the prepubertal range; height standard deviation scores, height age/bone age ratio, and the predicted adult height did not change during therapy. After discontinuation of therapy, pubertal progress resumed in all patients. The major adverse event of treatment was the disappointment of the patients at the lack of effect of the GnRH analogue on their growth potential.

Lindner D, et al. *Eur J Pediatr* 1993;152:393-396.

**Editor's comment:** Although administration of analogues of GnRH to selected children with central precocious puberty may increase predicted and attained height,<sup>1-3</sup> present data indicate that such therapy does not affect the ultimate growth of otherwise normal, short adolescents. Whether co-administration of GH and GnRH analogue would affect adult stature in such subjects is as yet unknown but, even if so, the cost/benefit ratio of such therapy must be carefully considered. In the opinion of this writer, such therapy is not justified in the majority of such subjects until there is unequivocal proof that it is both effective and of psychosocial, educational, and economic benefit. The disappointment of the present subjects over the lack of benefit of treatment is an avoidable complication. Most such patients are best managed by reassurance of their basic normality and individual support.

Allan W. Root, MD

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## Evolution of the Sex-Determining Gene

Mammals require a sex-determining gene (SRY) on the Y chromosome for male sex determination and testis differentiation. The SRY gene encodes a protein with a central *high mobility group* domain (HMG box) of about 78 amino acids. In mice, this HMG box consists of an HMG DNA-binding domain flanked by an N- and a C-terminal region.

SRY belongs to a family of genes that are related by sequence homology within the DNA-binding domain. The genes most similar to SRY are called SRY box or SOX genes and are homologous to SRY only in the HMG box sequence. These HMG boxes are found in many proteins, and although SRY is an essential developmental regulator apart from the HMG box domain, the SRY sequence is poorly conserved among species. The conservation of the HMG box in mammals was recently reviewed by Whitfield et al.<sup>1</sup>

The authors studied and compared predicted SRY protein sequences in primate (human), lagomorph (rabbit), rodent (mouse), and marsupial (dunnart). Then, by comparing the frequency of DNA point mutations at synonymous and nonsynonymous positions in 8 primates including the human, the authors were able to ascertain the relative frequency of mutations that alter the amino acid sequence and to correct for the evolutionary separation of the compared species.

Whitfield and associates found that there is a high degree of divergence in the flanking sequences of the HMG box, but that the HMG box domain is highly conserved in all species. The results obtained by comparing the differences in the HMG box and the flanking sequences in various species showed that the evolution for the flanking sequences either has been very rapid or may have been the result of directional selection.

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Further studies were conducted by Tucker and Lundrigan<sup>2</sup> who examined the rate and pattern of evolution of the SRY coding sequence in 7 species of Old World mice and rats. They found varying degrees of differences in the flanking SRY sequences; the HMG box sequences, however, were highly conserved.

These findings suggest that the majority of the noncoding SRY flanking areas (ie, the nonbox regions) may have no functional significance or may have been the result of directional selection. Although unclear, the substitution of amino acids in the flanking sequences must have given the primate SRY gene a selection advantage. It is clear that SRY is constantly being challenged by selective forces and since it plays such a major role in male sex determination, populations with different

SRY sequences may be at risk for reproductive isolation.

1. Whitfield LS, et al. *Nature* 1993;364:713-715.
2. Tucker PK, Lundrigan BL. *Nature* 1993;364:715-717.

**Editor's comment:** Studying specific genes in evolution allows for further understanding of the critical functional area of each gene. This is especially true in the SRY gene. Clearly the SOX region (the DNA-binding domain) is critical for sex determination, and all evidence so far suggests that sex is sex, regardless of the species.

Judith G. Hall, MD

## The Role of Estrogens in Disorders of the Male Reproductive Tract

Diethylstilbestrol (DES) was a widely used synthetic estrogen administered to pregnant women from 1945 through 1971. It was later found to have an in utero teratogenic and a later carcinogenic effect in the daughters of the pregnant women who were exposed to it.

Recent evidence has shown that in utero exposure of males to DES led to an increased incidence of cryptorchidism, hypospadias, falling sperm counts, and testicular cancer.<sup>1</sup> Animal studies also have shown that exposure to synthetic or natural estrogens may lead to male and female reproductive disorders.<sup>2,3</sup>

Several investigators reported that the incidence of disorders of the human male reproductive tract has increased greatly in the last 40 to 50 years.<sup>4,5</sup> Sharpe and Skakkebaek<sup>6</sup> recently wrote a review of the changes that have occurred in the sources of estrogen exposure in humans since 1940. Although an accurate measurement of estrogen exposure levels in humans is not available, this review suggested that the changes that have occurred in estrogen exposure in the past 4 decades may have led to the increase in male reproductive malformations.

Some of the suggestions reported in the review include the possibility that low-fiber diets may lead to the recycling of excreted estrogen, and increased body fat may convert other steroids into excessive amounts of estrogens. Furthermore, the use of synthetic oral estrogens, or the so-called pill for family planning, was not so common 40 years ago. These synthetic estrogens are excreted in the urine and may find their way into drinking water thus increasing human exposure. Orally active estrogens were commonly used in livestock from 1950 to 1970, but were banned in Europe in 1981, and may have been another mechanism for major estrogenic effects.

In addition, increased consumption of dairy products containing estrogens and the increased distribution of environmental estrogenic chemicals<sup>7</sup> are phenomena that have occurred during the last 50 years and may have altered estrogen exposure of humans.

These changes in the environmental content of estrogens become particularly important for pregnant women. The evidence of male and female reproductive disorders and exposure to DES in utero is very clear.<sup>1,8</sup> If pregnant women are overexposed to estrogens, then the increase in male reproductive disorders is not surprising.

Because of the association of DES with male reproductive tract abnormalities, and because the routes for human exposure to estrogens have changed so dramatically over time, the

suggestion has been made that the incidence of any disorder associated with estrogen exposure will greatly increase. However, better methods for measuring toxic and nontoxic exposure to estrogens are needed. A good animal model could help solve problems in quantification. Then the association between the increase in male reproductive disorders and the exposure to estrogens could be better understood.

**Editor's comment:** Sharpe and Skakkebaek's<sup>6</sup> excellent review of male fetal exposure to estrogens raises several important issues related to side effects of teratogens and the need for in depth evaluation in specific cases. This review also alerts physicians to carefully ask questions about maternal exposure in XY intersex cases and in males with reproductive malformations and malignancies.

Judith G. Hall, MD

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# GROWTH

## Genetics & Hormones

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### Neuroendocrinology of Growth Hormone Secretion

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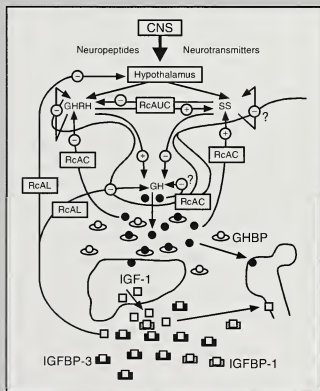
Fundación Endocrinología y Nutrición

Madrid, Spain

The physiology and pathophysiology of growth hormone (GH) was first a matter of discussion in *GROWTH, Genetics, & Hormones* in 1985, with an update of this topic published in 1991. Since this latter article, important advances in our understanding of the GH axis have taken place. Pituitary-specific transcription factors involved in the expression of the GH gene have been identified; the growth hormone-releasing hormone (GHRH) receptor gene has been cloned, as well as a number of somatostatin (SS) receptor genes; pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors have been described; and advances in our understanding of the insulin-like growth factor binding proteins (IGFBPs), growth hormone-binding proteins (GHBPs), and growth hormone-releasing peptides (GHRPs) have been made (Figure 1). Therefore, the purpose of this article is to review our current

understanding of the control of the GH axis, with a special emphasis on those topics that have come to the forefront since the last review.

Figure 1  
Schematic Representation of the  
Growth Hormone Axis



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#### Abbreviations

CNS = central nervous system  
GHRH = growth hormone-releasing hormone  
GHBP = growth hormone-binding proteins  
SS = somatostatin  
RcAUC = ultrashort loop  
RcAC = short loop  
RcAL = long loop  
IGF-1 = insulin-like growth factor 1  
IGFBP = insulin-like growth factor binding protein

## NEUROENDOCRINE CONTROL OF GH SYNTHESIS AND RELEASE

It has been more than a decade since the discovery of GHRH and SS, 2 hypothalamic peptides known to play an important role in controlling the synthesis and secretion of GH. Released into the hypophyseal-portal system in a reciprocal manner, GHRH stimulates and SS inhibits GH release from the anterior pituitary. Hence, the pulsatile secretion of GH is thought to result from an increase in GHRH neuronal activity and a coincident decrease in SS release, thus effecting a GH surge, while the opposite (ie, a decrease in GHRH activity and a rise in SS secretion) suppresses the baseline, or nadir. However, how this reciprocal pattern of neuropeptide is generated at the level of the hypothalamus remains an area of intense investigation.

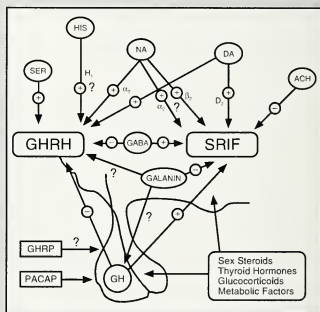
Many neurotransmitters and neuropeptides have been implicated in the control of GHRH and SS release. However, the role of many of these, such as serotonin,  $\gamma$ -amino butyric acid, and dopamine, is still a matter of discussion. For example, dopamine agonists have been shown to both stimulate and inhibit GH release. This depends on the conditions under which these drugs are administered and which agonist is used. One possible explanation for this conundrum is that dopaminergic actions on GH may be mediated through the stress axis or by adrenergic metabolites. The adrenergic system is thought to stimulate GH secretion, acting via  $\alpha_2$ -adrenergic receptors, by increasing the release of GHRH. Clonidine, an  $\alpha_2$ -adrenergic agonist, stimulates GH in humans and in experimental animals, both in vivo and in vitro. However, recent evidence indicates that clonidine may be acting, at least in part, through the inhibition of SS secretion.<sup>1</sup> On the contrary,  $\beta$ -adrenergic agonists inhibit GH secretion, presumably by increasing SS release. The cholinergic system appears to play an important role in modulating GH secretion. Muscarinic cholinergic blockers obliterate both spontaneous and GHRH-stimulated GH release. Cholinergic agonists stimulate GH secretion, most likely by inhibiting SS release. Hence, instead of affecting GHRH secretion, many of the higher pathways appear to modulate basal hypothalamic SS release.

Over the years, a number of neuropeptides have been implicated in the control of GH secretion, both at the level of the hypothalamus and the anterior pituitary (Figure 2). Thyroid-releasing hormone, vasoactive intestinal peptide (VIP), gastrin, neurotensin, and substance P, among others, have been suggested as stimulators of GH secretion, while calcitonin, neuropeptide Y, and corticotropin-releasing hormone (CRH) have been implicated in the diminution of GH secretion. Galanin, a 29 amino

acid peptide found in considerable amounts in the hypothalamus, both stimulates GH release and enhances the GH response to GHRH release. This peptide is thought to act by diminishing the naturally occurring SS inhibitory tone. Interestingly, galanin is also produced by a percentage of GHRH neurons and can be found in the median eminence, suggesting that this peptide may have a biologic function in the control of GH secretion.

As a newly isolated hypothalamic peptide with a possible role in the control of GH secretion, PACAP has received considerable attention.<sup>2</sup> This polypeptide is a member of the secretin-glucagon-VIP family, with the greatest homology to VIP. It is present in 2 amidated forms, one with 38 residues

**Figure 2**  
**Effects of Hypothalamic Neurotransmitters on the Release of GHRH and SRIF and on the Secretion of GH**



Metabolic factors (hypoglycemia or hyperglycemia, fasting) as well as sex, thyroid, and glucocorticoid hormones act in both the hypothalamus and anterior pituitary to modify the secretion of GH.

### Abbreviations

|       |   |
|-------|---|
| SER   | = serotonin   |
| HIS   | = histamine   |
| NA    | = noradrenalin (norepinephrine)                         |
| DA    | = dopamine  |
| ACH   | = acetylcholine   |
| GABA  | = $\gamma$ -amino butyric acid                          |
| GHRH  | = growth hormone-releasing hormone                      |
| GHRP  | = growth hormone-releasing peptide                      |
| SRIF  | = somatotropin release-inhibiting factor (somatostatin) |
| PACAP | = pituitary adenylate cyclase-activating peptide        |
| GH    | = growth hormone  |

(PACAP38) and one with 27 residues (PACAP27); PACAP38 is the more abundant form. As its name suggests, this polypeptide acts to stimulate cyclic adenosine monophosphate (cAMP) activity, but not only in the pituitary. A number of different tissues, including the hypothalamus, express receptors for PACAP. To date, 2 types of PACAP receptors have been identified. Type I has 2 subforms. Type A binds to both PACAP38 and PACAP27, with a slight preference for the latter; type IB has a greater preference for PACAP38. The type II receptor binds both PACAP and VIP with similar affinities, and may be identical to the VIP receptor. This receptor is found in lung, liver, intestine, and other tissues; the type I PACAP receptor is found in high concentrations in brain, spinal cord, anterior pituitary, and adrenal medulla. Although the precise role that this peptide plays in the regulation of GH secretion remains to be elucidated, evidence is accumulating to suggest that it may indeed be another hypothalamic factor involved in this phenomenon. Not only does the anterior pituitary contain type I PACAP receptors, but immunoreactivity for PACAP can be found both in the hypothalamus and median eminence. Furthermore, studies have demonstrated that this peptide can increase the calcium concentration in somatotropes and stimulate the release of GH from anterior pituitary cell cultures.<sup>3</sup> However, a possible role for PACAP in human short stature remains to be elucidated.<sup>4</sup>

Synthetic hexapeptides that stimulate GH release, GH-releasing peptides 1, 2, and 6 (GHRP-1, GHRP-2, and GHRP-6), have been available for a number of years.<sup>5,6</sup> These peptides are not homologous to GHRH and now are known to work through anterior pituitary receptors distinct from those of both GHRH and PACAP. In normal children, these peptides stimulate the release of GH, acting directly at the pituitary level. Although the mechanism is not yet understood, GHRPs do not activate GHRH receptors.<sup>7</sup> In addition, evidence has accumulated in the literature indicating that GHRPs and GHRH act in a synergistic manner when administered simultaneously. Administered orally, GHRP-2 can be used as a potent drug to liberate GH from somatotropes. Recently, a nonpeptidyl substituted benzolactam has been developed that acts in a manner similar to that of GHRP-6 and is biologically active in humans. A possible role for GHRPs in the treatment of human short stature remains to be demonstrated.

Many circulating hormones and metabolic substances affect GH secretion, a number of which act, at least in part, at the level of the hypothalamus. Thyroid hormone, acting at the level of both the pituitary and the hypothalamus, plays an important role in the regulation of GH production and secretion. The promoter of the rat GH gene contains a

thyroid hormone-response element (TRE) and is induced by thyroid hormone. However, this triiodothyronine response element has not been identified in the human GH promoter; moreover, human GH gene transcription appears to be negatively regulated by triiodothyronine ( $T_3$ ). In humans, both hypothyroidism and hyperthyroidism can lead to decreased GH secretion. This may be the result of a dual action of thyroid hormones, decreasing hypothalamic somatostatinergic tone and antagonizing GHRH action at the level of the pituitary.

In humans, glucocorticoids are important regulators of GH secretion. Administration of glucocorticoids to both humans and laboratory animals has been shown to blunt GH secretion and to inhibit somatic growth. This observation is counterintuitive when one considers more recent evidence showing that glucocorticoids stimulate the GH gene at a specific corticoid-response element. Indeed, glucocorticoids also appear to have dual effects on the GH system. Although a single dose of dexamethasone is capable of blocking GHRH-stimulated GH secretion for several days, glucocorticoids potentiate GH secretion over the short term (3 hours). It may therefore be that the stimulatory and inhibitory effects of glucocorticoids are mediated at different levels of the GH axis.

It has long been known that there is an interrelationship between sex steroids and GH. The spontaneous elevation of sex steroids during puberty or the administration of small or moderate doses of androgens or estrogens during the prepubertal period results in a marked increase in the response of GH to pharmacologic stimuli, in addition to augmenting the spontaneous basal secretion of this hormone. At least in the laboratory rat, androgens stimulate the synthesis of both hypothalamic GHRH and SS,<sup>8-10</sup> suggesting that the effects of sex steroids are at least partly mediated at the hypothalamic level. Gonadal steroid effects at the pituitary level are less clear, although they may exist.

GH secretion is profoundly modified in metabolic diseases such as anorexia nervosa, obesity, malnutrition, and diabetes mellitus. This is not surprising considering the number of metabolic substances that modulate GH secretion. One commonly used diagnostic test for GH secretion abnormalities involves inducing hypoglycemia. Hypoglycemia itself normally produces a rise in GH secretion, while insulin inhibits GH release, an action that may be mediated at the hypothalamic level since insulin receptors can be found in this tissue. Furthermore, free fatty acids inhibit and amino acids stimulate GH release. Delineating the precise mechanisms underlying abnormal GH secretion in these metabolic diseases remains an area of active research.



The identification of the pituitary-specific transcription factor, Pit-1 (or GHF-1), in 1988 has contributed substantially to our understanding of the physiology and pathophysiology of GH synthesis.<sup>11,12</sup> This transcription factor is involved in the developmental generation of somatotropes, lactotropes, and thyrotropes, as well as in the regulation of the genes for GH, prolactin, and possibly the  $\beta$ -chain of thyroid-stimulating hormone.<sup>13,14</sup> Some effects of GHRH and SS, both of which act by modulating cAMP levels in the somatotrope, may be mediated through Pit-1. Although no cAMP-response element (CRE) has been demonstrated in the GH gene, the promoter region of the Pit-1 gene contains a CRE, and the transcription of this gene is augmented with increasing levels of cAMP. Furthermore, the interaction of this protein with other DNA-binding factors, such as sex steroid receptors, thyroid hormone receptors, and glucocorticoid receptors, may also modulate the response of somatotropes to these other circulating factors. Alternative splicing of the messenger RNA for Pit-1 results in a related transcription factor, Pit-2.<sup>15</sup> This peptide is also endogenous to the anterior pituitary, although it is found in a concentration 7-fold less than that of Pit-1. The physiologic role of Pit-2 is even less well understood than that of Pit-1, although it appears to be much more potent in stimulating the transcription of the GH gene than of the prolactin gene.

The cloning of the human cDNA for this transcription factor has led to the clinical classification of a new type of pituitary insufficiency. During the past year, a small number of patients has been described in whom the underlying deficit includes different mutations involving most of the exons in the gene for Pit-1.<sup>16-19</sup> These patients present with severe short stature, an absence of GH and prolactin production, and different degrees of thyroid deficiencies and pituitary hypoplasia. The demonstration of this new combined pituitary hormone deficiency syndrome will allow pediatric endocrinologists to identify the underlying deficit in these patients. An excellent review has been recently published by Parks and colleagues.<sup>20</sup>

Because the GHRH receptor gene has been cloned,<sup>21</sup> molecular diagnosis of short stature, including GHRH receptor abnormalities, can be made. Although, this defect has not yet been demonstrated, possible candidates were recently presented at the Fourth Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society/European Society of Pediatric Endocrinologists (LWPES/ESPE) in San Francisco. These extremely short-statured children do not respond to exogenous GHRH and exhibit very low levels of IGFbPs. Mutations in or near the GH-1 gene were excluded.<sup>22</sup>

The insulin-like growth factors (IGFs) circulate in serum bound to IGFbPs. During the last few years new biochemical parameters, including the IGFbPs and the GHbPs, have become available to the clinician in order to better evaluate normal growth and growth disorders during childhood.

The IGFbPs are circulating proteins that modulate IGF actions.<sup>23,24</sup> Based upon protein and DNA sequence analysis, 6 different human IGFbPs have been classified so far: IGFbP-1 through IGFbP-6.<sup>25</sup> The major circulating IGFbP can be detected in serum as a 150-kilodalton (kd) ternary complex. The IGFbP subunit ( $\beta$ ) of this complex is IGFbP-3, a 45-kd glycosylated protein with a core molecular mass of 29 kd. The  $\alpha$ -subunit is a glycosylated protein of 85 kd that is unable to bind IGFs. The third component, or  $\gamma$ -subunit, is IGF-1 or IGF-2. Changes in IGFbP-3 levels during childhood have been described. IGFbP-1 is generating much interest because it possesses properties that are atypical for a classic binding protein.<sup>26</sup> Its physiologic role remains unknown, although IGFbP-1 could be the endocrine element secreted by the liver under insulin regulation in order to modulate IGF activity in humans. IGFbP-1 levels vary markedly during the day.<sup>27</sup> This variation is related to metabolic status, with an inverse relationship existing between IGFbP-1 and insulin levels. In contrast, little is known about IGFbP-2, IGFbP-4, IGFbP-5, and IGFbP-6.

The identification and characterization of the GHbPs, as well as the cloning of the GH receptor,<sup>28</sup> have provided new biochemical markers useful in understanding the physiology of GH and growth disorders.<sup>29-31</sup> Serum GHbP levels correlate inversely with 24-hour GH secretion in healthy boys of normal stature.<sup>32</sup> In addition, there is a strong positive correlation between the body mass index (BMI) and serum levels of high-affinity GHbP in normal boys. Furthermore, there is a significant direct relationship between BMI and responsiveness to exogenous GH,<sup>33</sup> suggesting a relationship between GH function and circulating GHbP levels.

## SUMMARY

The rapid advances that have occurred in biochemistry and molecular biology have led to an enormously increased understanding of the GH axis. However, this increase in knowledge has led to even more questions and has complicated our theories regarding the control of GH secretion. Nonetheless, these new diagnostic tools have helped to define new diseases, such as the combined pituitary hormone deficiency syndrome, and to improve our understanding of the variety of diseases involving growth abnormalities. As new genes

involved in the control of the GH axis are cloned, the possibilities for future diagnosis and identification of underlying defects will improve. Our increasing understanding of peptides such as PACAP and the GHRPs will likely play an important role in the years to come regarding the diagnosis and treatment of GH abnormalities.

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# Serum Polypeptide Hormone-Binding Proteins Part 1: Growth Hormone-Binding Proteins

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Serum proteins that bind polypeptide hormones have only recently been identified. The recognition of these serum proteins has necessitated revision of concepts concerning the mechanism of action of their ligands, as these binding proteins alter the metabolism and influence the association of the polypeptide hormones with their cell membrane receptors. They also may enhance or inhibit the bioactivity of their ligands. The most well-characterized binding proteins are those for growth hormone (GHBP) and the insulin-like growth factors (IGFBPs).

## GROWTH HORMONE-BINDING PROTEINS

Serum proteins with GH-binding activity have been identified in humans, rabbits, mice, rats, swine, and higher primates.<sup>1,2</sup> Two GHBPs are found in human serum, one with low affinity and the other with high affinity for GH. The low-affinity, high-capacity GHBP with a molecular weight (MW) of 165 kilodaltons (kd) specifically binds the 20-kd variant of GH. The high-affinity human GHBP is discussed here unless otherwise indicated. This protein binds primarily the more abundant 22-kd form of GH. Structurally it is

identical to the extracellular domain of the membrane receptor for GH.

In rodents, GHBP is synthesized from an alternately spliced mRNA transcribed from the gene for the GH receptor; it contains an extra hydrophilic sequence that replaces the transmembrane and intracellular domains of the GH receptor. However, in rabbits, and probably in humans, GHBP is synthesized by proteolytic cleavage of a single transcriptional/translational product of the GH receptor gene<sup>3</sup> and is a 239 to 246 amino acid glycoprotein (MW 61 kd) primarily of hepatic origin.

Serum or plasma GHBP values have been estimated by determining the percentage of protein-bound radiolabeled GH after incubation of radiolabeled GH with serum and separation of protein-bound from free GH by exclusion or high performance liquid chromatography. Methods for separation of protein-bound and free GH by adsorption of free GH to charcoal or immunoprecipitation of protein-bound GH with monoclonal antibody to GHBP or to the GH receptor as well as a radioimmunoassay for (rodent) GHBP have also been described.<sup>4,5</sup> Both total GHBP (free and GH-bound) and the endogenous complex of GH and GHBP (GH/GHBP complex) may be measured by utilizing the ligand-mediated immunofunctional assay (LIFA).<sup>6,7</sup> There is a reasonable correlation between LIFAs and radiolabeled GH-binding assays, although absolute GHBP measurements by LIFA are one third of the values obtained by the radiolabeled GH binding assay.<sup>8</sup>

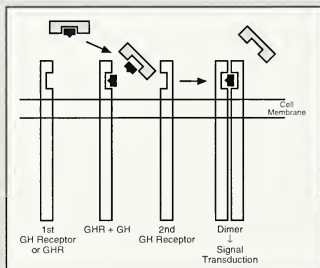
In both males and females, approximately 40% to 50% of circulating GH is bound to high-affinity GHBP and 5% to 8% to low-affinity GHBP, leaving 42% to 55% free. Total GHBP is relatively constant throughout a 24-hour period, although slightly lower at night than during the day. Levels of GH/GHBP complex fluctuate throughout the day, paralleling the changes in endogenous GH secretion.<sup>7</sup> In the fetus and neonate, GHBP values are low, increase 2-fold by 6 years of age, and continue to rise throughout childhood. During adolescence, GHBP values do not change, although values may fluctuate 3-fold over time in an individual.<sup>9</sup> In children and adolescents the level of GHBP is directly related to height, weight, and therefore, body mass index. Serum levels of GHBP are low in malnourished subjects, high in obese individuals, and decline with weight reduction.<sup>5</sup> GHBP levels are similar in normal, GH-deficient, and acromegalic adults. Administration of GH does not alter GHBP values in normal or GH-deficient adults,<sup>2,10</sup> suggesting that GH does not influence the production of GHBP. However, in GH-deficient children an increase in GHBP values after administration of GH has been reported. Whether this response is due to the effect of GH itself or to the increase in body size is unclear.<sup>11,12</sup> Testosterone decreases and estradiol increases GHBP levels, which is consistent with its hepatic origin.<sup>10</sup>

Although GH probably does not directly regulate the production of GHBP, GHBP does appear to influence the secretion and biologic activity of GH. Thus, there is an inverse relationship between the GHBP level and the mean 24-hour GH concentration, mean amplitude of the GH pulse, and the sum of the amplitudes of the GH pulse in normal children and adolescents. In GH-deficient children, the linear growth response to a constant dose of GH is inversely related to the pretreatment GHBP value.<sup>9,13,14</sup>

GHBP serves as a reservoir for secreted GH; the half-life of free GH in serum is approximately 7 minutes, and that of bound GH/GHBP complex is 27 minutes. The half-life of total GH is 18 minutes. In this dynamic system, the minute-to-minute concentrations of free GH, GHBP-bound GH, total GH, and the percentages of free GH, GHBP-bound GH, and occupancy of GHBP vary with the half-life of free GH, the instantaneous secretion rate of GH, and the capacity and affinity of GHBP.<sup>15</sup> Although the GH receptor is a single-chain polypeptide, effective induction of the intracellular GH signal requires that 2 separate sites of the circulating GH molecule interact with 1 specific receptor binding site, which is near the amino terminal of the extracellular domains on 2 GH receptor molecules.<sup>16,17</sup> GHBP may facilitate the exposure of (GHBP-bound) GH to the extracellular domain of the cellular GH receptor (Figure 1). In vitro, GHBP inhibits the binding of GH to its receptor and attenuates the biologic effectiveness of GH.<sup>18</sup> In vivo, GHBP appears to enhance the growth-promoting effects of GH in humans and rats.<sup>14,19</sup> Clark et al<sup>9</sup> demonstrated that recombinant human GHBP had no effect on the growth of GH-deficient dwarf mice, but more than tripled the effect of a constant dose of human GH on bone growth and weight gain, an effect attributed to prolongation of the effective biologic half-life of GH. These observations are difficult to integrate into the concept that pulsatile secretion of GH is important for optimal GH effect. Thus, excellent growth and a final height consistent with the genetic background can be achieved by administration of GH once daily to GH-deficient subjects. Interestingly, continuous infusion of GH stimulates linear growth to the same extent as intermittent injection,<sup>12</sup> and overgrowth occurs in patients with GH-secreting tumors in whom GH levels may be relatively constant. The exposure of tissues to coordinated levels of constant basal and intermittently increased amounts of free GH could conceivably be optimal for cell growth and function.

In many subjects with GH insensitivity due to an abnormality in the gene for the GH receptor, such as in the Laron syndrome, serum GHBP activity is low

Figure 1



Growth hormone-binding protein (light gray symbol) delivers growth hormone (GH, solid symbol) to a single binding site on the extracellular domain of 1 cell membrane GH receptor (GHR). Two molecules of GHR then dimerize. The univalent binding site on the extracellular domain of the 2 receptors binds to 2 different portions of the GH molecule. Dimerization is essential for signal transduction of the GHR.<sup>16,17</sup>

or absent. GHBP values may also be low in patients with cirrhosis of the liver, chronic renal disease, insulin-dependent diabetes mellitus, malnutrition, and severe acute illness.<sup>2,20-22</sup>

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# Serum Polypeptide Hormone-Binding Proteins

## Part 2: Insulin-Like Growth Factor-Binding Proteins

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The family of serum proteins that specifically bind 7.5-kilodalton (kd) insulin-like growth factor (IGF) 1 and 2 (but not insulin) whose gene structures and amino acid sequences have been identified currently numbers 6 members (Table 1).<sup>1-3</sup> The amino terminal, cysteine-rich 80-90 and the carboxyl terminal 80-100 amino acids of the IGF-binding

Table 1  
The Insulin-Like Growth Factor-Binding Proteins

| IGFBP   | Chromosome | Mature Protein | Source   | IGF-Binding Characteristics |
|---------|------------|----------------|--|-----------------------------|
| IGFBP-1 | 7p12-14    | 234* 25 kd     | Amniotic fluid<br>Human serum<br>Placenta<br>Endometrium             | IGF-1=IGF-2                 |
| IGFBP-2 | 2q33-34    | 289 31 kd      | Human/rat serum<br>Fetal serum<br>Cerebrospinal fluid                | IGF-2>IGF-1 (10:1)          |
| IGFBP-3 | 7p12-14    | 264 29 kd      | Human/rat serum<br>Liver<br>Fibroblasts<br>Follicular fluid          | IGF-1=IGF-2                 |
| IGFBP-4 | 17q12-21   | 237 26 kd      | Osteosarcoma<br>Prostatic carcinoma<br>Human/rat serum               | IGF-1=IGF-2                 |
| IGFBP-5 | 5          | 252 29 kd      | Osteosarcoma<br>Human bone<br>Cerebrospinal fluid<br>Human/rat serum | IGF-2>IGF-1 (5-15:1)        |
| IGFBP-6 | 12         | 216 23 kd      | Fibroblasts<br>Cerebrospinal fluid<br>Human/rat serum                | IGF-2>IGF-1 (70:1)          |

\* Number of amino acid residues  
Adapted from references 1-3.



proteins (IGFBPs) have substantial (50% to 80%) sequence homology within and between species. IGF-binding regions of IGFBP are located near cysteine residues in both the amino and carboxyl terminals, depending on the individual IGFBP. The affinity of an IGFBP for IGF is enhanced by increased phosphorylation of the IGFBP. Only 1% to 5% of the total serum IGF level is free; and 95% to 99% is bound to IGFBP – 90% to IGFBP-3 in a 150-kd complex, and ~5% to IGFBP-1, -2, and -4 as 30- to 50-kd complexes.

The IGFBPs both inhibit and enhance the bioactivity of the IGFs by controlling their clearance from blood and delivery to the target cell. The IGFBPs transport, serve as a reservoir, inhibit the degradation, and consequently prolong the half-life of the IGFs. The 150-kd IGFBP-3/IGF complex has a serum half-life of 14 to 18 hours and is unable to cross the vascular membrane; the half-life of the 40- to 50-kd, capillary permeable IGFBP-IGF complexes is 0.5 hours and that of free IGF is 0.2 hours. The smaller IGFBP-IGF complexes may enhance transcapillary transport of IGF, its delivery to the cell membrane, and interaction with its cellular receptors. The IGFBPs also protect against some of the potential adverse effects of IGF such as hypoglycemia and possibly excessive cell and tissue growth. In addition, the binding proteins have intrinsic biologic activity; thus, IGFBP-1 inhibits DNA synthesis in chick embryo fibroblasts and perhaps ovarian steroidogenesis independently of IGF.<sup>1</sup> IGFBP-3 inhibits insulin as well as IGF-1-mediated growth of IGFBP-3 transfected Balb/c 3T3 fibroblasts.<sup>4</sup>

## IGFBP-1

IGFBP-1 is synthesized by human secretory and late proliferative endometrium, placental decidua, granulosa cells, and liver. Near its carboxyl terminal IGFBP-1 has a tripeptide recognition sequence (Arg-Gly-Asp) for the integrins, a group of cell membrane

receptors, suggesting that IGFBP-1 may bind to the cell membrane. IGFBP-1 is present in follicular and amniotic fluid and in human serum, where its concentrations are highest at birth and decline progressively to adolescence. Serum values rise acutely during hypoglycemia and fasting and are high in patients with hypoinsulinemic diabetes mellitus and in those with growth hormone (GH) deficiency. The production of IGFBP-1 is depressed by insulin but stimulated by IGF-1; serum concentrations of IGFBP-1 are inversely related to those of insulin. Insulin also enhances translocation of IGFBP-1 to the extravascular space.<sup>5</sup> There is a diurnal variation in serum concentrations of IGFBP-1, with highest values at night, reflecting the reciprocal relationship between IGFBP-1 and insulin secretion. Primarily, IGFBP-1 inhibits the metabolic/growth-promoting actions of IGF. It has been suggested that in periods of nutrient deprivation such as hypoglycemia, increased levels of IGFBP-1 bind the IGFs, thus inhibiting their hypoglycemic and anabolic effects and permitting remaining metabolic fuels to maintain or restore cellular energy homeostasis. IGFBP-1 is the major IGFBP synthesized by human granulosa-luteal cells.<sup>6</sup> It inhibits the proliferative, differentiating, and functional effects of IGF-1 on granulosa-luteal cells, while its production is inhibited by follicle-stimulating hormone (FSH) and IGF-1.<sup>7</sup> In the polycystic ovary syndrome associated with hyperinsulinism in both lean and obese women, serum concentrations of IGFBP-1 are depressed.<sup>7</sup> It has been hypothesized that lowered ovarian IGFBP-1 production by hyperinsulinism leads to increased ovarian free IGF-1 and enhanced gonadotropin-mediated androgen production, in turn inhibiting follicular development and leading to follicular atresia.<sup>5</sup>

## IGFBP-2

IGFBP-2 is synthesized in the liver, brain, ovary, and endometrium and binds IGF-2 approximately 10-fold more avidly than IGF-1. It too has the recognition sequence (Arg-Gly-Asp) for binding to the cell membrane. Serum concentrations of IGFBP-2 are high in fetal and cord serum and in elderly and GH-deficient patients; they are lowest in pubertal individuals. IGFBP-2 values are increased by administration of insulin and IGF-1.<sup>2</sup> Serum concentrations of IGFBP-2 are also increased by hypoglycemia, hepatic and renal failure, and leukemia.<sup>8</sup> IGFBP-2 primarily inhibits IGF action. In contrast to IGFBP-1, IGFBP-2 is synthesized by human granulosa-luteal cells in response to IGF-2 and inhibited by human chorionic gonadotropin.<sup>9</sup> Serum concentrations of IGFBP-2 are slightly increased in patients with prostate cancer.<sup>10</sup>

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## IGFBP-3

IGFBP-3 is the major circulating IGFBP; it is associated with an acid-labile glycosylated subunit and with either IGF-1 or IGF-2, a 150-kd complex is formed (Figure 1).<sup>11</sup> IGFBP-3 is synthesized in the liver and many other tissues (fibroblasts, ovary, placenta) under the direction of GH. In experimental animal models, serum levels of IGFBP-3 fall after hypophysectomy, but tissue levels of mRNA for IGFBP-3 do not decline, suggesting that the decrease in IGFBP-3 is perhaps due to a decrease in its translation or an increase in its metabolism. In GH-resistant patients, IGF-1 has a biphasic effect on serum IGFBP-3 levels – decreasing values when administered for 7 days but increasing them after 6 months of therapy.<sup>12,13</sup> Circulating values of IGFBP-3 are constant over a 24-hour period and do not change rapidly in response to metabolic perturbation. IGFBP-3 levels are low in the fetus and neonate and increase throughout childhood; they reach maximum values in midpuberty, are stable during adulthood, and decline with aging. In children and adolescents IGFBP-3 concentrations reflect

the GH secretion rate.<sup>14</sup> IGFBP-3 values are low in GH-deficient subjects and high in hypersomatotropic patients, and are increased by administration of GH. IGFBP-3 both inhibits by sequestering IGF, and enhances the action of IGF by prolonging its half-life, sustaining its delivery to tissue and increasing its cellular binding through a cell surface associated IGFBP-3 molecule, thus perhaps facilitating interaction of IGF with its receptor.<sup>15</sup> Fibroblast-derived cell surface associated IGFBP-3 may be dissociated from the cell membrane by IGF. Transforming growth factor, type  $\beta_1$ , stimulates transcription of the fibroblast IGFBP-3 gene.<sup>16</sup>

The human acid labile subunit of the 150-kd IGF-1 binding complex (Figure 1) is an 84- to 86-kd glycosylated protein of hepatic origin and is composed of 552 amino acids, 22% of which are leucine. It does not bind to IGF, but binds only to IGFBP-3 to which IGF has bound. In serum the acid-labile subunit is present in the free and complexed state. Serum levels of the acid-labile subunit increase between birth and puberty, and decline in older subjects.<sup>2</sup> Values are high in acromegalic and low in hypsomatotropic patients. GH may directly regulate the synthesis of this material. The biologic role of the acid-labile subunit is uncertain; it may maintain the 150-kd IGF-binding complex, thus decreasing the rate of transfer of IGF with IGFBP-3 to the extravascular space and preventing the hypoglycemic effect of IGF.

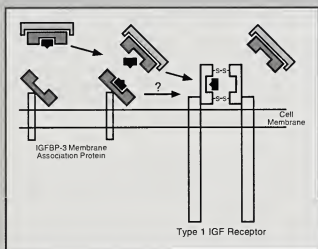
## IGFBP-4, IGFBP-5, AND IGFBP-6

IGFBP-4 is synthesized by human fetal and adult liver, brain, ovary, and bone, partly in response to IGF-1.<sup>17</sup> IGFBP-5 has been identified in follicular fluid; it binds IGF-2 severalfold more strongly than IGF-1. IGFBP-6 is synthesized by human adult liver but not by fetal liver, and binds IGF-2 ~70 times more avidly than IGF-1. IGFBP-4, -5, and -6 inhibit IGF-1 and -2 bioactivity.

## IGFBP PROTEASES

Serum IGFBP proteases degrade the IGFBPs and alter their binding affinity for and distribution of IGF. The protease for IGFBP-3 is present in the serum of pregnant women and in patients with severe debilitating illness, GH insensitivity, and various neoplastic diseases.<sup>8</sup> The prostate-specific antigen, a marker of prostatic neoplasia, is the IGFBP-3 protease in seminal plasma.<sup>12</sup> Serum concentrations of IGFBP-3 are decreased in patients with prostate cancer, suggesting that it may have been degraded by its protease, releasing IGF-1 and enhancing the effect of IGF-1 on growth of the neoplasm.<sup>10</sup> IGF-1 does

Figure 1



The circulating 150-kd complex of IGFBP-3 (light gray symbol), acid-labile subunit (dark gray symbol), and IGF (solid symbol) may deliver IGF directly to the IGF receptor or to a membrane-bound molecule of IGFBP-3 (possibly bound to a receptor for IGFBP-3) that, in turn, presents IGF to the IGF receptor.<sup>15</sup> Although each heterodimer of the type 1 IGF receptor can bind one molecule of IGF, the dimerized complex binds but one molecule of GH.

### Abbreviations

IGF = insulin-like growth factor  
IGFBP = IGF-binding protein

not affect IGFBP-3 protease activity in subjects with GH insensitivity.<sup>13</sup> A protease specific for IGFBP-4 activated primarily by IGF-2 is produced by adult human fibroblasts.<sup>19</sup> While intact IGFBP-4 inhibits IGF action, the proteolyzed form of IGFBP-4 does not. Thus, the IGFBP proteases may be another important factor regulating IGFBP binding of IGF and the bioavailability of IGF.

## OTHER BINDING PROTEINS

A binding protein for IGF-2 distinct from the IGFBPs discussed above has been found in human serum and urine. It has been characterized as the extracellular domain of the IGF-2/mannose-6-phosphate receptor.<sup>20,21</sup> The physiologic significance of this circulating protein is uncertain.

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## Abstracts From the Literature

### Influence of the High-Affinity Growth Hormone (GH)-Binding Protein on Plasma Profiles of Free and Bound GH and on the Apparent Half-Life of GH: Modeling Analysis and Clinical Applications

Growth hormone (GH) in serum is approximately equally divided between the free form and that bound to a high-affinity GH-binding protein (GHBP) that is the extracellular portion of the transmembrane GH receptor. Although GH is secreted in an episodic fashion (and it is this pattern of GH release that is most effective in stimulating growth in experimental animals), GHBP provides a circulating reservoir of GH. It also may be important for the presentation of GH to the cellular GH receptor.

Previously, Veldhuis et al demonstrated an apparent inverse relationship between the GH-binding capacity of GHBP and the secretion rate of GH. These workers have now established mathematical models to examine the interaction of GHBP with GH under both equilibrium conditions and in the more physiologic nonequilibrium state. Under equilibrium conditions, as might apply when GH is constantly infused or secreted by some tumors, variation of the secretion rate of constantly infused GH alters the total amount of GH in serum and the amount of bound and free hormone, as well as the percentage of the GHBP occupied by GH. Altering the affinity or capacity of GHBP does not affect the concentration of free GH. Thus, the amount of free GH in serum is directly related to its secretion rate and half-life and inversely related to the volume of distribution of GH, but it is little affected by GHBP itself. The authors' calculations yielded estimated GH half-lives for GHBP-bound GH, free GH, and total GH of 29, 7 to 9, and 18 minutes, respectively.

Applying these mathematical models to the nonequilibrium state, the investigators demonstrated that initially following a pulse of GH secretion, free GH levels increase and then decrease quickly as free GH is removed by both binding GHBP and by catabolic processes. The amount of GH bound to GHBP also increases rapidly, but declines more slowly than free GH levels. In this system, GHBP can serve as a reservoir of GH potentially available for delivery to target cells during intervals between pulses of GH release.

**Editor's comment:** The fact that some protein hormones such as GH and the insulin-like growth factors (IGFs) circulate bound to carrier proteins suggests that these carrier proteins might be physiologically important for the biologic activity of the hormone. That the serum GHBP is the extracellular domain of the GH receptor rather than a nonreceptor-related protein (as are IGF-binding proteins) implies that GHBP may interface with GH at the GH receptor. Perhaps this facilitates contact of GH with its receptor. Since *in vivo* GHBP enhances the growth-promoting effects of GH,<sup>1</sup> it is likely that at least GHBP increases the biologic half-life of GH, although it may well have some more specific effect.

*I have observed an occasional hyposomatotropic subject in whom antibodies to exogenous GH have developed, and in whom the linear growth response to GH has remained good to excellent over a period of years without increase in GH dosage. This suggests that in some patients antibodies to GH may also serve to increase the biologic effectiveness of GH.*

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## A Linkage Between DNA Markers on the X Chromosome and Male Sexual Orientation

The relationship between the genetic and environmental components of homosexuality is complex and has not been clearly elucidated. Studies of twins and adoptive siblings have shown the possibility of a large genetic component<sup>1</sup> and the search for a "homosexuality gene" has recently been undertaken. If sexual orientation is somehow programmed by the genes, then homosexuality may finally have a valid scientific explanation.

This recent study by Hamer et al reported linkage analysis of homosexual brothers that suggested there is a gene on the X chromosome that may be responsible for some component of male sexual orientation. The data come from the analysis of 76 homosexual men. The pedigree analysis showed that 13.5% of the gay men's brothers were homosexual; this is much higher than the 2% expected from the general population. It also showed that there were more gay relatives on the maternal side of the family, particularly those uncles and cousins who were sons of maternal aunts. This finding suggested that homosexuality could be a trait passed on through the females and so the possibility of a "gay gene" on the X chromosome.

Random X chromosome markers were identified in 40 pairs of homosexual brothers chosen because there was evidence that homosexuality was being inherited from the maternal side of the family. In order to assess how the markers were inherited, their mother's X chromosomes were also typed. For any X-linked genetic marker, the chance that 2 brothers inherited the same allele from their mother is 0.5. If the brothers inherited the same maternal X-linked markers more frequently than expected by chance, it is thought that they share a trait due to a gene in the region of the shared marker.

The results showed that except for 1 marker on Xq28, all markers along the X chromosome had been inherited as often as expected by chance. At the Xq28 marker, however, 33 of the

40 pairs had inherited the same allele from their mothers. The other 7 pairs of twins were discordant at that marker. Linkage of these markers gave a multipoint lod score of 4.0; this represents a 99.5% chance that there is a gene (or genes) in this area of chromosome X that predisposes to at least one subtype of male homosexuality.

Because Hamer et al did not study the group of homosexual men in whom the trait seems to be inherited from the father, the Xq28 site is not expected to explain all homosexual males. Even if it does, the site of a gene predisposing to male homosexuality may be difficult to find since the Xq28 region of chromosome X is known to contain several hundred unidentified genes.

Needless to say the results of this study will have to be replicated with a different population. If Hamer and colleagues have found the first genetic clue to male homosexuality, it will be interesting to see whether the Xq28 locus plays a role in female homosexuality.

Hamer DH, Hu S, Magnuson VL, et al. *Science* 1993;261:321-327.

**Editor's comment:** *The possibility that a gene may exist that predisposes to homosexuality is of considerable consequence. It reinforces the possibility that sexual orientation has a genetic basis rather than its being completely determined by the environment. Replication of this study should be easy and may provide further insight into the association between Xq28 and homosexuality.*

Judith G. Hall, MD

1. Holden C. *Science* 1992;255:33.

## Exclusively Paternal X Chromosomes in a Girl With Short Stature

Uniparental disomy (UPD) occurs when the 2 chromosomes of a pair have been inherited from only 1 parent (Engel, 1980). It is now recognized as a mechanism producing disease with certain chromosomes. Paternal UPD for sex chromosomes in humans has been reported in only 2 cases. The first was a case of paternal disomy of sex chromosomes in a father-to-son transmission of hemophilia A (Vidaud et al, 1989). The second is this recent report by Schinzel et al (1993) documenting paternal UPD for the X chromosome in a girl with a 45,X/46,XX karyotype and some features of Turner syndrome.

The patient reported by Schinzel and colleagues had growth retardation, short neck, broad chest, cubitus valgus, and gonadal dysfunction. Cytogenetic analysis on lymphoblasts showed 45,X/46,XX mosaicism. At age 11 7/12 there were 2/15 cells with a 45,X line; at age 13, 2/25 cells demonstrated a 45,X line, with the other cells being 46,XX; and by age 13 7/12, all 50 cells were 46,XX. DNA analysis on blood showed that both X chromosomes of the 46,XX line were of paternal origin. No other tissue (eg, fibroblasts) had been analyzed at the time of the report.

This report suggests that the most likely mechanism for

paternal XX UPD would be the loss or absence of the maternal X (before, during, or after meiosis) and early postmeiotic non-disjunction with complementation of the paternal X. This would allow for some degree of mosaicism with the 45,X line. It is possible that the phenotypic features of Turner syndrome were due to a higher degree of mosaicism with a predominant 45,X line in other tissues (ie, skin). From the studies in blood it is clear to see that the 46,XX line has a selective advantage; this may not be true for other tissues, in which the predominant line may be 45,X.

New molecular techniques allow the easy recognition of the parental origin of a specific chromosome. Maternal and paternal UPD has been reported for a number of autosomes with different disorders. Maternal UPD of chromosome 15 has been associated with Prader-Willi syndrome (Nicholls et al, 1989), paternal UPD of 15 has been reported with Angelman syndrome (Knoll et al, 1989), and segmental UPD of chromosome 11 (specifically the 11p15 region) has been shown in some cases of Beckwith-Wiedemann syndrome. UPD for chromosomes 22 and 21, however, has no known phenotypic effect (Schinzel et al, 1992).



As mentioned previously, paternal UPD for sex chromosomes has been reported in a few cases (Vidaud et al, 1989; Schinzel et al, 1993). XY mice with paternal UPD for sex chromosomes are known to be normal (Handel et al, 1990). This would suggest that this condition has very few or no phenotypic effects. Maternal XX UPD in female mice has never been reported, but XO female mice survive longer if the single X chromosome is maternally derived (Hunt, 1990). No explanation has been given for the differences in survival. Maternal disomy of the X chromosome in humans has been documented in normal fertile females (Avivi et al, 1992) and does not have any known phenotypic effect.

Maternal UPD for chromosome 7, maternal and paternal UPD for chromosome 16, and now paternal UPD for X chromosome have been associated with growth retardation. The patient reported by Schinzel et al (1993) had growth failure and minimal dysmorphic features suggestive of Turner syndrome. Due to the findings suggestive of Turner syndrome, further study of 8 families with a proband demonstrating growth failure and a 46,XX Turner-like syndrome was undertaken. However, all had

normal paternal and maternal sex chromosome contribution.

It is clear that more research is needed to establish the true incidence of paternal XX UPD and its phenotypic effects. So far the evidence has shown that the X chromosome has very few or no paternally imprinted regions.

Schinzel AA, Robinson WP, Binkert F, et al. *Hum Genet* 1993; 92:175-178.

**Editor's comment:** In this particular patient, the short stature may be related to being mosaic for the 45,X Turner syndrome cell line. The question is whether the loss of abnormal cell lines is the usual situation or if 46,XX has sufficient selective advantage to outgrow the 45,X cells. It will be interesting to look at the report of the fibroblast studies in this patient when they are done. They might give a clue to the origin of the 2 cell lines (ie, did the zygote start as 46,XpXp or 45,Xp). The frequency of UPD for sex chromosomes in the "normal" population will be quite interesting to determine.

Judith G. Hall, MD

## In Vivo Gene Therapy of Hemophilia B: Sustained Partial Correction in Factor IX Deficient Dogs

Hemophilia B is an X-linked clotting disorder affecting about 1 in 30,000 males. It can be managed successfully by infusion of virus-free factor IX preparations; however, the high cost of such preparations has limited their use and has led investigators to search for alternative therapies. This report from Savio Woo's team in Houston provides insight into potential gene therapy for this condition.

The investigators had previously demonstrated that retroviruses could successfully be used to deliver recombinant genes to liver cells in mice. In this study they extended their work to larger animals, targeting hepatocytes in dogs manifesting hemophilia B.

First, to show that a foreign gene could be transferred successfully to canine liver cells, they infused the portal veins of normal dogs with a retroviral vector containing the *Escherichia coli*  $\beta$ -galactosidase gene. Analysis of liver tissue and cells 2 weeks later revealed enzyme activity in some cells. Since this enzyme is not normally present in mammalian cells, its presence in this instance indicates that gene transfer was successful.

Next, they prepared a retroviral vector containing the coding sequences (cDNA) of canine factor IX and infused it into the portal veins of 3 hemophilia B dogs. The dogs harbored a missense mutation in the catalytic domain of the factor IX gene that abolished the antigenicity and severely disturbed the function of the clotting factor. The animals were then monitored for up to 9 months by immunoassays of factor IX antigen and by functional assays for the intrinsic pathway for clotting in which factor IX participates.

Plasma factor IX levels rose from undetectable to 2 to 10 ng/mL, where they remained for 6 months in 1 dog and for 9 months in another. More importantly, whole blood clotting times ranged from 15 to 25 minutes after treatment, compared with 45 to 55 minute values for untreated factor IX deficient littermates. Normal values for dogs range from 6 to 8 minutes.

Similarly, partial thromboplastin times decreased substantially after treatment, compared with pretreatment values. The improvement in clotting assay times continued through the follow-up period as noted above.

The authors acknowledge that the factor IX levels achieved by gene transfer were only about 0.1% of normal. However, they emphasize that these levels resulted in a substantial improvement in standard clotting parameters. They feel that the results demonstrate the feasibility of in vivo retroviral-mediated gene transfer into the livers of large animals. With technical advances in vector construction, they foresee potential uses of this approach for treatment of hemophilia B and other metabolic disorders secondary to hepatic deficiencies in humans.

Kay MA, Rothenberg S, Landen CN, et al. *Science* 1993;262: 117-119.

**Editor's comment:** It is ironic that when gene therapy was first contemplated, many wondered how the genes of interest would be assembled, what regulatory elements would be used to control their expression, and how the regulatory elements would be incorporated into vectors. Although construction of vectors that permit therapeutic genes to be expressed appropriately is still a formidable task, it is clear that delivery of such vectors to where they are needed (ie, cells, tissues, organs, etc) is also a major problem – perhaps an even bigger challenge. In other words, the greatest obstacle to gene therapy may lie in its cell biology rather than in its molecular biology. This paper reflects the recent attention that the delivery aspects of gene therapy are beginning to receive, which in turn reflects the rapid evolution of this field.

William A. Horton, MD

## Molecular Basis of the *Little* Mouse Phenotype and Implications for Cell Type-Specific Growth

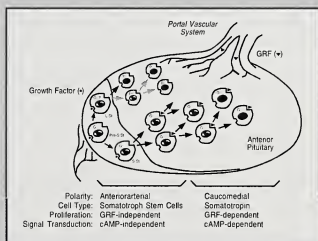
The investigators demonstrate that in the growth hormone (GH)-deficient *little* mouse the somatotrope receptor for GH-releasing hormone (GHRH) is abnormal. There is substitution of adenine by guanine (A→G) in the second nucleotide of codon 60 resulting in replacement of a highly conserved aspartic acid by glycine (Asp 60→Gly) in the amino terminal, extracellular, ligand-binding domain of the GHRHr. GHRH was unable to stimulate intracellular production of cyclic adenosine monophosphate in a CV1 cell line transfected with and expressing the abnormal GHRHr, whereas expression of wild-type GHRHr in these cells revealed normal GHRH activity, consistent with a functionally defective GHRHr in the *little* mouse. Examination of the microscopic structure of the anterior pituitary of these animals revealed hypoplasia and substantial loss of GHRHr and GH-expressing somatotropes but a normal population of lactotropes. Those pituitary cells expressing GHRHr and GH were confined to the periphery of the anterolateral regions of the adult gland. (In the normal mouse pituitary gland GHRHr and GH-expressing cells are widespread and generally distributed.) Pit-1, a transcription factor necessary for differentiation and function of somatotropes and other cells, was normally expressed in the pituitary of the *little* mouse. The authors conclude that Pit-1 is important for initial differentiation of stem cells and for differentiation of the common somatomammotrope into lactotropes and somatotropes, and that GHRH (requiring normal GHRHr) is responsible for further differentiation, distribution, and function of the mature GH-producing cell. Thus, there would appear to be a reservoir of somatotropes that, under the guidance of GHRH, divide and migrate to the caudomedial region of the normal mature anterior pituitary gland.

Lin S-C, Lin CR, Gukovsky I, et al. *Nature* 1993;364:208-213.

**Editor's comment:** Pit-1 is a tissue-specific POU domain protein that serves as a transcription-activating factor for genes, leading to differentiation of somatotropes, lactotropes, and thyrotropes, and for expression of the GH and prolactin genes. Absence of a functional Pit-1 protein (in the Snell rat and in humans) leads to pituitary hypoplasia and deficiencies of GH, prolactin, and thyrotropin secretion. The present article offers good evidence to suggest that after initial differentiation of stem cells, GHRH (and normal GHRHr) is required for further differentiation and function of the somatotrope. The list of possible causes of GH deficiency grows longer and now includes defective GHRHr. One suspects that human examples of the *little* mouse animal model of hypopituitarism will be detected.

Allen W. Root, MD

Figure 1



**Pit-1 Mediated GRF Regulation of Somatotrope Proliferation Model of growth hormone-releasing factor (GRF) receptor modulation of somatotrope proliferation lineage.** A presomatotrope stem cell (Pre-S St) is proposed to give rise to either a somatotrope-stem cell (S St) or lactotrope-stem cell (L-St) that at some point must express both growth hormone (GH) and prolactin (G<sup>+</sup>, P<sup>+</sup>). Somatotrope stem cells (S St) express GH (G<sup>+</sup>), and at least a subset are GRF-receptor positive (Y). Stem cells are located in the anterolateral circumference of the gland. Caudomedial proliferation of somatotropes requires GRF receptor and GRF, which is supplied by the portal circulation from median eminence. Somatotropes proliferate (indicated by mitotic spindle) across the entire anterior pituitary. In contrast, lactotropes, once differentiated, proliferate little, if at all (gray arrows and nucleus). The 2 zones of proliferation, representing stem cells or mature somatotropes, are cyclic adenosine monophosphate (cAMP)-independent or cAMP-dependent, respectively.

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### In Future Issues

#### Insulin-Like Growth Factor 2 and Growth by Yves Le Bouc, MD

#### Osteochondrodysplasias With Mild Clinical Manifestations: A Clinician's Guide by Richard M. Pauli, MD

#### Noonan Syndrome: A Review by Michael A. Patton, MA, MSc, MD

#### Prader-Willi Syndrome: The Unfolding Genetic Story by Uta Francke, MD

#### Treatment of Craniopharyngioma: End Results by Edward Laws, MD

## Overexpression of Dystrophin in Transgenic *mdx* Mice Eliminates Dystrophic Symptoms Without Toxicity

The development of gene therapy for human diseases has been gathering momentum on many fronts. Cox and colleagues cite a good example by reporting correction of many of the pathologic manifestations in a mouse model of Duchenne muscular dystrophy (DMD).

The investigators studied the *mdx* mouse, a mouse strain with a mutation in the dystrophin gene that eliminates expression of the muscle and brain forms of the protein. Although *mdx* mice do not exhibit the severe muscle weakness typical of DMD, they manifest marked impairment of diaphragm function and progressive degeneration and fibrosis of muscle fibers similar to that found in DMD.

Cox et al introduced a dystrophin transgene into *mdx* mice using standard microinjection techniques. The transgene contained the full-length mouse dystrophin cDNA controlled in a tissue-specific manner by regulatory elements of the mouse creatine kinase gene to obtain expression in muscle. Extensive comparisons were made between control mice, *mdx* (nontransgenic) mice, and *mdx* transgenic mice.

Several assays revealed expression of dystrophin in transgenic *mdx* muscle tissues. Immunoblot (western) analysis identified a skeletal and cardiac muscle-specific protein of appropriate size for dystrophin in transgenic *mdx* muscle but not in muscles from nontransgenic *mdx* mice. The protein was present in amounts about 50 times normal. Immunostaining confirmed these findings and localized the dystrophin to its normal site, the sarcolemma, as seen in control but not *mdx* mice. Histologic examination of muscles showed that the degenerative changes seen in the *mdx* mouse were virtually absent from the transgenic *mdx* muscles.

The investigators also examined the functional effects of the dystrophin transgene expression. Analysis of the contractile properties of the diaphragm revealed no differences between control and transgenic *mdx* muscle, whereas the power of *mdx* diaphragm to contract was reduced about one third from normal. Normally, dystrophin is associated with and induces the synthesis of sarcolemmal glycoproteins called dystrophin-associated proteins. These proteins are absent from DMD and *mdx* muscle; however, they were detected in transgenic *mdx* muscle.

Thus, the authors demonstrated correction of many of the pathologic markers and the functional sequelae of the *mdx* mutation without causing obvious harm to the mice. As emphasized by the investigators and in an accompanying editorial by Blau, one of the more interesting observations is that the considerable overexpression of apparently normal dystrophin did not appear to have deleterious effects on muscle structure or function.

Cox GA, Cole NM, Matsumura K, et al. *Nature* 1993;364:725-729. Blau HM. *Nature* 1993;364:673-675.

**Editor's comment:** A long-term concern of potential gene therapists has been that genes introduced therapeutically must be precisely regulated to be effective and safe. This suggested that entire genes, including their intervening sequences and upstream and downstream regulatory elements, may need to be packaged in therapeutic vectors. Such restrictions would eliminate many large genes, such as the dystrophin gene, from consideration for commonly used vectors, such as retroviruses, on the basis of size alone.

This paper reports that coding sequences alone, controlled by a promoter different from its own, can produce a tissue-specific functional protein that interrupts the natural course of the disease. This is technically a very important accomplishment. Surprising was the fact that the very large amount of gene product was still effective. As acknowledged by both Cox et al and Blau, this raises the possibility that regulation of "artificial" genes introduced for treatment may not need to be as tight as once believed, at least in some instances.

The strategy used to introduce the dystrophin cDNA in this investigation is obviously not suitable for humans. Nevertheless, the observations are quite relevant and will add to the growing body of knowledge needed to apply gene therapy to humans.

William A. Horton, MD

## The Y Chromosome in Turner Syndrome

The most common (40% to 60%) chromosomal constitution in patients with Turner syndrome is 45,X. With a 45,X karyotype in Turner syndrome it was initially thought that all patients lacked the entire second sex chromosome (either X or Y). However, the high lethality of a "pure" 45,X karyotype has led to the suggestion that most, if not all, live-born 45,X Turner patients are mosaic 45,X/45,XY or 45,X/46,XX.

The main functions of the mammalian Y chromosome are sex determination, early sexual differentiation, and the control of spermatogenesis and spermiogenesis. Recent DNA molecular studies have shown that some patients with 45,X – and even mosaic 45,X/46,XX – have residual cytogenetically undetectable Y chromosomal material in blood.<sup>1,2</sup> Further studies have shown that although the Y chromosome material may not be present in

peripheral blood, there is a probability of its being present in skin.

Some Turner syndrome patients have residual testicular tissue, and if there is some degree of mosaicism for Y chromosome DNA sequences, patients may experience excessive virilization and may be at increased risk for gonadoblastoma. This is why it is important to use reliable and sensitive diagnostic methods to ascertain the presence of Y chromosome material in Turner syndrome.

The study by Kocova et al evaluated 18 patients with clinical features of Turner syndrome. Ten patients had a 45,X karyotype, 7 were mosaic with a numeric or structural anomaly of the X chromosome, and 1 was 45,X/46,XX. None of these patients had any evidence of Y chromosome material in blood cytogenetic studies.

Polymerase chain reaction (PCR) and Southern blot techniques were used to detect the sex-determining region (SRY gene) and a repetitive sequence DY23 near the centromere of the Y chromosome in blood cells. Their results showed that 6 of 18 patients had positive Y chromosome signals. Three were readily detected on Southern blot and the other 3 showed chromosome Y material only after PCR amplification. They concluded that a "pure" 45,X karyotype is probably less common than is usually reported on cytogenetic studies and that Y mosaicism may go unnoticed unless PCR amplification is done.

Previous investigators<sup>1</sup> have been successful in using Southern blot to detect Y chromosome material. PCR amplification is a very sensitive method, and it is a more accurate method for determining the presence of Y sequences that may not be present in high-resolution karyotyping. Because of its high sensitivity, this method significantly increases the ability to look for micromosaicism in lymphocytes or skin fibroblasts.

The combination of Southern blotting and PCR techniques is helpful in identifying Y chromosome sequences in patients with Turner syndrome. These techniques may be required for some Turner syndrome patients with monosomy X or mosaic karyotype who have residual testicular tissue X. This will enable us to offer better counseling and clinical management.

Kocova M, Siegel SF, Wenger SL, et al. Detection of Y chromosome sequences in Turner's syndrome by Southern blot analysis of amplified DNA. *Lancet* 1993;342:140-143.

**Editor's comment:** In view of this evidence, the present challenge for practitioners is in deciding how far to go in looking

for Y chromosome material. PCR is obviously a helpful tool; however, it is important to remember that blood may not be enough to rule out mosaicism. We may need to look at fibroblasts. It may be necessary to establish new guidelines for managing Turner patients at risk of malignancies.

Judith G. Hall, MD

1. Muller U, et al. *Hum Genet* 1987;75:109-113.

2. Gemmill RM, et al. *Am J Hum Genet* 1987;41:157-167.

**2nd Editor's comment:** Approximately 50% of patients with Turner syndrome have the 45,X karyotype; the remainder have either X chromosome mosaicism and/or abnormal X chromosome formation. Since the majority of 45,X conceptions abort spontaneously, it has been suggested that many of the surviving 45,X patients may have subtle mosaicism. The present report demonstrates that many patients with the Turner phenotype carry the SRY sequence, but the clinical relevance of this observation is as yet unknown. As Held (Lancet 1993;342:128-129) suggests, the development of a 45,X-SRY+ subject could be the consequence of XY interchange followed by loss of 1 X chromosome by anaphase lag. Since the presence of Y chromosomal material places the Turner syndrome patient at increased risk for gonadoblastoma, he recommends that, when indicated, initial search for a Y chromosome fragment not detectable cytogenetically be conducted by fluorescence in situ hybridization (FISH) until the methodology of Kocova et al has been validated and the significance of the finding ascertained.

Allen W. Root, MD

## A Nonpeptidyl Growth Hormone Secretagogue and Stimulation of Growth Hormone Release From Rat Pituitary Cells by L-692,429, a Novel Non-Peptidyl GH Secretagogue

The investigators describe a benzolactam derivative chemically engineered by analysis of the structure-activity relationships of the growth hormone-releasing hexapeptide (GHRP-6 = His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>) that suggested that the aromatic amino acids and the NH<sub>2</sub>-terminal amine were the important bioactive sites of this molecule. The active R enantiomer, designated L-692,429, functions in a manner similar to GHRP-6. First, as does GHRP-6, it has a synergistic effect with growth hormone-releasing hormone (GHRH) on GH release and intracellular levels of cyclic adenosine 3',5'-monophosphate (cAMP). Second, it has no added effect on GH release when paired with maximal amounts of GHRP-6. Third, its effect on GH secretion is antagonized by an inhibitor of GHRP-6 activity, and the effect of GHRP-6 is inhibited by an antagonist of L-692,429. Fourth, rat pituitary cells desensitized to either GHRP-6 or L-692,429 are insensitive to the GH-releasing effect of the other compound but not to GHRH. Fifth, both GHRP-6 and L-692,429 block potassium currents and increase intracellular calcium levels, leading to depolarization of pituitary cells and GH secretion. Sixth, both GHRP-6 and L-692,429 activate protein kinase C. When administered intravenously, L-692,429 stimulates GH release in primates (including humans) and in rats, dogs, pigs, and sheep. Serum concentrations of corticotropin and cortisol

increase slightly after its administration, but serum levels of luteinizing hormone, prolactin, insulin, and thyroxine do not change. Somatostatin inhibits the effect of L-692,429 by preventing depolarization of the pituitary cell.

Smith RG, Cheng K, Schoen WR, et al. *Science* 1993;260:1640-1643.

Cheng K, Chan WW-S, Butler B, et al. *Endocrinology* 1993;132:2729-2731.

**Editor's comment:** The authors have introduced a nonpeptidyl compound that is likely to be absorbed from the gastrointestinal tract without digestion/degradation; thus it is orally active, permitting development of an enteral therapeutic agent. Furthermore, these data complement those implied by the identification of the specific GH-releasing property of GHRP-6 (an analogue of enkephalin, but one devoid of opioid activity) regarding the presence of an endogenous ligand with GH-releasing activity distinct from GHRH that has yet to be identified. Analysis of basic structure-function relationships is likely to have again proven valuable in the development of clinically therapeutic agent(s).

Allen W. Root, MD



## Influence of Spontaneous or Induced Puberty on the Growth Promoting Effect of Treatment With Growth Hormone in Girls With Turner's Syndrome

Among 36 girls with Turner syndrome treated for 3 years with recombinant human growth hormone (rhGH) 1 IU/kg/wk in daily subcutaneous injections, 15 (aged  $9.2 \pm 2.2$  years) remained prepubertal during the 3 years of treatment, 4 (aged  $12.0 \pm 1.2$  years) showed spontaneous breast development, and 17 (aged  $12.9 \pm 1.7$  years) received ethinyl estradiol 0.1 µg/kg/d during the third year on rhGH.

Height velocity increased significantly during the first rhGH treatment year in all patients, then decreased but remained above baseline values. Height velocity was higher in the few patients who developed spontaneous puberty than in age-matched controls:  $8.9 \pm 1.2$  cm/y vs  $7.4 \pm 1.2$  cm/y,  $P < 0.05$ . Estradiol 0.1 µg/kg/d seemed to significantly reduce the growth velocity during the third year:  $4.0 \pm 1.6$  cm/y vs  $5.6 \pm 1.2$  cm/y in the nonpubertal patients. However, this difference related mainly to age, since the growth rate expressed in standard deviation score (SDS) for age was not different in the 2 groups:  $2.2 \pm 1.3$  vs  $2.2 \pm 1.0$  SDS.

Multivariate regression analysis showed some discrepancies – mainly a positive effect of estradiol on apparent bone maturation in patients receiving it during the second year of treatment but not during the third year.

The authors conclude: "The onset of spontaneous puberty during the first years of rhGH treatment seems to have an additive effect to rhGH on height velocity. Induction of puberty with oral administration of 100 ng/kg/d ethinyl oestradiol did not

have any beneficial effect on height velocity and seems therefore not to be the optimal way to induce puberty with an adequate pubertal growth spurt in girls with Turner's syndrome under rhGH therapy. Different doses and routes of oestrogen administration have to be evaluated in order to mimic the growth promoting effect of spontaneous puberty as well as possible."

Massa G, Maes M, Heinrichs C, et al. *Clin Endocrinol* 1993;38: 253-260.

**Editor's comment:** Among several recent reports of the possible effects of associating estrogens with GH in Turner syndrome at the age of physiologic puberty, this one seems particularly relevant. Our own data (not yet published) are in good accordance with those of the present authors: a very low dose of estradiol given at an age of approximately 12 years, or rather at a bone age close to 11 years, has no significant positive or negative effects on the growth rate obtained with GH. At a higher dose, or given earlier, estradiol could reduce the growth rate and/or accelerate bone maturation. The positive effect of spontaneous sexual development on growth rate in Turner girls, documented in a small number of patients in the present study, seemed less certain in our data, and will have to be established with larger groups of patients compared with controls.

Jean-Claude Job, MD

## Hazards of Pharmacological Tests of Growth Hormone Secretion in Childhood

Three patients underwent growth hormone (GH) stimulation tests with insulin-induced hypoglycemia and developed severe complications. Two died and 1 sustained neurologic damage.

A 4½-year-old girl was tested with 0.1 U/kg of insulin given intravenously. Nausea, sweating, and tachycardia occurred within 35 minutes, and she became unresponsive. She was given 50% dextrose and 100 mg hydrocortisone intravenously, and 1 mg of glucagon intramuscularly. Blood glucose measured for the first time 1 hour after insulin was greater than 44 mmol/L. Generalized convulsions occurred, which were controlled with IV diazepam and phenytoin. The blood glucose increased to 130 mmol/L and plasma osmolality rose to 388 mmol/L. Treatment with IV insulin was given, and plasma osmolality fell to 339 mmol/L. She became hypotensive and acidotic (arterial pH of 7.02), and then comatose with fixed dilated pupils and no brain-stem reflexes. Cerebral edema was demonstrated by computed tomography (CT). No definite cortical activity was noted by electroencephalography (EEG). Renal failure with anuria ensued, and death occurred 24 hours after the start of the test.

A 9-year-old girl with developmental delay was admitted for investigation of short stature and polyuria. The septum pellucidum was absent on the CT scan, consistent with de Morsier syndrome. Insulin (0.1 U/kg), thyrotropin-releasing hormone, and luteinizing hormone were given IV. At 20 minutes, her blood glucose concentration was 2.0 mmol/L, and the patient became drowsy. With IV dextrose and hydrocortisone, her blood

glucose increased to 17 mmol/L, but she developed generalized convulsions, which were treated with diazepam, paraldehyde, phenytoin, and thiopentone. Two hours post insulin, the plasma sodium concentration was 110 mmol/L and her potassium concentration was 2.7 mmol/L. Repeat CT scans revealed a right-sided subdural hematoma with a shift of the midline. Subsequent CT scans revealed considerable cerebral edema with tentorial herniation and intracerebral hemorrhages. Diabetes insipidus responded to desmopressin treatment. The patient recovered, but seizures remained a problem.

The third patient, a 2-year-old girl, was admitted to investigate short stature and secondary hypothyroidism. After an overnight fast, glucagon 100 µg/kg was given IM. Three hours later, she ate a partial lunch, and was awake and crying at discharge. However, plasma glucose concentrations after the administration of glucagon were later found to be 0.5 to 1.0 mmol/L. At home, vomiting began; she progressively deteriorated and became unconscious. In a local emergency department, her blood glucose concentration was 1.0 mmol/L. External cardiac massage was given, and she was intubated and treated with intravenous bicarbonate, adrenaline, hydrocortisone, and dextrose. When transferred to a referral center, she became hypotensive with fixed dilated pupils, and the EEG showed total electrocerebral inactivity. A small pituitary gland and an atrophic thyroid gland were found at postmortem examination. The tolerance test revealed deficiencies of thyrotropin-stimulating hormone and GH. Cortisol secretion was normal.

The authors concluded that given the risks of pharmacologic testing for GH deficiency, these tests should be avoided; the selection of patients for GH treatment should not be determined by GH response to a pharmacologic test but by the growth rate of the patient before and during treatment.

Shah A, Stanhope R, Matthew D. *Br Med J* 1992;304:173-174.

**Editor's comment:** In this paper, 3 cases with major tragic complications associated with pharmacologic diagnostic tests for GH stimulation were presented. This paper is reported now, although it was published in 1992, because it encourages all endocrinologists to exercise caution while performing stimulation tests, and prompts us to reconsider the validity of such assessments. As was clearly presented in the paper, the complications described were preventable with appropriate management; they were not solely due to the intrinsic risks of the test. Two patients developed hyperglycemic hyperosmolar coma, in addition to complications, as a result of their management after the test, and 1 developed severe hypoglycemia that was not recognized nor treated properly.

In this era of the "gatekeeper" in medical care, this paper emphasizes that only experienced and qualified individuals should perform provocative stimulation tests for diagnosing GH deficiency. They should also be qualified to effectively manage the possible complications.

These tests should be performed in a hospital setting, and with qualified personnel and the equipment necessary to treat unexpected episodes such as hypoglycemia. Blood glucose

should be monitored by a glucometer throughout the test. Finally, overtreatment should be avoided. The dose of glucose infused when hypoglycemia ensues should be 0.5 to 1.0 g/kg. This amount of glucose should be infused even when no adverse effects are seen at the end of the test. This ensures rapid restoration of blood glucose in case there is a delay in oral intake.

Regarding the validity of the instruments used for diagnosis of short stature children, it is worth questioning the necessity of potentially risky tests. These tests may not necessarily qualify the condition of the patient to a more precise degree, nor do they determine the treatment to be used any better than less risky tests. The so-called gold standard for assessment of GH deficiency – the insulin tolerance test – needs to be challenged, not only because it may cause harm but also because it may yield inappropriate information. Throughout the country, pediatric endocrinologists waiver on the levels of GH elicited by insulin-induced hypoglycemia to diagnose hypopituitarism: is it more than 7, 8, or 10 mg/dL of serum GH? What is the minimal response needed to avoid GH treatment? Do the levels achieved after pharmacologic testing determine the response to treatment? Isn't it better to make such decisions based on accurate physical measurements and monitoring and assessment of the growth rate of short children?

In this era of cost containment, pediatric endocrinologists need to reexamine the validity of their procedures and be sure that we continue to do what we have always done best: treat patients, not test results.

Fima Lifshitz, MD

## Life With Turner's Syndrome: A Psychosocial Report From 22 Middle-Aged Women

Sylvén and colleagues utilized a semistructured interview to assess social functioning, emotional development, sexuality, and coping style in a group of 22 middle-aged women with a median age of 44.5 years (range, 39-63 years) with Turner syndrome. These individuals included 10 (45.5%) with a 45,X karyotype and 12 (54.5%) who were mosaic. The interview included questions concerning family background, social identity, emotional development, relationships, female identity, sexuality, and Turner syndrome. The median age at diagnosis was 17.5 years. The majority (12) were diagnosed when puberty failed to occur. Complaints of short stature prompted 7 individuals to seek medical care. Seventeen (77%) of the women reported good contact with their parents and had left home at an average age of 19 years to pursue work, studies, or to marry. All completed elementary school, but only 3 had completed upper secondary schools. Work was considered important for self-esteem and self-confidence, and no woman had been unemployed for a long period. Six were unable to attain desired employment because of their body height, including 1 woman who was not admitted to a nursing school because of her height. Three stated that their parents had suggested they not pursue a physically demanding career. Nineteen (86%) of the women felt the need for improved self-esteem, and of the 16 (72.7%) that had suffered from depression, half (50%) cited infertility as the key factor. For these women, the most important relationship was with their mothers. Ten of the 22 had few friends during their school years, even though 17 presently have very close friendships. Female identity was defined by most in

terms of being in a relationship, but many felt that something was missing psychologically and in their appearance. The median age at which female hormone replacement therapy was begun was 18 years. This may have contributed to the lack of friends and isolation from peers during their school years. Thirteen of the 22 stated that menstruation was a positive experience but unfortunately reminded them of their infertility. During their teenage years all 22 experienced romantic fantasies. Fifteen were presently married, and their median age at first sexual experience was 19.5 years. Eleven (50%) of these adult women were reasonably satisfied with their body; however, 12 (54%) stated that short stature had been psychologically

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upsetting to them. Their first thought at diagnosis usually concerned infertility, and this was the most painful aspect of the diagnosis. Thirteen women (60%) felt that they had received insufficient information concerning their diagnosis from their health-care team. Nineteen of the 22 women (86%) were satisfied with their present life.

In analyzing their findings, the authors identified 4 different ways of coping. Seven of the women were judged to act in an adolescent manner. An additional 7 expressed a chronic feeling of inferiority. Four expressed grief that they had not been able to discuss their diagnosis. Four women were felt to be unaffected by their disorder. The authors point out that these women had been deprived of important psychosocial development and maturation, and many did not understand the significance of their diagnosis.

Sylvén L, Magnusson C, Hagenfeldt K, et al. *Acta Endocrinol* 1993;129:188-194.

**Editor's comment:** This is an interesting report. There are few data available on the lifetime adjustment of middle-aged women with Turner syndrome. Most psychosocial studies have been performed with relatively young women. Unfortunately, the authors failed to interview a control group of similarly aged women, and it may be that much of the data reported by these women would be similar to those of other women without chromosomal disorders. Unfortunately, these women were diagnosed at a relatively late age, and, therefore, hormone replacement therapy was begun at an age when most women would have been completing their formal education. Growth hormone therapy was obviously not available to improve their stature 27 years ago. One can only wonder whether earlier treatment of short stature and appropriately timed hormonal replacement therapy will alter the adult psychosocial status of girls currently being diagnosed with Turner syndrome.

William L. Clarke, MD

## Final Height in Patients Treated for Childhood Acute Lymphoblastic Leukemia

Sklar et al determined the adult (females >18 years; males >21 years) heights of 127 patients treated (in 4 institutions) before 12 years of age for acute lymphoblastic leukemia (ALL) who were disease free, had received no growth-stimulating therapy, and had undergone spontaneous sexual maturation. Various chemotherapeutic regimens were utilized in these patients, but the type and intensity of chemotherapy did not affect final height. Growth data in this patient population were analyzed based on exposure to irradiation of the central nervous system (CNS-XRT). The mean ( $\pm$ SEM) height standard deviation score (SDS) at diagnosis was within the normal range in all groups ( $+0.28 \pm 0.12$ ). Height SDS declined in all subjects during treatment, but final height SDS was less than height SDS at diagnosis in 75% of patients. In all groups of patients, adult stature SDS was significantly less ( $P < 0.01$ ) than the height SDS at diagnosis:

| Patient Groups     | n  | Adult Height SDS |
|--------------------|----|------------------|
| Chemotherapy alone | 38 | 0.49 $\pm$ 0.14  |
| 1800 cGy CNS-XRT   | 36 | 0.65 $\pm$ 0.15  |
| 2400 cGy CNS-XRT   | 53 | 1.38 $\pm$ 0.16  |

Patients receiving CNS-XRT lost more height than those who did not, and the higher the dose of CNS-XRT the greater the loss of final height. Much of the loss of adult stature was recorded in females  $\leq 4$  years of age at diagnosis who also received CNS-XRT (1800 cGy, -1.38 SDS; 2400 cGy, -2.68 SDS), attributable in part to early pubertal development and partial deficiency of growth hormone secretion in these subjects, although no data concerning these phenomena were reported.

As reported by Katz et al, the Pediatric Oncology Group (POG) has gathered data on adult stature (females >16 years; males >18 years) in 109 subjects treated in 2 similar multiarm POG chemotherapeutic protocols for children with ALL, where 51 patients also received 2400 cGy cranial irradiation and 58 patients received no XRT. All of the patients entered puberty spontaneously, and none received growth hormone (GH) therapy. Mean height SDS at diagnosis was  $-0.06 \pm 1.4$ .

The adult stature of subjects receiving only chemotherapy was -0.14 SDS, while the final height of the group who also received CNS-XRT was -1.04 SDS ( $P < 0.001$ ). Final height SDS was lower in female patients than in male patients, whether or not they had also received CNS-XRT.

Sklar C, Mertens A, Walter A, et al. Final height after treatment for childhood acute lymphoblastic leukemia: comparison of no cranial irradiation with 1800 and 2400 centigrays of cranial irradiation. *J Pediatr* 1993;123(1):59-64.

Katz JA, Pollack BH, Jacaruso D, et al. Final attained height in patients successfully treated for childhood acute lymphoblastic leukemia. *J Pediatr* 1993;123(4):546-552.

**Editor's comment:** The adverse effect of CNS-XRT on linear growth in children in the first several years after treatment has been long recognized, but these 2 articles now report long-term, final height data in 246 subjects. In children with ALL not receiving CNS-XRT, growth may be somewhat compromised (although the 2 studies disagree on this point), primarily due to the severity of the illness, its treatment (often with glucocorticoids), and inanition. However, once therapy is completed further loss in height does not occur (Sklar et al). Similarly for children receiving 1800 cGy CNS-XRT, there is no incremental height loss after completion of therapy. However, in children receiving 2400 cGy CNS-XRT, adult height may be substantially compromised (due both to partial deficiency of GH and to early or precocious puberty), although most children will have an adult stature within  $\pm 2$  SD of the mean.

Two groups have reported data on the growth of children undergoing bone marrow transplantation (BMT) and total body irradiation (TBI). Bozzola et al<sup>1</sup> administered 1200 cGy TBI (in 6 fractions over 3 days) to 18 children whose growth rates were normal prior to therapy. They observed an immediate decline in growth rate in 9 children who had received prior CNS-XRT (1800 cGy); growth rate remained normal in 9 children who had not received CNS-XRT for the first 2 years after BMT, but declined in the third year. Sixteen of the 18 patients had subnormal GH secretion (peak  $< 10$  ng/dL) by provocative testing. Five of 7



subjects treated with GH responded with an increase in growth velocity. Thomas et al<sup>2</sup> compared the growth of 66 children undergoing BMT, preconditioned with TBI administered as a single dose (900 to 1000 cGy) or in fractions over 3 days (1200 cGy in 6 fractions; 1440 cGy in 8 fractions). Height SDSs prior to BMT were near zero (as were midparental heights). In patients receiving a single TBI fraction, growth rate fell immediately after BMT and height SDS continued to decline over the next 3 years ( $-0.90 \pm 0.90$ ). In children undergoing fractionated TBI, growth velocity declined less rapidly; however, 3 years after BMT, height SDS was significantly lower ( $-0.22 \pm 1.02$ ) than it had been before BMT. Children who received CNS-XRT prior to BMT had a more profound decline in growth rate with either TBI regimen. Patients who received only fractionated TBI did not lose significant height within 3 years after BMT, whereas those without prior CNS-XRT who received single-dose TBI did lose substantial height (SDS,  $-0.74 \pm 1.1$ ). The growth in sitting height was more impaired by TBI (no matter how fractionated) than was growth in leg length, suggesting that the adverse effects of TBI on vertebral epiphyses may be more profound

than on the femoral and tibial epiphyses. In this study, 17 children received GH, with no significant effect on height SDS over 3 years of therapy.

These reports lead to the following conclusions: (1) Growth can be impaired by ALL and its primary treatment; (2) CNS-XRT at a dose of 1800 cGy impairs growth acutely but results in only slightly more loss of adult stature than does the primary disease and its treatment; (3) TBI delivered in fractionated doses does not impair growth (for 3 years after BMT); (4) CNS-XRT at a dose of 1800 cGy followed by TBI for BMT leads to further growth retardation; (5) CNS-XRT at a dose of 2400 cGy significantly impairs growth; (6) TBI impairs vertebral growth to a greater extent than limb growth; (7) females receiving CNS-XRT lose significantly more adult height than do males; and (8) the efficacy of GH therapy in children with BMT is questionable.

Allan W. Root, MD

1. Bozzola M, et al. *Horm Res* 1993;39:122-126.

2. Thomas BC, et al. *Eur J Pediatr* 1993;152:888-892.

## Growth and Growth Hormone Secretion After Bone Marrow Transplantation

Growth and its hormonal factors were studied in 29 children (14 males, 15 females) having undergone bone marrow transplantation (BMT) prepared according to 4 different protocols: total body irradiation (TBI) of 10 grays (Gy) in a single exposure (group 1, 11 children aged  $7.3 \pm 1$  years); TBI of 8 Gy in a single exposure (group 2, 4 children aged  $3.1 \pm 0.6$  years); TBI of 12 Gy given as 6 fractionated doses (group 3, 7 children aged  $5.3 \pm 0.2$  years); or chemotherapy alone (group 4, 7 children aged  $1.3 \pm 0.4$  years). Growth hormone (GH) secretion was first evaluated, 2 to 7.5 years after transplantation, by combined arginine-insulin stimulation test, with a peak above 10 ng/mL in 26 patients, and 6.9 to 8.9 ng/mL in 3 patients of group 1. A second evaluation was performed 2 to 5 years later in 10 patients, with normal results in 8, a subnormal GH peak value in 2 patients from group 1, but no significant change from the first evaluation, and no deficiency of the nocturnal GH plasma levels. Plasma IGF-1, at the time of first evaluation, was normal for sex and age in 18 patients, subnormal in 11, including 2 low GH-responders, and not correlated with the body mass index. At the time of the second evaluation, the mean plasma IGF-1 level was not significantly changed, but 3 of the 4 patients having low IGF-1 at the first evaluation had a normal value at the second. Plasma free thyroxine was decreased in 3 patients, who were therefore given a replacement dose of L-thyroxine.

Clinical follow-up showed a decrease of height SDS for age in the 3 irradiated groups, the mean cumulated change in the 3 years following BMT being  $-1.4 \pm 0.2$  SD in group 1,  $-0.1 \pm 0.4$  in group 2, and  $-0.4 \pm 0.2$  in group 3, while group 4 had a catch-up of  $1.5 \pm 0.5$  SD. The changes were significant in groups 1 and 4 only. Comparing growth of one monozygotic twin of group 1 with that of his brother (donor) showed a difference of 17.5 cm in final height. Among 8 patients with congenital immune deficiency and growth retardation at the time of BMT, those conditioned by chemotherapy alone had significant catch-up growth while those conditioned by X-rays (a single 8 Gy exposure) did not.

The authors conclude that the total radiation dose is critical for growth evolution, as is the fractionation schedule. For the TBI doses and the interval since BMT studied, they did not find correlation between GH or IGF-1 and the height loss. The rapidity of decreased growth velocity after TBI and the comparison between monozygotic twins lead them to suggest that radiation-induced skeletal lesions are partly responsible for the decreased growth.

Brauner R, et al. *Arch Dis Child* 1993;68:458-463.

**Editor's comment:** This protracted study of a large group of children who underwent BMT points out the discrepancy between their growth and the results of repeated measurements of GH secretion or plasma IGF-1, and the clear relationships between transplantation conditioning and growth in the following years. Only the group of children who had been exposed to a single exposure of 10 Gy failed to grow. Those given a single dose of 8 Gy or a fractionated dose of 12 Gy had no serious growth decrease, and those conditioned by chemotherapy alone had catch-up growth. In the group given 10 Gy, there was no good agreement between growth and the results of GH and IGF-1 measurements.

Regarding GH values, the study demonstrated that GH levels are seldom subnormal in children subjected to BMT, and that a second evaluation several years later usually does not evidence delayed changes. However, the data suggest that the protocol of conditioning with 10 Gy in a single dose poses a serious risk of partial GH deficiency and that GH-deficient patients given GH at standard doses normalized their growth velocity, a fact that favors the contribution of hormonal deficiency to the decreased growth of these patients. Conditioning by a fractionated schedule of irradiation or the use of chemotherapy is certainly preferable.

Jean-Claude Job, MD



## Growth and Growth Hormone in Children During and After Therapy for Acute Lymphoblastic Leukaemia

Caruso-Nicoletti et al studied growth hormone (GH) secretion prospectively in (1) a group of 50 children with acute lymphoblastic leukemia (ALL) over a 2- to 5-year period following diagnosis and (2) in a group of 12 long-term survivors. Subjects in the longitudinal group had a median age at diagnosis of 5 years and were treated according to protocols of the Italian Group of Paediatric Haematology Oncology with combined chemotherapy as induction and post-remission therapy over 2 years. Children labeled as high or average risk received preventive central nervous system therapy, including intrathecal methotrexate, plus cranial irradiation up to 18 Gy, divided in 10 fractions over a 2-week period. Height was measured at diagnosis, every 3 months during treatment, and every 6 months post-treatment using a Harpenden stadiometer. In patients receiving only chemotherapy (n=8) height was measured yearly. Bone age was determined according to the TW2 method in 31 patients at diagnosis and 19 at the end of therapy. Patients older than 8 years underwent Tanner staging every 6 months. In addition, parents' heights were measured. Arginine-insulin tolerance tests (AITTs) were performed in 19 of the children who underwent cranial irradiation. Ten of these underwent evaluation prior to and immediately after radiation while the remaining 9 underwent a single AITT 2 years after the 2-year cycle of therapy. The group of 12 long-term survivors was evaluated 9.2  $\pm$  2.3 years after diagnosis at a median age of 13.6 years (range, 9.4 to 20.5 years). All long-term survivors received 24 Gy of prophylactic cranial radiations.

All children had a decrease in growth velocity during the first year of therapy. Mean growth velocity standard deviation score (SDS) at 6 months was -2.48 in patients who received radiotherapy (RT) and -1.22 at 1 year. During the second year of treatment, growth velocity returned to near normal, and during the third year children showed a catch-up growth with a mean growth velocity SDS of +1.08 in children receiving RT and +1.19

in the others (NRT). Mean height SDS decreased to +0.58 and +0.04 in RT and NRT patients, respectively, during the fourth and fifth years. Bone age after discontinuation of therapy had increased from that at diagnosis in a linear manner. All patients tested had positive GH responses to at least 1 stimulation test, both at diagnosis and after RT. In addition, all patients tested 3 to 5 years after diagnosis had normal GH responses. In the group of long-term survivors the mean height SDS was +0.23  $\pm$  0.83 at diagnosis and -0.25  $\pm$  0.69 at follow-up. Bone age SDS at follow-up was 0.97  $\pm$  0.2 and pubertal development was normal. Mean age of menarche in the long-term survivors was 10.6  $\pm$  1.5 years.

Caruso-Nicoletti M, Mancuso M, Spadaro G, et al. *Eur J Pediatr* 1993;152:730-733.

**Editor's comment:** This is an interesting study and its results need to be contrasted with those of Schriock et al reported in GGH (1991;7[4]:15). In that study, a significant mean final height decrement in survivors of ALL who were less than 12 years at diagnosis was observed. Similar findings were recently reported by Katz et al (*J Pediatr* 1993;123:546-552) and attributed to cranial irradiation. The authors of the present study suggest the differences between their results and other studies may be due to the dose of radiation, the interval during which the radiation was administered, or patient age at the time of radiation. It is interesting that they found normal GH secretion in their long-term survivors despite their receiving 24 Gy radiation. The growth deceleration with chemotherapy and radiation followed by increased velocity associated with the cessation of therapy is encouraging. The mean age of menarche is somewhat surprising as it is somewhat lower than might be expected in American girls.

William L. Clarke, MD

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# GROWTH

## Genetics & Hormones

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### Insulin-Like Growth Factor 2 and Growth

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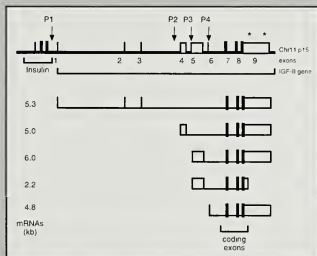
Insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) are polypeptides involved in metabolism, growth, and cell differentiation.<sup>1</sup> IGF-1 is mainly produced during the postnatal growth period. It is involved in statural growth, exerting an endocrine mechanism of action on certain tissues, including cartilage. Furthermore, autocrine/paracrine actions are described in various tissues expressing high levels of IGF-1.<sup>1</sup>

In contrast to IGF-1, IGF-2 seems to play a role predominantly during fetal development. However, in human as well as in other mammals, elevated IGF-2 levels persist in serum after birth and even increase during childhood and in young animals. This suggests an endocrine role for IGF-2. IGF-2 is also produced in many tissues, where it has an autocrine/paracrine mechanism of action.<sup>1</sup> The locus of the IGF-2 gene is chromosome 11p15.5, which contains 9 exons. Different IGF-2 mRNAs are expressed, depending on the promoter used among the 4 promoters (P1 through P4) of the IGF-2 gene and on tissue type and stage of development (Figure 1).<sup>2,3</sup>

#### IGF-2 AND FETAL GROWTH

IGF-2 plays an important role in fetal growth and differentiation. It is expressed very early in development. In mice, transcripts are detected as early as the blastocyst stage.<sup>4</sup> IGF-2 expression is found in different types of cells, such as muscle, cartilage, spinal ganglia, hepatocyte, and lung. During fetal life, IGF-2 expression varies relative to the developmental stage. The regulation of IGF-2 expression in the fetus is poorly defined. However, nutrition does not appear to play a central role.<sup>5</sup> Direct evidence for the physiologic role of IGF-2 in embryonic growth was provided by the work of De Chiara et al.<sup>6</sup>

Figure 1



Structure of the human IGF-2 gene and mRNA species.<sup>1-3</sup> Arrows indicate the 4 promoters (P1-P4) alternatively used depending on the tissue and developmental stage concerned. The 5.3 kb mRNA P1 is expressed only in the adult liver. The other mRNA (P2-P4) are expressed in all fetal tissues and in nonhepatic adult tissues.

\* Indicates 2 alternative polyadenylated sites.

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Chimeric mice were obtained by gene targeting; IGF-2 gene function was abolished for only 1 allele. The results demonstrated the relation between the loss of function of this 1 allele and a growth-deficiency phenotype, at least at a stage as early as embryonic day 16 and persisting after birth. The size of the heterozygous mice was approximately 60% of the normal size. Moreover, IGF-2 mRNA expression of the intact allele was 10-fold less in heterozygotes than in wild-type embryos. Further studies using these transgenic mice were extended to several generations.<sup>7</sup> The homozygote mutant mice, in which both alleles were disrupted, did not exhibit any difference from heterozygotes. This indicated no IGF-2 gene-dose-dependent effect on growth. Furthermore, genetic evidence is provided indicating that the IGF-2 locus is subject to parental imprinting: only the paternally derived IGF-2 allele is expressible.<sup>7</sup>

## IGF-2, CELLULAR GROWTH, AND DIFFERENTIATION

The IGFs stimulate cell differentiation and DNA synthesis in a large number of cell types, including chondrocytes, fibroblasts, steroidogenic cells, muscle, and lung cells. IGF-1 is more often a better mitogen than IGF-2.<sup>1</sup> Therefore, IGFs have an important role in many organs. For example, IGFs play a major role in muscle differentiation. They have been shown to regulate expression of 3 specific genes: skeletal muscle myogenin, smooth muscle, aortic elastin, and cardiac  $\beta$ -myosin heavy chain genes.<sup>8</sup> Interestingly, autocrine production of IGF-2 has been detected during skeletal myogenic differentiation.<sup>8</sup> In lung, they are also involved in organogenesis, compensatory hypertrophy, and repair following injury.<sup>9</sup> Although IGF-1 and IGF-2 are produced by human lung cells at various stages of development, IGF-2 seems to be more abundant, especially in the fetus.<sup>9</sup> The development and maintenance of differentiated cell function suggests the role of IGF-1 and IGF-2, particularly in the adrenal gland and in the ovary.<sup>10</sup>

## IGF-2 AND TUMOR

Increased IGF-2 mRNA expression has been detected in numerous human tumors, including osteosarcomas, nephroblastomas, colon carcinomas, liposarcomas, mammary carcinomas, hepatoblastomas, liver carcinomas, leiomyomas, and leiomyosarcomas.<sup>1,2,11-14</sup> Autocrine / paracrine IGF-2 production can be involved in both tumorigenesis and tumor growth. Activated P1, usually detected only in adult liver, disappeared in

hepatocarcinoma, while P3 and P4 are activated, as in fetal liver.<sup>13</sup> IGF-2 DNA demethylation could play a role in IGF-2 gene expression in some tumors such as hepatocarcinoma and leiomyoma.<sup>13,14</sup>

Chromosomal abnormalities have been reported in some tumors (Wilms' tumors, rhabdomyosarcomas, hepatoblastomas, hepatocellular carcinomas, breast tumors) where loss of an allele was detected in the short arm of chromosome 11.<sup>15</sup> Most chromosomal abnormalities occurred in embryonal tumors, which are often associated with the Beckwith-Wiedemann syndrome.<sup>13,16-19</sup> These anomalies are found in the 11p13-15 region, suggesting the presence of a tumor suppressor gene. A tumor suppressor gene associated with Wilms' tumor (WT1) was isolated, and mapped to the 11p13 locus.<sup>20,21</sup> In these tumors, overexpression of IGF-2 was detected; this may have an autocrine effect in tumor progression. Drummond et al<sup>22</sup> also have shown by in vitro studies a direct effect of WT1 on the transcription of IGF-2 from the proximal region of P3. These results suggested that functional loss of WT1 may result in increased expression of IGF-2. In cases of sporadic tumors, a preferential 11p15 loss of maternal alleles was detected.<sup>23</sup> In other tumors associated with the Beckwith-Wiedemann syndrome loss of heterozygosity in the 11p15 maternally derived chromosome was found, along with a paternal isodisomy. This suggests the role of parental imprinting and, also, an apparent 2-fold increase in gene dosage of the active IGF-2 allele.<sup>19,24</sup> A recent report<sup>25</sup> demonstrated that the IGF-2 gene was expressed from the paternal allele in human fetal tissues, and expression can occur biallelically in Wilms' tumor. Therefore, a relaxation of imprinting may play a role in cancer development.

## IGF-2 IMPRINTING AND METHYLATION\*

DNA methylations are heritable, and reversible modifications are implicated in gene control. A link was observed between IGF-2 gene expression and DNA demethylation depending on tissue types.<sup>13,14</sup>

Genomic imprinting controls the expression of several genes from only 1 allele, either maternally or paternally derived. For example, the IGF-2 gene is paternally expressed<sup>7</sup> and the IGF-2 receptor is maternally expressed.<sup>26</sup> Regulation of imprinting is still an unknown phenomenon. However, DNA methylation was shown to be variable for maternally versus paternally derived chromosomes.<sup>27</sup>

As mentioned above, it was reported that in mice, as in humans, the IGF-2 gene was subject to a

\*See Genetics Glossary insert in *GGH* Vol. 9, No. 1.

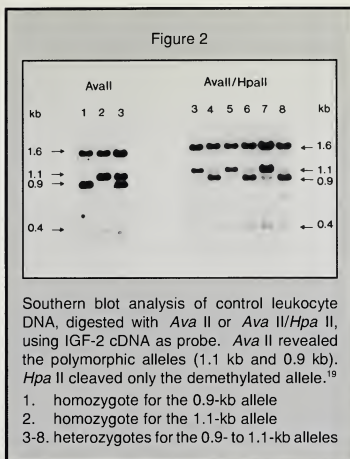
specific parental imprinting.<sup>7,25</sup> A parental methylation difference was detected for the mouse IGF-2 gene, suggesting that the paternal chromosome, which had the active allele, was more extensively methylated.<sup>28</sup> Moreover, only specific DNA sites located in the coding region, but not in the promoter region, were determined to be the target of such methylation modification.

In order to determine whether DNA methylation was linked to differential imprinting, Southern blot analyses were done using leukocyte DNA from unrelated controls. We determined that only 1 allele was always demethylated on the IGF-2 gene (Figure 2).<sup>19</sup> We located this particular phenomenon in exon 9 of the IGF-2 gene. This was extended to familial analysis, and we observed that the maternally derived allele was always demethylated, whereas the paternally derived allele was always methylated. This was an IGF-2-specific phenomenon that was not detected for insulin or calcitonin genes, which also are located on the short arm of chromosome 11. Thus, it appeared that the IGF-2 gene was subjected to a parental allele-specific methylation.<sup>19</sup> Although no direct evidence for a link between imprinting and DNA methylation has been provided, an increasing number of studies support the fact that DNA methylation may be the controlling factor for imprinting.<sup>29</sup>

## GENOMIC IMPRINTING IN HUMAN DISEASE AND IGF-2

In specific syndromes such as the Angelman syndrome and Prader-Willi syndrome, the same 15q11-13 locus has been implicated. This is a typical example of pathology in which allele-specific imprinting leads to different anomalies. De novo deletions occur in both cases for the same locus, but exclusively on the paternal chromosome in the Prader-Willi syndrome and exclusively on the maternal chromosome in the Angelman syndrome.<sup>30</sup>

It is likely that abnormalities in IGF-2 imprinting also are present in pathologic processes. The observation that IGF-2 imprinting is altered in some Wilms' tumors<sup>25</sup> suggests that this event also may occur in the Beckwith-Wiedemann syndrome. Moreover, family studies have indicated that there is a link between Beckwith-Wiedemann syndrome and the chromosome 11p15 region where the IGF-2 gene is localized.<sup>31</sup> Both loss of maternal 11p15 heterozygosity and paternal isodisomy have been described in Beckwith-Wiedemann syndrome patients.<sup>13,19,24,32</sup> We analyzed genomic DNA in 29 Beckwith-Wiedemann syndrome patients. Loss of heterozygosity was detected in 5 of the 22



informative cases, the maternally inherited allele was always the lost allele.<sup>19</sup> In most of the cases, mosaicism of cells was observed, with both normal cells and cells with loss of heterozygosity present. This supports the concept of a postzygotic event. Loss of heterozygosity was detected in tumor tissues and in nontumor tissues such as leukocytes and lingual tissue obtained after partial glossectomy because of invalidant macroglossia. This revealed that loss of heterozygosity is not a unique element needed for tumorigenesis. However, detection of loss of heterozygosity in leukocytes could be a useful tumor prognostic parameter for regular follow-up of these Beckwith-Wiedemann syndrome patients. Effectively, among the 5 cases where loss of heterozygosity was observed, 4 had a tumor.<sup>19</sup> For the 29 Beckwith-Wiedemann syndrome patients, we also analyzed the IGF-2 parental allelic methylation. Abnormal results were found only in pathologic tissues, not in leukocyte DNA.<sup>19</sup> Therefore, a relationship between IGF-2 gene expression, methylation, imprinting, and loss of heterozygosity seems to exist in the pathology of the Beckwith-Wiedemann syndrome, but the sequence of events remains to be established.

In the future, other pathologic conditions in which IGF-2 could be implicated, such as intrauterine growth retardation, should be examined in regard to these new findings and concepts.



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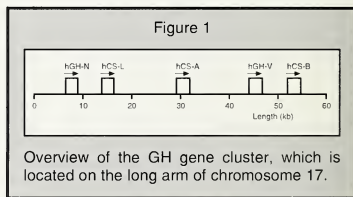
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# Placental Growth Hormone Variant: A Specific Marker of Pregnancy With Still Unknown Functions

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The human placenta has recently been shown to express the GH-V gene specifically, leading to the production of placental growth hormone (placental GH). The GH-V gene belongs to a family of 5 GH/chorionic somatomammotropin (CS) genes located in a 58-kbp cluster on the long arm of chromosome 17. These 5 GH/CS genes are aligned in the same transcriptional orientation (GH-N, CS-L, CS-A, GH-V, and CS-B from 5' to 3'). They show a high degree of sequence identity (91% to 99%) and have the same structure (4 introns/5 exons) (Figure 1). GH-N gene expression in the pituitary yields human GH. The CS-A and CS-B genes encode for the same protein known as placental lactogen (PL) or CS. PL is secreted at very high levels during pregnancy, with maternal serum concentrations of up to 10 µg/mL at term. Although the CS-L gene was presumed to be nonfunctional, recent evidence for its expression in the placenta, as well as in the pituitary, have been reported.<sup>1</sup> Two different size transcripts are generated from the GH-V gene: a major GH-V mRNA translated into the 22-kd placental GH<sup>2</sup>; and a minor one, named GH-V2 mRNA, resulting from an alternate splicing with the retention of intron 4 in the mature mRNA. The translated protein has neither been defined nor shown to be secreted. The carboxyl-terminal configuration of the GH-V2 protein is consistent with a membrane spanning region, suggesting the possibility that GH-V2 may be an integral membrane protein.<sup>3</sup>



The major GH-V mRNA is translated into a mature secreted protein: the placental GH. The amino acid sequence of placental GH differs from that of the GH-N protein by 13 residues. Placental GH is more basic than pituitary GH and contains a unique N-linked glycosylation site at asparagine 140. It is produced as 2 different size variants corresponding to a glycosylated 25-kd form and a nonglycosylated 22-kd form.<sup>4,6</sup> Placental GH is produced by the syncytiotrophoblast in vivo and in vitro.<sup>7-9</sup>

## ASSAYS FOR PLACENTAL GH

Placental GH can be detected in the maternal blood and is distinguishable from pituitary GH on the basis of its reactivity with 2 monoclonal antibodies (MAbs: K24 and 5B4) raised against purified pituitary GH.<sup>4</sup> The 5B4 Mab reacts with the N-terminal epitope of both pituitary GH and placental GH. The K24 Mab reacts with an internal epitope and recognizes pituitary GH exclusively. Therefore the difference between the results obtained with the 2 assays provides an estimation of the concentration of placental GH

present in the serum. Cross-reactivity with CS is  $<0.005\%$  in both systems. Recently, the laboratory of G. Hennen (Liège, Belgium) has produced a monoclonal antibody (Mab E8) against purified placental GH from *Escherichia coli*. It recognizes an internal epitope of the molecule and is strictly specific for placental GH. A very good correlation ( $r=0.93$ ) between the levels of placental GH measured with the 5B4 radioimmunoassay and the E8 radioimmunoassay is observed in the maternal plasma near term (38 weeks of amenorrhea).

#### PLACENTAL GH LEVELS IN MATERNAL SERUM DURING NORMAL PREGNANCY

Studies of GH physiology in pregnant women have revealed that in the early stages of pregnancy (up to 15 to 20 weeks), pituitary GH is present in significant amounts in the maternal circulation, displaying a highly pulsatile 24-hour serum concentration profile. Later on, from 15 to 20 weeks up to term, increasing concentrations of placental GH replace pituitary GH, the levels of which decrease progressively to the point of undetectability (Figure 2).<sup>4,10</sup> Placental GH is not detected in the fetus or in the cord blood of the newborn.<sup>4</sup> In contrast to pituitary GH, placental GH is secreted in maternal serum in a nonpulsatile manner and declines rapidly following delivery, as expected for a placental hormone.<sup>11</sup> There is a drastic fall in placental GH at the onset of labor, probably due to the decrease in uteroplacental blood flow and the release of placental proteases.

#### PLACENTAL GH IN MATERNAL SERUM DURING ABNORMAL PREGNANCIES

Recently, we have measured placental GH levels in normal pregnancy and in pregnancies complicated by intrauterine growth retardation.<sup>10</sup> Interestingly, maternal plasma samples obtained after 31 weeks of amenorrhea until the initiation of labor in cases of intrauterine growth retardation contained significantly ( $P<0.001$ ) low levels of placental GH. Plasma insulin-like growth factor 1 (IGF-1) levels also were lower than normal ( $156.0 \pm 25.5 \mu\text{g/L}$  vs  $285.1 \pm 40.8 \mu\text{g/L}$ ). These results suggest a relationship between placental GH levels in the maternal plasma and the development of the fetoplacental unit. In contrast, the levels of placental GH were within the normal range in the sera of pregnant women with anencephalic fetuses.

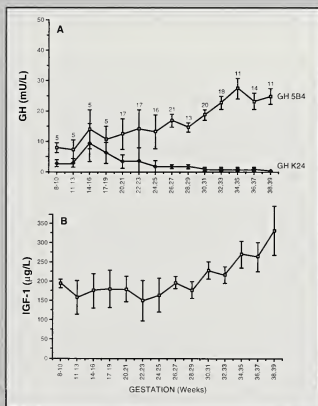
#### PHYSIOLOGIC ROLE OF PLACENTAL GH

The precise functions of placental GH during pregnancy still are poorly known. A physiologic role for a placental GH variant is suggested since it binds to a

GH-binding protein and to GH receptors from pregnant rabbit liver cells, as well as to human placental tissues.<sup>12,13</sup> Placental GH may be biologically active mainly as a somatogen and less as a lactogen,<sup>14</sup> since its somatotrophic activity on human GH-V has been illustrated by a 40% to 90% increase in size of transgenic mice bearing the human GH-V gene, as compared with controls.

However, direct action of placental GH on fetal growth seems very unlikely because placental GH is not detected in the fetal circulation. A metabolic role in the mother is suggested by the positive correlation observed between placental GH levels and IGF-1 levels in maternal blood in late pregnancy, when pituitary GH is no longer secreted.<sup>15</sup> In addition, maternal IGF-1 levels do not seem to be

Figure 2



Transverse study: maternal plasma GH (A) and IGF-1 (B) levels during pregnancy ( $n=186$ ).<sup>10</sup> Results with GH 5B4 reflect the concentrations of placental GH, while results with GH K24 reflect pituitary GH. Each point represents the mean  $\pm$  SEM of values from individual samples obtained in pregnant women at the indicated periods of pregnancy, expressed as weeks of amenorrhea. Each period consists of 3 weeks until the 20th week and of 2 weeks thereafter. The number of individual assays for GH and IGF-1 for each gestational stage is indicated in panel A of the figure on top of the vertical bars.

solely under the control of pituitary GH during pregnancy, as shown by studies of acromegalic women in whom, despite the apparent stability of pituitary GH levels, serum IGF-1 levels increase during pregnancy.<sup>16</sup> Through its somatogenic activity, placental GH may be involved in maintaining high levels of energy-yielding nutrients in the mother-to-be transferred into the fetoplacental unit. Indeed, during the last trimester of pregnancy, fetal growth is normally constrained by maternal factors. A direct role of placental GH on placental development and on its multiple endocrine and immunologic functions (and, therefore, indirectly on fetal growth) is suggested by the fact that placental GH is subject to autocrine control within the syncytiotrophoblast, which produces placental GH and expresses GH receptors.<sup>7-9,13</sup>

In conclusion, the further development of assays for detecting placental GH will be of help in elucidating the physiologic role of this hormone, which is specific for pregnancy. The lower levels of placental

GH observed in most cases of intrauterine growth retardation suggest that placental GH levels reflect placental biologic activity and may be useful in assessing chronic fetal distress resulting from abnormal placental development and function.

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## The Diagnosis and Management of Craniopharyngioma

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The profound effects upon growth and development produced by craniopharyngiomas make these lesions among the most dramatic and challenging abnormalities encountered in medical practice. As early as 1900, Babinski<sup>1</sup> described a patient with sexual infantilism and dystrophic obesity who had a cystic sellar-suprasellar lesion, almost certainly a craniopharyngioma.

#### ETIOLOGY AND PATHOLOGIC ANATOMY

Craniopharyngiomas are generally believed to be developmental lesions, believed to arise from remnants of Rathke's pouch. These embryonic remnants occur as epithelial rests, deposited between the tuber cinereum and the pituitary gland itself, along the tract of an incompletely involuted hypophyseal-pharyngeal duct; craniopharyngiomas can arise anywhere along this pathway. It has also been postulated that craniopharyngiomas might arise from squamous metaplasia of normal cells of the pars

intermedia situated along the pituitary stalk.<sup>2</sup> From the standpoint of size, location, contents, pathologic appearance, and overall clinical behavior, craniopharyngiomas encompass a broad biologic spectrum. At the one extreme are minute tumors of microscopic proportions situated wholly within a normal pituitary gland. At the other and more common extreme, there are larger tumors whose progressive growth enables them to compress the pituitary gland and stalk, optic apparatus, and hypothalamic structures. These larger lesions may extend into the third ventricle, causing hydrocephalus. Craniopharyngiomas can be solid or cystic, and the overwhelming majority exhibit both features. The cyst contents, although classically described as "machinery oil" in appearance and consistency, can range from a shimmering cholesterol-laden fluid to a brown-black purulent sludge admixed with desquamated debris. Calcification is a common feature of craniopharyngiomas, ranging from microscopic specks to palpable and even bone-like concretions of considerable size. Pathologically, the epithelial elements comprising these tumors range from cuboidal to columnar to squamous in appearance.

Topologically, approximately 60% to 80% of craniopharyngiomas arise in the suprasellar region.<sup>3</sup> Approximately 30% to 40% of craniopharyngiomas originate within the sella, resulting in its enlargement in a fashion similar to that seen with pituitary

**Table 1**  
**Symptoms and Signs of 82 Pediatric Patients With Craniopharyngiomas**

| <b>Symptoms</b>                | <b>Incidence (%)</b> | <b>Signs</b>         | <b>Incidence (%)</b> |
|--------------------------------|----------------------|----------------------|----------------------|
| Headache                       | 71                   | Papilledema          | 35                   |
| Visual loss                    | 55                   | Visual field deficit | 52                   |
| Endocrine deficiency           | 45                   | Somatic retardation  | 45                   |
| Polydipsia/polyuria            | 21                   | Sexual retardation   | 44                   |
| Personality and mental changes | 12                   | Diabetes insipidus   | 21                   |

adenomas.<sup>4</sup> Rare examples of craniopharyngiomas wholly situated within the third ventricle, optic chiasm, or sphenoid bone have been reported.

### EPIDEMIOLOGY

In the United States, craniopharyngiomas represent approximately 3% of intracranial tumors. They are more frequent in children than adults, comprising approximately 9% of childhood brain tumors. There is an incidence peak in childhood (ages 5 to 10 years), and then the frequency for the 3rd to 7th decades is relatively constant; however, there is a tendency towards a second smaller peak at 50 to 60 years of age. Sex distribution is nearly equal, with a slight male predominance.

### CLINICAL PRESENTATION

The clinical presentation of craniopharyngiomas is determined by the age of the patient and the size and location of the tumor.<sup>5-7</sup> In general, symptoms can be categorized as endocrine, visual, cognitive, and those deriving from increased intracranial pressure (Table 1).

Virtually all children with craniopharyngiomas will have an abnormal growth curve and an absence or delay in development of secondary sexual characteristics. In young adults, endocrine symptoms are often subtle, particularly those related to partial

hypopituitarism. The effects of moderate hyperprolactinemia from infundibular stalk or hypothalamic compression are generally more obvious, especially in young women, in whom prolactin (PRL) elevations manifest as amenorrhea and galactorrhea. Diabetes insipidus may occur as part of the presenting symptom complex in both children and young adults.

### ENDOCRINE DIAGNOSIS

The endocrine diagnosis of craniopharyngioma rests on physical signs and laboratory studies (Table 2). Laboratory measurements include basal and provoked tests of pituitary-hypothalamic function as clinically indicated. These include basal determinations of growth hormone (GH), insulin-like growth factor type 1 (IGF-1), PRL, cortisol, thyroid function (thyrotropin and thyroxine [ $T_4$ ]), gonadotropes (follicle-stimulating hormone [FSH], luteinizing hormone [LH]), testosterone, and estradiol. Determination of alpha subunit levels and corticotropin can occasionally be helpful. The major GH-dependent IGF-binding protein, IGFBP-3, can also be measured.

An insulin tolerance test with measurement of cortisol and GH offers a helpful dynamic evaluation of the pituitary-hypothalamic axis. If diabetes insipidus is suspected, urine and serum osmolality determinations or a water-deprivation test may be helpful.

**Table 2**  
**Deficiencies in Endocrine Testing: 82 Pediatric Patients Presenting With Craniopharyngiomas**

| <b>Hormone Deficiency</b>                   | <b>Patients<br/>No. Abnormal/No. Tested</b> | <b>Incidence</b> |
|---|---|------------------|
| Growth hormone                              | 10/40                                       | 25%              |
| Corticotropin                               | 30/71                                       | 42%              |
| Thyrotropin                                 | 19/69                                       | 28%              |
| Gonadotropin                                | 23/49                                       | 47%              |
| Vasopressin                                 | 10/45                                       | 21%              |
| Patients with at least 1 hormone deficiency | 40/77                                       | 52%              |



## IMAGING DIAGNOSIS

Magnetic resonance imaging (MRI) is the diagnostic procedure of choice for craniopharyngioma (Figures 1A and 1B). Both solid and cystic components are identified, along with important anatomic relationships and various extensions of tumor and cyst outside of the immediate suprasellar region. With high-resolution MRI, cerebral angiography is generally reserved for cases of presumed vascular tumors or those that have a particularly difficult relationship to blood vessels in the region. Computed tomography (CT) still plays some role in the anatomic diagnosis of craniopharyngiomas and has the advantage of showing calcifications and some aspects of bony distortion associated with the tumor more effectively than does MRI.

## MANAGEMENT

In newly diagnosed patients, maximum safe tumor resection is often a reasonable initial goal. In many instances, this can be achieved via craniotomy,<sup>8,9</sup> although in selected cases transsphenoidal resection provides safer access.<sup>4,10</sup> In some cases, complete excision will require a combination of both approaches. Tumors not associated with sellar enlargement generally arise and remain suprasellar, and therefore are best managed by a transcranial (pterional or subfrontal) route. During the course of tumor resection it will usually become evident whether complete removal is a safe and feasible strategy.<sup>11</sup> Aggressive attempts to remove tumor fragments that are tenaciously adherent to neural and vascular structures will be accompanied by an unacceptable functional cost.<sup>12,13</sup> Other tumors will be less adherent and complete excision can be safely achieved.

As a rule, craniopharyngiomas associated with sellar enlargement can be regarded as subdiaphragmatic in origin. Even though such tumors may exhibit significant intracranial extension, they invariably maintain an extrapial and extra-arachnoid disposition. Accordingly, they remain amenable to complete excision via a transsphenoidal route.

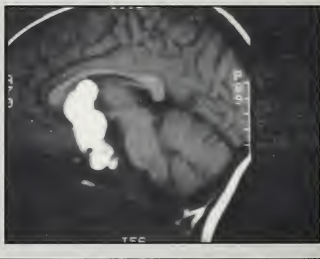
Completeness of surgical removal can usually be confirmed by postoperative imaging studies. When complete removal is not feasible, some form of postoperative radiation therapy is generally recommended, except in very young children.<sup>14</sup>

The management of recurrent craniopharyngiomas is considerably more complex, for therapeutic goals must be especially well defined. In some cases – and despite the technical demands of reoperation – total resection can still be achieved. For many recurrent tumors, however, palliative surgery is often the most realistic goal. Recurrent

Figure 1A  
**Sagittal View of a Craniopharyngioma With Retrosellar Extension**



Figure 1B  
**Sagittal MRI View of a Craniopharyngioma With a Complex Suprasellar Extension Into the Third Ventricle**



lesions with a significant cystic component can often be treated by repetitive aspiration. This can be achieved by inserting a silastic tube attached to an Ommaya reservoir into the cyst cavity. Alternatively, the transsphenoidal insertion of a silastic tube from the tumor cavity into the posterior nasal space can provide prolonged drainage, or bleomycin sulfate may be instilled to shrink and toughen the cyst wall.

Stereotactic needle aspiration of fluid contents may be remarkably effective in reversing symptoms and signs. In some cases a radioactive isotope (colloidal <sup>32</sup>P, yttrium, or gold) may be instilled into the cyst cavity to provide local beta particle-mediated radiotherapy. Radiosurgery, delivered by gamma knife or by stereotactic linear accelerator, may also provide dramatic results in patients who are not suitable surgical candidates.

## RESULTS OF SURGERY

This analysis will focus on a series of pediatric patients with craniopharyngiomas treated at the Mayo Clinic in Rochester, Minnesota and followed for at least 5 years.

The study group consisted of 82 children operated upon between 1950 and 1983. The age range was from 2 to 20 years, with a mean age of 12 years. Fifteen of the children were younger than 8 years of age at the time of surgery. All had developed symptoms and signs before age 16. The children were followed for a mean of 9.7 years.

The symptoms, signs, and endocrine deficiencies of the entire group at the time of surgery are presented in Tables 1 and 2 (page 7). Data on follow-up status are presented in Figure 2 and percent survival in Figure 3.

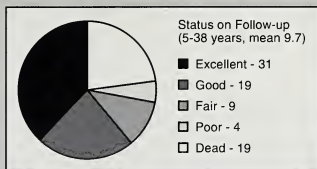
Management of the 82 children was categorized as follows:

- Total surgical resection only was performed on 31; total surgical resection followed by radiotherapy was performed on 6; subtotal resection only on 17; subtotal resection followed by radiotherapy on 17; minimal surgery (biopsy or cerebrospinal fluid shunt) on 8, and minimal surgery followed by radiation therapy on 3.
  - Of the 37 patients with total surgical resection, 6 had postoperative radiotherapy. Satisfactory outcome, defined as long-term survival with good or excellent quality of life, was achieved in 78% (29 children). There were 2 operative deaths, and 6 recurrences (16%) in spite of total resection. The 5- and 10-year survival rates were identical at 90%. On long-term follow-up, 4 of the 37 children (11%) had died.
  - Of the 34 patients with subtotal surgical resection, 17 had postoperative radiation therapy. A satisfactory outcome, as defined above, was achieved in 35% (12 children). Recurrent disease developed in 17 (50%). There was 1 operative death. The 5-year survival rate was 75%; the 10-year survival rate fell to 65%. On long-term follow-up, 14 of the 34 children (41%) had died.
  - Radiotherapy consisted of conventional teletherapy to a mean dose of 4,450 Gy, usually in 180-Gy daily fractions. The dose range was 2,650 to 6,300 Gy.
- These data support a policy of maximum safe tumor resection as the initial recommendation for management of children with craniopharyngioma.

## CONTROVERSIES IN MANAGEMENT

Various forms of subtotal removal of craniopharyngiomas or their contents, followed by radiation therapy, have been advocated.<sup>15</sup> Although radiation therapy is of proven benefit in delaying recurrence and controlling tumor growth, most forms of radiation

Figure 2  
Results of Management of  
82 Craniopharyngioma Patients



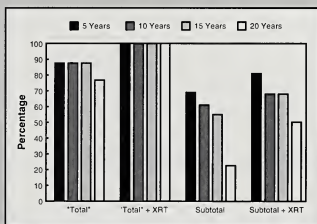
Excellent = independent, no neurologic deficit

Good = independent, stable neurologic or visual deficit

Fair = dependent but functional, with neurologic or visual deficit

Poor = dependent, nonfunctional

Figure 3  
Overall Survival Statistics in  
71 Pediatric Patients



|  |               |               |
|--|---------------|---------------|
| Total surgical resection                     | = 31 patients | } 37 patients |
| Total surgical resection and radiotherapy    | = 6 patients  |               |
| Subtotal removal                             | = 17 patients | } 34 patients |
| Subtotal surgical resection and radiotherapy | = 17 patients |               |

29 of the 37 total surgical resection patients (78%) had satisfactory outcome (defined as long-term survival with good or excellent quality of life) versus 12 of the 34 subtotal surgical resection patients (35%)

therapy currently given for craniopharyngioma produce hypothalamic and pituitary damage.<sup>16,17</sup> There is also a small but constant risk of radiation-induced optic neuropathy leading to blindness; diffuse brain damage leading to dementia; focal brain necrosis affecting the hypothalamus or septal region; vascular pathology leading to occlusion and a moyamoya type vasculopathy; and the late induction of secondary tumors, many of which are malignant. New forms of radiation therapy are being evaluated, and should represent a major advance in the avoidance of such complications. It is also hoped that they will be more effective in destroying the tumor and in reducing its potential for recurrence.

One must recognize the selection bias that affects previously reported retrospective studies. Rapidly growing large tumors that cause progressive visual loss and hydrocephalus demand surgical management, and the outcome of many of those patients reflects the aggressiveness of the tumor.<sup>18</sup> Those patients with small, indolent, relatively asymptomatic tumors are overrepresented in the group of patients treated by less radical surgery and radiation. Naturally, the end results reflect the less aggressive nature of their tumors.

Because each craniopharyngioma is different, it is important to individualize the plan of management for each patient, taking into account what is known about the anatomy and biology of the tumor. In previously untreated patients, several issues should be addressed. Total removal remains a reasonable goal for many tumors, especially those with enlargement of the sella and no major involvement of or attachment to the hypothalamus or the optic apparatus. In infants and children who are growing

normally, it is occasionally prudent to delay therapy until growth is complete.

## CONCLUSION

The goal of treatment – a neurologically intact patient living as normal a life as possible – is accomplished by using a judicious combination of careful surgery, meticulous medical and endocrine management, and appropriate radiation therapy. Improvements in diagnosis and in the technical and conceptual aspects of medical, surgical, and radiotherapeutic management should lead to continuing improvement in the prognosis for patients with craniopharyngiomas.

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## Abstracts From the Literature

### Transsphenoidal Surgery for Pituitary Adenomas in Children

The authors report the surgical results of transsphenoidal surgery for pituitary adenomas done on 66 patients; all were under 16 years of age at the time of surgery. The sex incidence was equal. Ninety-four percent (62 of 66) clinically showed evidence of hormonal hypersecretion; 36 secreted excessive ACTH producing Cushing's syndrome; 18 patients had hyperprolactinemia; 8 patients had gigantism.

ACTH producing adenomas (36) accounted for 55% of the cases. Thirty-three of these had normal sellar X-rays and the other 3 had Nelson's syndrome. In addition, neither CT or MRI scans proved very accurate in localizing this type of adenoma as these scans proved helpful in only 7 of 24 cases, in which at least 1 of the 2 types of scans was done. Eighteen of 26 with selective removal of the tumors and 5 of 8 requiring subtotal hypophysectomy were cured (70%). Ten failures occurred initially, but 5 of 6 with repeat subsequent surgery were successful. The overall surgical success rate was 78% (28 of 36), although 5 of the 28 required 2 operations. Temporary diabetes insipidus (12 cases)

was common, but permanent diabetes insipidus was uncommon (1 case). In this series, ACTH microadenomas occurred within the confines of a normal sella in 86% (31 of 36) of cases.

Prolactinomas occurred in 7 boys and 11 girls; only 3 girls had galactorrhea. Pubertal delay was common in both sexes. Fifteen of 18 patients with prolactin (PRL) adenomas had some evidence of sellar enlargement by X-ray or tomography. Thirteen of these had suprasellar extension. Parlodel® (bromocriptine mesylate) was often but not uniformly helpful preoperatively or postoperatively. The 4 patients in whom Parlodel was minimally effective, or in whom obvious invasion was present, received radiotherapy. Prolactinomas in boys were particularly difficult management problems, with 6 of 7 showing suprasellar extension at admission and 5 of 7 requiring Parlodel and/or radiotherapy postoperatively. Overall, only 1 (11%) of 9 patients with significant pubertal delay at admission subsequently had adolescent development. Patients with high PRL levels (>500 ng/mL) did poorly in contrast to those with values between 200 to 500 ng/mL.

The 8 children with growth hormone (GH)-secreting adenomas did dismally in contrast to the usual 60% to 80% cure rates in adults. Only 1 patient fared well. Five of the 8 had suprasellar extension. Six of the 8 had enlarged sellas.

Nonsecreting adenomas occurred in 4 patients, 2 of whom had suprasellar extension with visual field changes. The lesions in the other 2 were localized. There was no operative morbidity or mortality in this group.

Twelve (18%) of the 66 required drilling of an incompletely pneumatized sphenoid sinus to reach the sella, which did not in any case limit the surgical procedure. The authors state that second operations by the transsphenoidal route can be done in children with results approaching those seen in adults for the debulking or removal of recurrent or residual lesions. For those lesions not controlled by surgery, the authors recommend medical intervention, not radiotherapy.

Dyer EH, et al. *Neurosurgery* 1994;34:207-212.

**Editor's comment:** *The data in this paper cover the period 1966 to 1992 and were reported from the Department of Neurosurgery at the Centre Medico-Chirurgical Foch in Suresnes, France. The authors document that transsphenoidal surgery is viable in the pediatric age group. Visual scans are helpful in*

*most instances of non-ACTH-producing adenomas, but of limited value in ACTH-producing adenomas. Unfortunately, the latter make up the majority of adenomas observed in childhood. Incidentally, we are indebted to Dr. Laws, who wrote the article regarding the treatment of craniopharyngioma in this issue, for bringing this article to our attention.*

Robert M. Blizzard, MD

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## Third International Symposium on Insulin-Like Growth Factors

February 6 - 10, 1994; Sydney, Australia

*A Meeting Review by Paul Saenger, MD, Professor of Pediatrics, Department of Pediatrics, Montefiore Medical Center, Bronx, New York*

The 3rd International Symposium on Insulin-Like Growth Factors was held in Sydney, Australia, February 6-10, 1994. Approximately 800 clinicians and investigators presented research data in the burgeoning field of insulin-like growth factor 1 (IGF-1) function and action.

Highlights of the talks focused on new data on IGF-1 function in normal embryonic development and the role of IGF-1 in the treatment of diseases of the central nervous system (CNS). Dr. Peter Gluckman, Auckland, New Zealand, and his group presented data suggesting that the paracrine IGF-1 system responds to CNS organ injury with changes in the expression of insulin-like growth factors and their respective binding proteins. In this model using 21-day-old rats, neuronal death is a mixture of necrosis and apoptosis (cell death) and is evident between 12 and 72 hours after CNS injury. IGF-1 mRNA is markedly induced within 24 hours. In contrast, IGF-2 expression is not seen for 7 to 10 days. In adult rats subjected to a hypoxic-ischemic CNS injury, IGF-1 given intraventricularly caused a

dose-dependent reduction in infarction and neuronal death in all areas studied. The effect appears to be mediated by the IGF-1 receptor. IGF-1 was effective only if given within 2 hours after injury and not if given prior to the injury, suggesting that it acts on mechanisms activated by the hypoxemia-ischemia.

Dr. Gluckman suggested that IGF-1 may act as a survival factor blocking apoptosis. The action of IGF-1 was further determined by local production of IGF-binding proteins (IGFBPs). The neuroprotective action of IGF-1 was blocked by the coadministration of IGF-2. Both are probably competing for the same receptor. In a fetal sheep ischemic injury model, intracerebroventricular IGF-1 was also neuroprotective at very low doses. The data suggest that in the brain IGF-1 acts as a survival factor for asphyxiated neurons and that the effect is dependent on the presence of IGFBP-2 and/or IGFBP-3. This is an endogenous protective mechanism clearly warranting further study as the clinical therapeutic potential is obvious.

Dr. Ron Rosenfeld, Portland, Oregon, reported the results of an IGF-1 treatment trial in patients with primary growth hormone insensitivity from Ecuador. This was the first double-blind, placebo-controlled trial with a cross-over at 6 months. The growth velocity increased from 3 cm/y to 8.5 cm/y at an IGF-1



dose of 120 µg/kg bid. There was little change in the serum levels of IGFBP-3 or the distribution of IGF peptides among IGFBPs while patients were on treatment. There also was little change in the pharmacokinetics of IGF-1 therapy during treatment. The measured increase in growth velocity is significant, but does not match the response in a naive growth hormone-deficient patient treated with daily growth hormone injections. Dr. Rosenfeld concluded that the optimal dose of IGF-1 is still elusive. A different injection schedule or administration of IGF-1 complexed with binding proteins are among the approaches to be explored in the future. Most importantly, the studies of Gluckman and Rosenfeld demonstrate the ability of IGF-1 peptides to act as classical endocrine hormones in clinical trials.

Insulin-like growth factors involve many aspects of growth and development. The study of IGF-1 action has also been carried out by administering IGF to animals by injection or mini-pumps. Transgenic technology addresses the question of overproduction or underproduction with a recently developed embryonal stem cell technology. Here, gene expression for specific hormones can be removed from animals and then the effects can be studied. Dr. Lyn Powell-Braxton, South San Francisco, described her exciting research data with mice lacking a functional IGF-1 gene. These mice are profoundly IGF-1 deficient. This enables investigators to study strains of mice deficient in IGF-1 ("knock-out" mice) from

conception on. Mice with 1 functional IGF-1 allele are 10% to 20% smaller than their normal littermates, and they have lower IGF-1 levels. Mice totally lacking a functional IGF-1 gene progress normally through prenatal development, but more than 95% die at birth. These IGF-1 knock-out mice are just over half the size of their normal littermates. They have severe muscle dystrophy affecting both cardiac and skeletal muscle. The mice have poorly developed diaphragms and lungs, and are unable to breathe. Surviving animals show reduced myelination in nervous tissue. Dr. Powell-Braxton concluded that IGF-1 knock-out mice show severe effects on the development of both the central and peripheral nervous systems and, additionally, produce pronounced dwarfism, muscle underdevelopment, and reduced longevity. Lung development also may be affected, as the lung is one of the organs with the most striking disproportional growth retardation.

The availability of these mouse strains illustrates the importance of the IGF-1 system not only in postnatal but also in embryonic growth. The availability of mouse strains with genetically defined lesions is an increasingly powerful tool in the study of the role of growth factors and hormones throughout development. The availability of these knock-out mice with defined deficits in IGF-1 gene expression permits investigators to dissect the role of IGF-1 not only in development but also in disease pathogenesis.

## The First International Meeting of the Growth Hormone Research Society

*June 1 - 4, 1994; Aarhus, Denmark*

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*A Meeting Review by Paul Saenger, MD, Professor of Pediatrics, Department of Pediatrics, Montefiore Medical Center, Bronx, New York*

Over 400 delegates attended this meeting, which was devoted to clinical and basic science aspects of growth hormone (GH) research. At the meeting, which had attracted a large number of adult endocrinologists, the importance of GH in adults with GH deficiency was examined. Particular emphasis was placed on further study of the regulation of GH secretion, diagnosis, and characteristics of adult GH deficiency, and the effects of GH replacement therapy in adults.

Professor Iain Robinson, London, England, spoke about peptidal and nonpeptidal GH-releasing substances and their interaction with the GH-releasing factor (GRF) neuron. The GRF neuron has to be

viewed as a clearinghouse for a wide variety of afferent neuronal information. He stressed that, in his view, the GH-releasing action of GRF is not its primary function.

The pulsatile GH release is most likely regulated by somatostatin. Somatostatin optimizes the pulse frequency pattern, thus stimulating growth by setting a pulse pattern that is most economical for achieving the maximum growth of peripheral tissues. He could show elegantly that 9 GH pulses per day give more bone growth in the rat than 1 large pulse of GH with a similar area under the curve. The GRF/somatostatin interplay has been studied not only in physiologic settings but also in disease, such as in patients with ectopic GRF production where, even under constant GRF exposure, GH release remains pulsatile.

Professor Robinson reported preliminary data on

12-hour GH sampling in premature infants. In collaboration with Dr. David Dunger, Oxford, England, he was able to show that in 34-week gestation premature infants, GH is already secreted in bursts. The striking difference compared with older children was that their GH level never declined to zero. Peaks of GH were superimposed over a baseline level of 5 to 10 ng/mL of GH. Dr. Robinson indicated that the high GH levels in premature infants suggest that GH probably has important metabolic functions in utero.

Professor Robinson stressed it is far from true that all GRF pulses are associated with a subsequent GH release. It is only due to pulsatile somatostatin that we achieve a GH release after GRF at all.

The major function of GRF, according to Professor Robinson, is to build up GH stores in the pituitary. Indeed, it has been shown that GRF does induce increased GH gene transcription. He cited the *little* mouse, which does not respond to GRF, as an intriguing animal model to study the physiology of GRF action. A single point mutation in the extracellular domain of the GRF receptor renders the *little* mouse resistant to GRF. This then leads to a total failure of postnatal GH cell proliferation in the pituitary and the pituitary GH cell population is near zero. GRF, therefore, exerts a trophic function for growth hormone secreting cells as well.

Professor Robinson stressed further that the pituitary has to be viewed as a plastic organ that can change the number of GH-producing cells in responses to afferent input, GRF being among them. Physiologic GRF production is then enmeshed in a feedback loop where GH release exercises a negative feedback. Furthermore, central GH receptors are equally responsive to circulating peripheral GH. There are several afferent inputs for the GRF neuron. These inputs come from GH itself, somatostatin, synthetic GH-releasing peptides (GHRPs), neurotransmitters, and possibly also IGF-1.

Professor Robinson concluded his talk by reviewing the current knowledge of GHRPs. Simply just the fact that GHRPs have an effect in man suggests that there may be endogenous, still elusive, GHRPs produced in the brain. Since synthetic GHRPs are effective, one has to postulate that there are receptors in the brain for these synthetic GHRPs. Whether they are identical to GHRP receptors for endogenous GHRP is not clear. GHRPs work through their own receptors, not through GRF receptors. Furthermore, GHRPs also have a hypothalamic target in addition to a pituitary target. GHRPs act in synergism with GRF and regularize the response to GRF. Elegant studies utilizing anti-GRF show that GRF-Abs interfere with GHRP action. A functioning hypothalamus is required for full GHRP action. Additional effects of GHRP may also influence the firing rate of the arcuate nucleus. In studies using

the pregnant ewe as a model, investigators could show that GHRP stimulates GRF and GH release as well as somatostatin by measuring efferent products in the effluent of portal blood of the pregnant ewe. Little is known yet about the effects of these compounds in chronic use.

Dr. C. Eschen, Copenhagen, Denmark, showed that the administration of GHRP-6, originally synthesized by Dr. Cyril Y. Bowers, to rats for 14 to 90 days had little effect on weight gain or IGF-1 levels. Several new GH secretagogues have been synthesized recently. These GHRP analogues were discussed by Professor Robinson, and were also the topic of several poster presentations at the meeting.

One of the analogues, hexarelin, was described as a potent GH releaser in children and laboratory animals such as dogs. Its usefulness in the more refined diagnosis of GH deficiency was proposed. It should be noted that only 0.3% of GHRP is absorbed via the oral route, thus limiting its potency considerably. This does not seem to be the case for newer nonpeptidal oral secretagogues such as L-692,429 and L-692,585, which have a manifold higher potency and also better absorption.

Dr. S.L. Dickson, Cambridge University, England, showed that GHRP L-692,585 was inducing *fos* protein in the arcuate nucleus. Elegant neurocytochemistry documented the induction of this key protein in the wall of the third ventricle.

The GRF neuron can best be characterized as a clearinghouse for the multiple afferent neurons. The pituitary has to be viewed as an organ with considerable plasticity. The primary function of the hypothalamus, according to Professor Robinson, is to regulate pituitary size and thus enable specific pituitary hormonal responses. In conclusion, GHRPs, which were thought to act directly on GH secretion cells in the pituitary, are now believed to produce many of their effects by interacting with somatostatin and by stimulation of GRF neurons in the hypothalamus.

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## Chromosomal Localization of the Human Renal Sodium-Phosphate Transporter to Chromosome 5: Implications for X-Linked Hypophosphatemia

Phosphorus and sodium are absorbed across the luminal membrane of the renal tubule utilizing a cotransporter protein whose gene has been cloned.<sup>1</sup> The present investigators employed 3 methods for localizing the chromosomal site of this gene: (1) probing of somatic cell hybrid panels with a radiolabeled DNA genomic fragment that revealed a signal on human chromosome 5; (2) PCR amplification of DNA from somatic cell hybrids to localize the sodium-phosphate cotransporter gene to chromosome 5; and (3) utilizing a fluorescein-labeled DNA probe for the sodium-phosphate cotransporter gene and fluorescent in situ hybridization on metaphase chromosome spreads from human peripheral blood lymphocytes, which located the gene at chromosome 5q13. The location of the sodium-phosphate cotransporter gene on chromosome 5 was unexpected because the trait for human familial hypophosphatemic rickets in which renal phosphorus reabsorption is decreased is X-linked and has been assigned to Xp22.1-p21.3. Therefore, there may be a second phosphorus transporter isoform whose gene is on the short arm of the X chromosome, or the X chromosome product may regulate expression of the gene on chromosome 5 or the function of its product.

*United States. Although it is clearly X-linked, the gene for the function that is defective in these patients (ie, renal phosphorus transport) is not on the X chromosome. In the animal model of this disorder, the X-linked Hyp mouse, there is a decrease in the renal tubular content of mRNA and protein for the sodium-phosphate cotransporter that probably accounts for decreased renal tubular transport of phosphorus.<sup>2</sup> The chromosomal site of the mouse sodium-phosphate cotransporter gene has not as yet been reported, but there are data suggesting the presence of a humoral factor (whose gene may possibly be on the X chromosome) in these animals that inhibits phosphorus transport, perhaps by downregulating transcription of the sodium-phosphate cotransporter gene.<sup>2</sup> A "knock-out" experiment in which the calcium-phosphate transporter gene is eliminated and the effect on phosphorus transport observed would be of interest.*

*A second study has been published that localizes the human sodium-phosphate cotransporter gene to chromosome 5q35.<sup>3</sup> Thus, both reports assign this gene to the long arm of chromosome 5, but its specific sublocation is not certain.*

Allen W. Root, MD

Chishan FK, et al. *Pediatr Res* 1994;35:510-513.

**Editor's comment:** Familial hypophosphatemic rickets is the most common form of rickets presently encountered in the

1. Magagnoli S, et al. *Proc Natl Acad Sci USA* 1993;90:5979-5983.

2. Tenenhouse HS, et al. *J Clin Invest* 1994;93:671-676.

3. Kos CH, et al. *Genomics* 1994;19:176-177.

## Turner Syndrome: Natural History, Ethnic and Genetic Influences, Methods for Evaluation of Growth

The natural history of growth in Turner syndrome (TS) has been described by several authors previously, who were mainly from North European countries. These reports have not always been consistent. In some instances, the methodology was open to criticism, so that evaluating the effects of growth hormone treatment relied on insufficient data. Four papers published in the same issue of a European journal<sup>1-4</sup> afford useful contributions to solve the discrepancies reported.

The first paper<sup>1</sup> gives data recorded from a multicentric, retrospective, nationwide study (29 pediatric endocrinology centers) of 772 cases of TS in Italy. A major purpose was to present standards and charts for birth weight and height, and weight from infancy to adulthood, appropriate for this area. The study took into account the parents' height and the birth length of TS patients. The size of the study permitted the calculation that a 10 cm difference in midparental heights between 2 groups, one shorter than the other, resulted in a 6.5 cm difference in the adult stature of a TS patient. The data also indicated that birth lengths and weights strongly correlated inversely with postnatal growth. Other data obtained from this study were that the span and frequencies of the karyotypes of the 772 cases of TS did not differ from that reported in other series. There was a slightly but significantly increased incidence of TS with the age of both parents and the mother's parity.

The second paper<sup>2</sup> reports longitudinal data from the 1st to

the 18th year of life, obtained from a subgroup of these Italian patients, with calculation of their annual growth velocity. Taking into account the different karyotypes, this study shows that most TS subjects with 46,XX mosaicism have some degree of spontaneous puberty, occurring in the same age range as in normal girls, and earlier than in other karyotypic groups of TS. There was a slight pubertal growth spurt followed by a deceleration, so that in this series the final height was a little below that of 45,X patients. Charts for height and for growth velocity have thus been established separately for TS subjects with karyotypes 45,X, 46,XX/45,XO mosaicism, and 46,XX with structural abnormalities of the second X chromosome.

The third paper<sup>3</sup> reports the spontaneous final heights of 216 TS subjects from the southern part of France. In this series, no significant differences between karyotypic groups were found. The correlation with midparental height was  $r=0.45$ , a little stronger with father's height than with mother's height. Pointing out the importance of genetic factors and studying the differences recorded in TS statistics from various countries, the authors conclude that the results of treatment with growth hormone in TS must take into account the midparental height of each patient.

The last paper<sup>4</sup> is a critical approach to the methods for evaluating growth in TS, especially when appreciating the effectiveness of treatment with growth hormone. Analyzing 13

different studies of growth velocity and height in TS, the authors stress their discrepancies and point out the influence of auxometric methodologies when calculating the standard deviations (SD) to the means. They show that the variations of SD applied to TS growth tables and charts affect the evaluation of therapeutic results in these patients to a large extent. Their conclusion is that, in the absence of a completely accurate method for appreciating changes in growth velocity, the investigations on the effects of GH on growth in TS should focus mainly on final heights after therapy.

1. Bernasconi S, et al. *Acta Paediatr Scand* 1994;83:292-298.
2. Mazzanti L, et al. *Acta Paediatr Scand* 1994;83:299-304.
3. Rochiccioli P, et al. *Acta Paediatr Scand* 1994;83:305-308.
4. Haeusler G, et al. *Acta Paediatr Scand* 1994;83:309-314.

**Editor's comment:** These 4 studies afford useful data and comments for improving the detailed knowledge of growth in Turner syndrome, its relationships with parents' height, with birth length and weight, with the spontaneous partial breast development occurring in certain Turner patients, with the karyotype, and for developing cooperative studies with the aim of a relevant evaluation of short-term therapeutic results. These publications can be considered as milestones in preparing appropriate methodologies for new longitudinal studies. The data included in the manuscripts are much more extensive and detailed than could be abstracted here. Readers who are particularly interested should make every attempt possible to read the original articles.

Jean-Claude Job, MD

## Perspectives of Longitudinal Growth in Cystic Fibrosis From Birth to Adult Age

Haeusler et al performed a retrospective longitudinal analysis of growth data of 139 patients (72 girls, 67 boys) with cystic fibrosis (CF) who received care at the University of Vienna between 1955 and 1989. Height was measured with a Harpenden stadiometer and 1,605 individual observations were utilized. The mean observation period was  $7.1 \pm 5.9$  years. The mean number of recorded height and weight measurements for each patient was  $10.3 \pm 7.6$ . Age at diagnosis was  $1.4 \pm 2.37$  years in girls and  $1.5 \pm 2.56$  years in boys. In all cases a demonstration of *Pseudomonas aeruginosa* was treated vigorously with antibiotics. Dietary management included 150% of daily allowance with high dose supplementation of pancreatic enzymes. Fat was not restricted. Quartiles of height, weight, and growth velocity were estimated by nonparametric measures.

Height and weight were available in 103 patients. At birth, weight and length of children with CF were decreased compared to healthy infants. As expected, there was a further decrease in weight SDS in both girls and boys between birth and the time of diagnosis. Length, moderately decreased at birth ( $-0.55 \pm 0.13$  SDS in girls;  $-0.39 \pm 0.13$  SDS in boys), declined until diagnosis. During the year after diagnosis, length SDS improved but remained decreased. In girls, height followed the 25th percentile until age 8, when it dropped to the 10th percentile until approximately age 14. At that time, it began to rise to the 25th to 50th percentile. Median height of 18 girls who were available for measurement at 19 years was between the 25th and 50th percentile. Weight in girls showed a similar course. In boys, height followed the 25th percentile with a nadir between 10 and 16 years. The median height of 13 boys available at 19 years was 173 cm (25th percentile). Weight was much more affected than height in girls than boys after age 12. Growth velocity was relatively normal during the prepubertal age. Puberty was delayed. The calculation of growth velocity during puberty was not possible due to too few data. However, the pubertal growth spurt appeared to be both small and delayed.

There was no significant change in height SDS or growth velocity in either boys or girls after the acquisition of latent or established respiratory infections.

**Editor's comment:** This is an interesting report particularly as it covers a time span of 34 years between 1955 and 1989. One might suspect that treatment of children with CF was vastly different nearly 30 years ago, and that height and growth velocity of these children would be markedly different from that of the normal population. In addition, one might anticipate that the acquisition of *P. aeruginosa* infection would significantly reduce growth velocity. Neither of these suppositions proved correct. It is noteworthy, however, that for this relatively large group of children, mean height was reduced from the 50th percentile. One can only speculate that current aggressive therapy might have produced improved growth in these individuals.

William L. Clarke, MD

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#### Rationale for Recommending that Recombinant Human Growth Hormone be Prescribed by Weight Rather Than Units

by Margaret H. MacGillivray, MD



## Calcium-Sensing Receptor Genes Mutate and Produce Metabolic Disease

$\text{Ca}^{2+}$  associated with a specific cell membrane component had been postulated for several years to explain, in part, the mechanism by which  $\text{Ca}^{2+}$  regulates the secretion of parathyroid hormone,<sup>1</sup> but the structure of the membrane component was unknown until Brown and coworkers isolated the bovine gene for this receptor from parathyroid tissue and expressed and characterized it. The bovine gene encodes a 120 kd, 1.085 amino acid, 7 transmembrane polypeptide characteristic of receptors that activate guanyl triphosphate (GTP) binding proteins. This activation initiates a cascade of intracellular signals that produce the characteristic biologic response of the cell to the ligand. The receptor is expressed in the bovine parathyroid gland, kidney, thyroid, and some areas of the brain.

Subsequently, the 6 exon of the human gene was isolated and mapped to chromosome 3q2. It encodes a 1,059 amino acid with an extremely long (613 amino acids) amino terminal extracellular region to which  $\text{Ca}^{2+}$  is thought to bind. Hypothesizing that the  $\text{Ca}^{2+}$  receptor gene was abnormal in patients with familial hypercalcemic hypocalciuria, the composition of this gene was analyzed in patients with this disorder and its more severe variant, neonatal severe hyperparathyroidism. All of the affected members of the families studied had base pair changes, although the genetic error varied in different families. Three variants were identified. In the amino terminal region, a G  $\rightarrow$  A mutation in codon 186 altered arginine to glutamine and a C  $\rightarrow$  T mutation in codon 298 changed wild-type glutamine to lysine. These mutations might affect  $\text{Ca}^{2+}$  binding to the receptor or alter polypeptide processing, receptor stability, or other necessary function. In the third intracellular domain, a C  $\rightarrow$  T mutation in codon 796 altered arginine to tryptophan; this amino acid is near the site of receptor coupling to the GTP-binding protein. One subject with severe, and often fatal,

neonatal hyperparathyroidism had 2 copies of the abnormal gene at codon 298, thus lacking 2 functional  $\text{Ca}^{2+}$  receptor molecules. These observations suggest that familial hypercalcemic hypocalciuria is due to abnormalities within the gene coding for the membrane  $\text{Ca}^{2+}$  receptor and is genetically heterogeneous.

### REFERENCES

1. Brown EM, et al. *Nature* 1993;366:575-580.
2. Pollak MR, et al. *Cell* 1993;75:1297-1303.

**Editor's comment:** These articles provide an important advance in our understanding of the manner in which  $\text{Ca}^{2+}$  regulates the secretion of parathyroid hormone. Acting through the membrane  $\text{Ca}^{2+}$  receptor,  $\text{Ca}^{2+}$  activates GTP-binding proteins that increase activity of phospholipase C, thus hydrolyzing membrane phosphoinositide and increasing intracellular concentrations of inositol triphosphate, thereby releasing  $\text{Ca}^{2+}$  from its storage sites in calciosomes;  $\text{Ca}^{2+}$  and the GTP-binding proteins may also "open"  $\text{Ca}^{2+}$  channels directly. As the intracellular concentration of  $\text{Ca}^{2+}$  increases, neutral proteases, termed "calpains," are activated and increase the rate of degradation of parathyroid hormone.<sup>2</sup> High intracellular  $\text{Ca}^{2+}$  levels also decrease the rate of transcription of the gene for parathyroid hormone. It is possible that an abnormality in the  $\text{Ca}^{2+}$  receptor may account for the parathyroid hyperplasia seen in patients with multiple endocrine neoplasia or in a clone of parathyroid cells leading to a parathyroid adenoma. These unique observations suggest that there may be membrane receptors for other ions as well (ie,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ).

Allen W. Root, MD

## Genetic Mapping of Quantitative Trait Loci for Growth Fatness in Pigs

Quantitative inheritance of a trait implies that the expression of that trait is dependent on the interaction of several genes at different loci and, often, environmental factors. In order to identify the chromosome(s) on which the traits for growth and fatness in pigs may reside, the investigators analyzed the quantitative trait loci (QTL) in second generation crossbred progeny of European domesticated pigs (selected for large growth and leanness) and the European wild boar (characterized by increased body fat content but smaller size) utilizing a linkage map and genetic markers for the porcine genome of 18 autosomes. The authors measured birth weight, growth rate, abdominal and back fat, and length of the small intestine (a trait that correlates positively with growth) and reported that wild boar alleles on chromosome 4 were associated with decreased growth, shorter small intestinal length, and increased body fat content. There was also a QTL for birth weight and early growth on chromosome 13. There was no relationship between the detected QTLs and sex or feeding. The precision of chromosomal location of these QTL is relatively low; therefore, the authors could not determine whether these chromosomal sites contained 1 or multiple genes affecting the quantitative trait.

The loci for the genes for growth hormone (chromosome 12), its receptor (chromosome 16), and insulin-like growth factor 1 (chromosome 5) were not related to the QTL for growth, body fat, or intestinal length. Loci corresponding to porcine chromosome 4 are found on chromosome 1 in humans.

Andersson L, et al. *Science* 1994;263:1771-1774.

**Editor's comment:** The relevance of these observations to obesity in humans is uncertain, but it is of interest to note that in the mouse there are 2 genetic mutations associated with an obese phenotype (diabetes on mouse chromosome 4 and fat on chromosome 8) linked to genes with homologues on the first human chromosome. This observation points to a potential focus of attention in the study of human obesity and growth. The lack of association of body fat content with feeding regimen points to the importance of genetic factors in fat accumulation. It was a bit surprising that growth was not genetically linked to the loci for growth hormone, its receptor, or IGF-1, particularly since growth hormone-deficient pigs are dwarfed.

Allen W. Root, MD

# GROWTH

## Genetics & Hormones

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### Noonan Syndrome: A Review

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Now a well-established entity, Noonan syndrome (NS) is a frequent cause of short stature. The syndrome is estimated to have an incidence of 1 in 2,500 to 1 in 1,000 individuals.<sup>1</sup> This incidence, which is not based on complete ascertainment in a defined population, may appear high; however, NS is being increasingly recognized and is likely to be the second commonest syndrome with congenital heart disease after Down syndrome. Unlike Down syndrome, NS has a familial basis.

The syndrome was clearly defined by Dr. Jacqueline Noonan in 1963.<sup>2</sup> In 1968, she subsequently described both male and female patients with a characteristic facies, pulmonary valvular stenosis, and short stature.<sup>3</sup> There had previously been confusion about the status of male patients with the apparent features of Turner syndrome, but it was then recognized that NS accounted for cases described as male Turner syndrome. Although the term NS is most frequently used, some authors still use the term Ullrich-Turner phenotype synonymously.

#### CLINICAL FEATURES (Table 1)

The most significant clinical abnormality in most children is the presence of congenital heart disease, which is found in up to 80% of patients. The most frequent heart defect is pulmonary valvular stenosis, which is usually isolated but may be associated with other structural abnormalities. The electrocardiogram shows a superior axis, which reflects an abnormality in the conducting system. Frequently there is a structural deformity of the chest wall, with pectus carinatum superiorly and pectus excavatum inferiorly.

Of considerable interest is the presence of hypertrophic cardiomyopathy in up to 20% of patients.<sup>4</sup> Histologic examination of cardiac muscle has shown

Table 1  
**Clinical Features of Noonan Syndrome**

|                               |     |
|-------------------------------|-----|
| Pulmonary valvular stenosis   | 62% |
| Hypertrophic cardiomyopathy   | 20% |
| Abnormal electrocardiogram    | 87% |
| Height (<10th centile)        | 70% |
| Undescended testes            | 77% |
| Feeding difficulties (severe) | 24% |
| Developmental delay (mild)    | 10% |
| Refractive errors             | 67% |
| Coagulation abnormalities     | 60% |
| Ptois (severe)                | 42% |
| Pterygium colli (webbed neck) | 23% |
| Pectus carinatum/excavatum    | 95% |

a pattern of myofibrillar disarray similar to that seen in other forms of inherited cardiomyopathy.<sup>5</sup> This would suggest that children with NS would be at increased risk from sudden cardiac arrhythmias, but a recent report by Burch et al<sup>6</sup> has not confirmed this. They demonstrated the presence of hypertrophic cardiomyopathy in the neonatal period, which may lead to cardiac failure and has up to a 20% mortality in this period. The overall natural history of cardiomyopathy in NS is unclear, and certainly a

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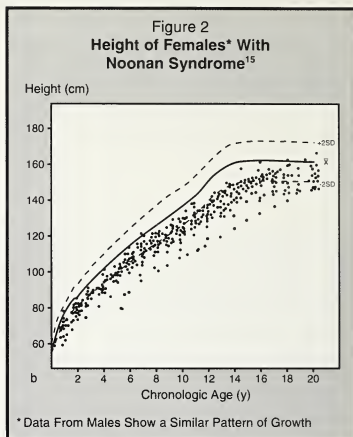
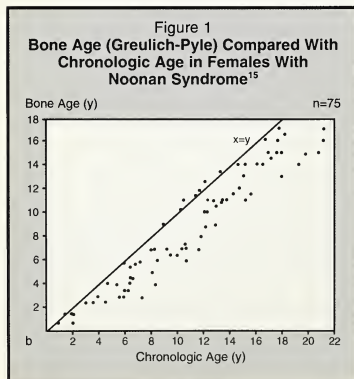
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few cases appear to improve or even resolve during childhood.

The characteristic facial features include hyper- telorism; down-slanting palpebral fissures; epicanthal folds; ptosis; low set, anteriorly rotated ears; and neck webbing. In addition, coarse curly hair and keratosis pilaris (excessive keratinization of the hair follicles) may occur.<sup>7</sup> The facial features change with age<sup>8</sup> and tend to become less marked in later childhood and adult life.<sup>9</sup>

One of the unusual and poorly understood features of this syndrome is the association with abnormal bleeding. Severe, life-threatening bleeding is fortunately rare, but a history of troublesome post-operative bleeding or easy bruising is found in 65% of patients.<sup>10</sup> The nature of the bleeding disorder differs among affected individuals. Witt et al<sup>11</sup> have shown a combination of clotting factor and platelet deficiencies in 19 patients with NS. De Haan et al<sup>12</sup> demonstrated partial factor XI deficiencies in 9 patients. In a more extensive study of 72 individuals with NS, Sharland et al<sup>10</sup> demonstrated defects in the intrinsic coagulation factors. They showed 33% had factor XI:C deficiency, 17% had factor VIII:C deficiency, and 14% had factor XII:C deficiency. In some cases there were combined deficiencies of 2 or more factors. These coagulation factor deficiencies were independent of the cardiac abnormality and were not associated with liver failure or excessive consumption of clotting factors. These intriguing observations have yet to be explained, and it remains to be seen how the gene mutation in NS can modify the levels of coagulation factors.

Feeding difficulties are frequent in infancy. Severe feeding difficulties (defined as tube feeding for 2 weeks or longer in a term infant) were present in



24% of cases.<sup>4</sup> These difficulties are independent of the cardiac abnormality and resolve during early childhood. Refractive errors and strabismus are also frequent and need to be identified early and corrected.<sup>13</sup>

## GROWTH AND DEVELOPMENT

The birth weight in NS falls within the normal range, although some babies may be generally edematous at birth. After birth, the growth in height and weight usually lie below the 3rd centile, while the head circumference continues to lie within the normal range. This gives the appearance of relative macrocephaly. The mean (standard deviation [SD]) delay in bone age, assessed by the Greulich and Pyle method, is -2 years (Figure 1).<sup>4</sup> Several studies on the growth of children with NS have been carried out, and growth charts are available in the papers by Witt et al<sup>14</sup> and Ranke et al<sup>15</sup> (Figure 2). Growth velocity as compared with Turner syndrome also is presented in the paper by Ranke et al<sup>15</sup> (Figure 3).

Growth hormone (GH) has been used to correct short stature in NS, but it has often been used in individual patients without standardized protocols. It may be important to monitor ventricular wall thickness in such cases, since there is some anecdotal evidence that cardiomyopathy may increase while on treatment. One study using GH 28 IU/m<sup>2</sup>/wk, in 14 children with NS has shown an increase in growth velocity from 3.8 to 10.5 cm/y after 1 year of treatment. Ventricular wall thickness was monitored in this study before and during treatment using

standardized echocardiographic sections, and no pathologic increase in ventricular wall thickness has been noted.<sup>16</sup> A further study of 5 children treated with GH for a period from 1.8 to 4.6 years has also shown a favorable response.<sup>17</sup> Puberty is delayed in both males and females with NS. The mean  $\pm$ SD age of menarche in 20 women with NS was  $14.6 \pm 1.17$  years. In males, the onset of puberty is more difficult to define, but it is frequently considerably delayed.<sup>18</sup> In addition, undescended testes are present in 60% to 80% of affected males. Fertility appears to be normal in females, but up to 50% of males are infertile. In one study of 8 adult males with NS, infertility was not associated with normally descended testes or a unilateral undescended testis but was associated with bilateral undescended testes, even following surgical correction.<sup>19</sup>

A number of earlier reports on NS suggested an association with mental deficits; however, recent series have shown this is rarely the case. Motor milestones are often delayed (eg, sitting unsupported, mean = 10 months; walking unsupported, mean = 21 months), but only approximately 10% of patients require special schooling for learning difficulties. The presence of mental retardation in NS indicates the need for careful chromosome studies, since a number of chromosomal abnormalities produce a similar phenotype.

## GENETICS AND ETIOLOGY

Clearly, NS is often an autosomal dominant disorder, since vertical transmission from father to son has frequently been demonstrated. There is considerable variation in expression, and on occasion it

may be milder in its expression in the parent or grandparent than in the child. This makes genetic counseling difficult if there are no striking features of NS in either parent. In the majority of such cases, the condition will have arisen as a new mutation; however, since there is a recurrence risk of 5% in those cases in which neither parent shows signs of NS, it is possible that some individuals have the mutation without any significant physical stigmata.

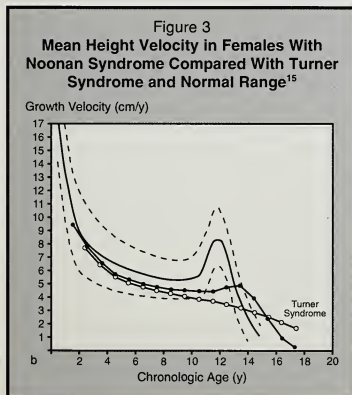
The gene locus for NS has not yet been mapped, and the gene product is not identified. The gene may exert part of its phenotypic effect through pre-natal hydrops, since this has been described in a number of case reports and also has been suggested in the pathogenesis of neck webbing in Turner syndrome. However, it is difficult to see how this mechanism alone could account for the diverse phenotypic features.

There are a number of syndromes to consider in the diagnosis of NS. These include cardiofaciocutaneous syndrome, leopard syndrome, Watson syndrome, and neurofibromatosis (NF)/NS (neurofibromatosis plus). In the Watson syndrome, patients have pulmonary valvular stenosis, café au lait patches, and reduced intelligence. Both Watson syndrome and NF/NS have been shown to be due to mutations (often with large deletions or rearrangements) in the NF1 gene on 17q11.<sup>20,21</sup> Interestingly, the NF1 locus was excluded early in the linkage studies on NS.<sup>22</sup> It may be that different loci are modifying similar embryologic pathways.

In conclusion, the diagnosis of NS still rests on its clinical features. A diagnostic scoring system<sup>23</sup> is available to assist in the diagnosis, but it is hoped that molecular genetic studies will ultimately help in confirming the diagnosis and elucidating the heterogeneity, if any, of the syndrome.

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# Prader-Willi Syndrome: Chromosomal and Gene Aberrations

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Prader-Willi syndrome (PWS) was first recognized as a clinical entity in 1956 by Andrea Prader, a pediatric endocrinologist in Zurich who, together with his colleagues Labhart and Willi, described 9 patients with obesity, short stature, cryptorchidism, and oligophrenia following severe hypotonia in the newborn period.<sup>1</sup> The etiology of the condition was unknown. Since the vast majority of cases are sporadic, the recurrence risk is estimated to be low (1.6%), making a mendelian pattern of inheritance unlikely. It was hypothesized that a primary developmental defect in the hypothalamus could be responsible for the clinical findings (Table 1). When chromosome studies were performed in these patients, the karyotypes were interpreted as normal in most cases; however, starting in 1976 various abnormalities involving chromosome 15 were reported. They were of different types, including robertsonian and reciprocal translocations, both balanced and unbalanced; isochromosomes for the long arm; and the presence of additional small metacentric markers derived from chromosome 15. Because of the rare occurrence and inconsistent nature of these karyotypic abnormalities, no straightforward hypothesis of PWS's being due to a chromosomal imbalance could be derived from these observations. Finally, in 1981 Ledbetter and colleagues,<sup>2,3</sup> using high-resolution chromosome analysis, reported finding small interstitial deletions of the 15q11-q13 region in a high proportion of these patients. Submicroscopic deletions or mosaicism were speculated to be present in those PWS cases whose karyotypes were apparently normal. However, structural abnormalities involving this region are difficult to interpret, particularly using very high-resolution chromosome analysis. There is often a difference in appearance between the maternal and paternal chromosomes due to differential condensation of the proximal 15q region. Therefore, under high-resolution analysis, chromosomes from normal individuals may show differences between the 2 chromosome 15 homologues in the number and size of subbands in this region. Molecular cytogenetic diagnosis, in particular in situ hybridization with probes from the commonly deleted region, provides more definitive evidence for deletions than cytogenetic analysis of elongated chromosomes.

The etiologic relationship between the apparent chromosome 15 deletion and the PWS phenotype was challenged when, in 1987, an identical deletion was reported in a number of patients with a neurologic disorder quite different from PWS, called Angelman syndrome (AS) after the author of the first report in 1965. The features include microcephaly; jerky movements; seizures; a peculiar face with prominent chin; large mouth with protruding tongue; and inappropriate laughter. Mental deficiency in AS patients is usually more severe than in PWS and

Table 1  
**Criteria for Clinical Diagnosis of Prader-Willi Syndrome<sup>19</sup>**

## Major Criteria

Neonatal/infantile central hypotonia  
Feeding problems/failure to thrive in infancy  
Rapid weight gain after 12 months  
Facial features: narrow bifrontal diameter, almond-shaped eyes  
Hypogonadism  
Mild/moderate developmental delay  
Hyperphagia

## Minor Criteria

Decreased fetal movement; infantile lethargy, improving with age  
Behavior problems; obsessive/compulsive, rigid, stubborn  
Sleep disturbance/apnea  
Short stature by age 15 years  
Hypopigmentation  
Small hands and feet for height age  
Narrow hands with straight ulnar border  
Esotropia, myopia  
Thick viscous saliva  
Speech articulation defects  
Skin picking

## Supportive Findings

High pain threshold  
Decreased vomiting  
Temperature control problems  
Scoliosis and/or kyphosis  
Early adrenarche  
Osteoporosis  
Unusual skill with jigsaw puzzles  
Normal neuromuscular studies

the dysmorphic, neurologic, and behavioral findings are quite distinct. There are more familial cases in AS, and the recurrence risk in siblings was estimated as 4%. The deletions of the 15q11-q13 region in PWS and AS appear cytogenetically similar if not identical.<sup>4</sup>

Resolution of this puzzle was provided in 1983 when it was discovered that deletions in PWS always involve the paternally derived chromosome 15<sup>5</sup> while deletions in AS always involve the maternally derived chromosome.<sup>6</sup> Furthermore, in a significant proportion of PWS patients whose chromosomes appear structurally normal, both copies of chromosome 15 are maternally derived; the paternal copy is missing (uniparental disomy, UPD).<sup>7</sup> In AS, a smaller fraction of cases have paternal UPD and about one third of AS cases have neither deletion nor UPD. Submicroscopic deletions that are maternally inherited have been demonstrated in some families with multiple affected sibs. In other families with inherited AS, no deletion has been demonstrated. It is likely, therefore, that only 1 or very few genes are responsible for the AS phenotype, that they are expressed exclusively from the maternal chromosome, and that they are silent on the paternally derived chromosome.

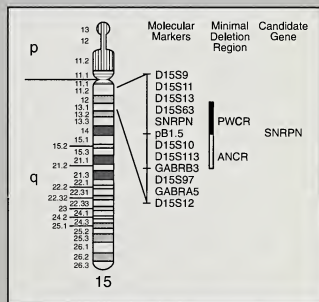
The situation for PWS is quite different, as there are very few familial cases and all sporadic cases have either UPD or deletions. It is likely, therefore, that PWS is a true microdeletion syndrome that requires the silencing or deletion of more than 1 locus from the paternally derived chromosome 15. Nevertheless, the extent of the deletions in the majority of PWS and AS cases is quite similar on the molecular level. The breakpoints are defined in intervals between molecular markers and the total region is approximately 3 to 5 mega-base pairs in size (Figure 1). This entire region, however, behaves differently on the maternal versus the paternal chromosome in normal individuals. For example, evidence is emerging that the region replicates earlier on the paternal than on the maternal chromosome<sup>8</sup> and that there are differential DNA methylation patterns.<sup>9</sup> Taken together with the different clinical features of the 2 deletion syndromes and the observation of UPD, the differential replication and methylation strongly suggest that there are genes in this region that are predominantly or exclusively expressed from only 1 chromosome 15, ie, they carry a parental-specific imprint.

Are the genes responsible for PWS and AS intermingled, or are there distinct subregions that may be responsible for the different phenotypes? The discovery of a few unusual patients who appear to have the full syndrome in the presence of submicroscopic partial deletions has allowed researchers to subdivide the region into a PWS minimal deletion region (PWCR) and a distally adjacent AS minimal

region (ANCR) (Figure 1). The submicroscopic deletion reported in a 3-generation Japanese family<sup>10</sup> is particularly informative because a woman who inherited this deletion from her father was phenotypically normal; in particular, she did not have any PWS features. In 3 of her children, who inherited the chromosome 15 with the deletion, the features of AS were present. The deletion in this family delineates the ANCR. The deletion junction fragment has been cloned, and the proximal fragment flanking the deletion (pB1.5) demarcates 1 border of the PWCR.<sup>11</sup> The proximal border is defined by another unusual patient with a partial deletion. The entire PWCR has been cloned in overlapping yeast artificial chromosomes. It is 320 kb in size.<sup>12</sup>

The first gene encoding a known protein mapped to the PWCR was SNRPN, the gene for small nuclear ribonucleoprotein (snRNP)-associated polypeptide SmN.<sup>13</sup> Expression of SNRPN appears to be limited to neuronal tissue.<sup>14</sup> The protein sequence is highly similar to SNRPB (gene on chromosome 20), which is ubiquitously expressed. In the brain, however, SNRPB expression is replaced by SNRPN. By studying DNA from PWS patients with partial deletions, SNRPN was mapped to the minimal PWS deletion region (Figure 1).<sup>13</sup> The

Figure 1



ideogram of chromosome 15<sup>26</sup> with ordered molecular markers within the common Prader-Willi syndrome/Angelman syndrome (PWS/AS) deletion region (15q11.2-q13.1). The minimal deletion region for PWS (PWCR) extends from a breakpoint between D15S63 (PW71) to pB1.5<sup>11</sup> and for AS (ANCR) from pB1.5 to a breakpoint within GABRB3, the gene for the  $\beta_3$ -GABA receptor.

homologous locus in mouse, *Snrpn*, was mapped to a region on mouse chromosome 7 that is evolutionarily related to the human 15q11-q13 region. In mouse brain, the *Snrpn* gene was expressed exclusively from the paternal allele, as one would expect for a gene involved in the PWS phenotype.<sup>15</sup> To determine whether the gene is also imprinted in humans, extensive sequencing of the human SNRPN gene was carried out in normal human samples and has revealed a common sequence polymorphism in exon 2, where either a C or T is present.<sup>16</sup> This polymorphism allows the determination of the parental origin of any transcripts. In brains from fetuses who were heterozygous at the DNA level, it could be shown that only the paternal allele is expressed.<sup>17</sup>

By virtue of its location in the PWR and its uniparental expression from the paternal chromosome, SNRPN is the premier candidate gene to explain the PWS phenotype. The cDNA is rather small, consisting of 720 nucleotides that encode 240 amino acids and are distributed over 7 coding exons. The total genomic size is less than 25 kb. To prove that SNRPN is a candidate for PWS, a mutation in the paternally derived copy of the gene needs to be demonstrated. However, PWS in the absence of either deletion or UPD is extremely rare. In 2 such rare families,<sup>18</sup> sequencing of the SNRPN coding regions from affected individuals has not yet turned up a mutation. Likewise, patients with some features of PWS but who do not meet the diagnostic criteria (Table 1)<sup>19</sup> have been studied for SNRPN deletions or gene rearrangements, with negative results.<sup>13</sup> Inactivation of SNRPN, however, could be due to mutations outside of the coding region.

SNRPN is also an attractive candidate gene based on functional considerations. It is a core protein of snRNP particles that also contain small nuclear RNA molecules and are involved in the processing of pre-mRNA or the transport of mRNA out of the nucleus. The highest expression of SNRPN is in the brain, which suggests that N-containing particles may have a brain-specific function, eg, brain-specific splicing of differentially spliced genes such as calcitonin. Speculations about the pathogenetic mechanism of SNRPN deficiency assume that in the absence of SNRPN expression, no N-containing snRNPs would be made. The absence of these particles might not affect all of the central nervous system neurons equally but may be particularly important for areas of the hypothalamus, where centers responsible for the regulation of muscle tone, growth, appetite control, temperature control, and pain sensitivity — all systems affected in PWS — are located.<sup>13,15</sup>

Diagnostic tests for PWS have progressed from high-resolution cytogenetics, which is tedious and

the results of which are often difficult to interpret, to the use of molecular technology. In particular, fluorescence in situ hybridization (FISH) is now routinely used in diagnostic cytogenetic laboratories. In this procedure, large insert probes that hybridize consistently to normal chromosome 15 and fail to hybridize to PWS chromosomes in which the hybridizing region is deleted are used. While probes from the larger commonly deleted region (Figure 1) can be used successfully in the majority of deletion cases, it is preferable to use probes containing the SNRPN gene to ensure detection of rare cases with partial deletions of the region. FISH results have to be correlated with the phenotype, since a deletion that is detected cannot be assigned to the maternal or paternal chromosome by this method. In the absence of a deletion, molecular genetic studies such as polymerase chain reaction (PCR) analysis of highly polymorphic dinucleotide repeats are necessary to determine the presence of UPD.<sup>20</sup> This requires parental samples. UPD can be detected by distinct restriction fragments generated by methylation-sensitive enzymes and hybridization with probes from the region.<sup>9,21</sup> Another novel direct test currently being developed makes use of blood samples to study the expression of SNRPN and another paternally expressed gene in the PWS region (IPW). By PCR analysis of reverse transcribed mRNA, expression is detected in normal controls but not in PWS patients with either deletion or UPD.<sup>22</sup>

As is true for much of the recent progress in human molecular genetics, the identification of a gene involved in a particular genetic disorder is only the first step towards understanding the pathogenesis of the disease manifestations. Such an understanding is necessary in order to devise rational treatment protocols that would interfere with the stepwise process that leads from the abnormal gene to the diseased brain. In the case of PWS, a promising candidate gene for the hypothalamic manifestations, SNRPN, has been identified. Most PWS patients, however, have deletions that extend much beyond the PWR and include many more genes. The hypopigmentation frequently observed<sup>23</sup> may somehow be related to hemizygosity at the (nonimprinted) p locus (D15S10, Figure 1), since individuals homozygous for mutations or deletions of this gene have tyrosinase-positive oculocutaneous albinism.<sup>24</sup> It is also possible that genes outside of the deleted region whose pattern of methylation, replication, and expression has been altered by the deletion contribute to the PWS phenotype.<sup>25,26</sup> It may be necessary, therefore, to identify all genes in the region that are potentially involved before one can propose hypotheses for pathogenesis and design experiments to address them.

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# Rationale for Dosing Recombinant Human Growth Hormone by Weight Rather Than Units

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The practice in Europe and Japan of prescribing growth hormone (GH) on the basis of units differs from that in the United States and Canada, where milligram dosing has been the standard procedure since 1985. The consequence of these 2 approaches to dosing human GH (hGH) is widespread confusion in the literature as well as in the medical community. Many clinicians are not familiar with the events that led to this dual approach to prescribing GH. This article will discuss the factors that influenced how hGH was initially prescribed and the new technologies that have greatly improved its purification and characterization. Fortunately, we now have a much clearer understanding about the relationship between unitage and weight for recombinant hGH (rhGH). Hence, dosing by milligrams per kilogram of body weight should become the universal norm for prescribing and writing about

rhGH. Failure to move in this direction will mean that it will be impossible to compare studies done in North America with those done elsewhere. Even in North America there was recent confusion caused by claims that one rhGH product was more potent than another on the basis of unitage comparisons; the erroneous basis for this conclusion will also be explained in this article.

In this report, the historical basis for dosing rhGH by units is reviewed along with the developments that have led to the current practice in North America of using milligrams.

## HISTORICAL ASPECTS

Although pituitary GH was first identified in 1921, it was not until 1956 that a GH preparation became available for clinical use in hypopituitary patients.<sup>1-5</sup> Much early effort had been dedicated to isolating bovine GH and porcine GH and to documenting their anabolic and metabolic properties in hypophysectomized rats. When these GH products were administered to humans, they were ineffective; the treatment failures were presumed to result from catabolic impurities or toxic contaminants.<sup>6,7</sup> However, studies in comparative endocrinology provided insight into species specificity of GH. Specifically, fish GH prepared by Wilhelmi was ineffective in rats but anabolic in fish. Bovine GH and porcine GH were anabolic in rats but ineffective in monkeys, while monkey GH led to nitrogen retention in hypophysectomized monkeys.<sup>8-10</sup> Successful collections of human pituitary glands by Li et al in San Francisco and Raben in Boston resulted in the availability of human pituitary GH (pGH), which proved to be efficacious in human hypopituitary patients.<sup>5,11</sup>

In 1963, the National Pituitary Agency was established to improve the collection, extraction, and distribution of hGH.<sup>12</sup> The method used by Wilhelmi and colleagues to extract native pGH from

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acetone-preserved or frozen pituitaries differed from the ones used by Raben (glacial acetic acid) and Li  $[(\text{NH}_4)_2\text{SO}_4]^{2,3,13,14}$ . Those early preparations of native pHGH contained variable amounts of impurities and degraded products. Additional purification by column chromatography was not undertaken until 1977 because losses from this additional step would have aggravated the existing severe shortages of hormone.<sup>15</sup> It was essential in this period to standardize pHGH using bioassays because of product variability; moreover, sensitive techniques for characterizing the physicochemical properties of hormones had not been developed. Hence, dosing of pHGH by arbitrarily defined units of activity derived from bioassay standardization was the only available choice at that time.

From 1956 to 1985, the main bioassays used to standardize pHGH were the hypophysectomized rat weight-gain assay and the tibial width assay.<sup>16-18</sup> In both bioassays, 2 doses of international GH standard (10  $\mu\text{g}$  and 50  $\mu\text{g}$ ) and 2 doses of "unknown" GH product were administered subcutaneously to hypophysectomized rats, and the estimated relative potency of the unknown was calculated versus the international standard using the mean gains in each group of test animals over the entire treatment period. In the weight-gain assay, the gain in body weight is proportional to the logarithm of the daily dose of GH over a range of 10 to 250  $\mu\text{g}/\text{d}$ . In the tibial width assay, the increased width of the proximal epiphysis of the tibia is proportional to the log of the total dose of GH administered in micrograms over a range of 15 to 400  $\mu\text{g}$ . Although other bioassays were developed during this period, the only one that is current and considered the *gold standard* is the rat weight-gain assay.

Then in 1985, the Food and Drug Administration (FDA) approved the first rhGH preparation (somatrem\*) for clinical use and mandated that dosing be based on the physicochemical property of weight (therefore, milligrams), provided the product had been standardized for biologic activity. The bioassay method employed was the weight-gain assay in young hypophysectomized rats. The potency of rhGH was estimated by comparing its effect to that of an international reference standard (bovine pituitary GH for bioassay, World Health Organization [WHO]). Using this approach, somatrem was assigned a biopotency of 2 IU/mg and the dose approved for clinical use was 0.3 mg/kg/wk.

Confusion about reported differences in the potency of the 2 available rhGH products, somatrem\* (methionyl hGH) and somatropin† can be traced to the use of different primary GH standards for standardization of their biologic activity. Historically, bovine pituitary GH standard was used until 1987 to

standardize pHGH as well as somatrem. The desirability of an international standard of like origin led to the development of a primary human pituitary standard 80/505 for bioassay in 1987 (potency 2.6 IU/mg). Unlike somatrem, somatropin, which was the second rhGH to be approved, was standardized using the pHGH standard 80/505. It was assigned a specific activity of 2.6 IU/mg, while somatrem, which had been initially standardized using the original bovine GH standard, had received a specific activity of 2.0 IU/mg. Restandardization of somatrem using the pHGH standard 80/505 proved that somatrem and somatropin are equipotent, ie, 2.6 IU/mg.

## CURRENT CONSIDERATIONS

Why is it still necessary to standardize rhGH for biologic activity? The FDA reasons that rhGH will be produced by different processes, which may or may not alter the biologic activity of the product, and has mandated that bioassay standardization will remain one of the prerequisites for drug approval.<sup>19</sup> Recently, the argument has been made that while bioassay standardization was essential for natural biologic preparations because they gave highly variable responses in animals or cultured cells, recombinant DNA-derived products have a much higher level of purity (>99%) and can be dosed accurately by weight alone. The FDA insists, however, that potency (activity determination) is still needed because physicochemical methods in themselves are unable at present to guarantee the biologic activity of a protein derived from natural or recombinant sources. It is likely that a further adjustment in the potency of rhGH products will occur because of the development of a new international WHO rhGH reference reagent for somatropin (88/624) by the National Institute for Biological Standards and Control (NIBSC).<sup>20</sup> The proposed specific activity of this rhGH reference reagent will be approximately 3.0 IU/mg. However, due to considerable disagreement within the scientific community about the validity of this specific activity, the new preparation will not be designated as a primary reference material but will be assigned a potency of 6.7 IU/ampule and 2.0 mg/ampule and serve as a reference reagent. Based on this issue, a separate United States Pharmacopeia (USP) standard for rhGH is presently being developed. It is also possible that more accurate biochemical methods will become available for determining potency and that the concept of units of activity based on relatively imprecise bioassays will eventually be eliminated. For example, sophisticated *in vitro* receptor binding assays or specific antibody epitope mapping methods may eventually prove to be acceptable alternatives to the current animal bioassays.

In January 1993, a panel of experts participated in a GH workshop convened by the FDA for the purpose

\*Protropin

†Humatrope

of selecting the most suitable tests for evaluating and labeling rhGH products.<sup>19</sup> The panel was asked to choose which tests should be used to determine the identity (proof of authentic hGH structure); the purity (lack of contaminants and degraded products); and the chemical strength (quantity of authentic hGH in milligrams of rhGH). A majority of the panel recommended reversed phase high-performance liquid chromatography (RP-HPLC) and peptide mapping as identity tests, although some panel members supported the use of SDS-PAGE and size exclusion chromatography (SEC) as alternative or additional methods of determining identity of hGH. There was unanimous disapproval of amino acid analysis as an identity test. With regard to purity testing, the panel agreed that RP-HPLC, SEC, ion exchange, and SDS-PAGE are satisfactory methods. The tests recommended for evaluating the chemical strength were ultraviolet spectrophotometry, SEC, and occasionally RP-HPLC in the event of ultraviolet interference. Many of the methods now available for characterizing the physicochemical properties of rhGH will ensure a level of hormone purity not possible in the pHGH era.

## CONCLUSION

Many countries (eg, European countries and Japan) continue to require rhGH dosing in units while vial contents are labeled in milligrams. Regardless of how rhGH is dosed elsewhere, the FDA will adhere to its policy that rhGH products be dosed in milligrams provided bioassay criteria are satisfied. Given the worldwide availability of precise and sensitive methods for ensuring production of equipotent rhGH products, a uniform approach to dosing by milligrams would eliminate the confusion that exists

when comparisons are made of treatment regimens in different countries.

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## Abstracts From the Literature

### Utah Growth Study: Growth Standards and the Prevalence of Growth Hormone Deficiency

This population study aimed to: (1) determine the feasibility of having nonmedical personnel accurately perform and record height measurements; (2) obtain serial height measurements on randomly selected school-age children to establish norms by age and sex for height and growth rates; (3) generate growth curves for comparison with standard growth curves; and (4) determine the prevalence of growth hormone deficiency (GHD) as a cause of growth failure in the study population.

In order to achieve these goals, a prospective investigation was designed in the state of Utah. In 251 elementary schools, randomly selected by computer, 114,881 children were measured the first year; 79,495 growth rates were calculated after the second measurements obtained 1 year later. The height and growth velocity curves generated were very similar to the currently used charts (National Center for Health Statistics growth charts). Subsequently, 555 children with short stature (<3rd percentile) and poor growth rates (<5 cm/y) were examined. The presence

of GHD (defined as peak level <10 ng/dL with 2 provocative tests) was found in 16 children who were previously undiagnosed; 17 children were already known to have GHD. The male:female ratio was 2.7: 1 ( $P=0.006$ ). Six girls with Turner syndrome were identified.

Among the authors' conclusions were that the growth curves generated in the 1960s and 1970s are valid for children of the 1990s and that most children growing <5 cm/y (a commonly used threshold rate) will not have an endocrine disorder. If the criterion were shifted to <4.5 cm/y, none of the children with GHD would have been missed and fewer children with normal variants of short stature would have been examined. In many children (48% in this study), GHD and Turner syndrome may currently be unrecognized and untreated. GHD appears to be more common in boys. The incidence of GHD in Utah and, presumably, in the United States is at least 1/3,480.

Lindsay R, et al. *J Pediatr* 1994;125:29-35.

**Editor's comment:** This very good and much needed study is based on a large population and offers an estimated incidence of GHD among elementary school children in Utah. It also confirms the validity of some of the currently used norms. However, there are several methodologic problems that limit the completeness of ascertainment, summarized by the authors as follows: (1) Volunteers, though carefully trained, may have committed undetected errors. (2) Growth rates were obtained for only 69% of the original base of 114,881 children. Therefore, some slow-growing children from the original group may have been missed. (3) Some children with growth problems were never examined because of lack of parental concern or refusal to allow follow-up. (4) The diagnostic evaluations made by the physicians were not uniform. (5) Children of normal stature and low growth rate were not examined.

Although these self-criticisms are well evaluated, probably the most important deficiency of the study is not mentioned. The weight and weight progression of the children were not measured and followed up. Weight and height are 2 parameters that need to be considered together for the evaluation of growth. Linear growth will not take place in the absence of appropriate weight gain. Since weight was not considered in this study, nutritional growth retardation was not diagnosed, nor was it considered by the authors.

In this study, primary care physicians seemed to miss 85% of

the children with growth failure. However, just as important is the failure of endocrinologists to look for and diagnose nutritional growth retardation, a condition that may be more prevalent than GHD.

Fima Lifshitz, MD

**Second Editor's comment:** Indeed, this is an important article. In my opinion, the most important aspect of this article is that it demonstrates the importance and value of screening programs to identify pathology that is not otherwise recognized. Objections to screening programs are running rampant, and even highly respected endocrinologists vary in their opinions. Concerns have been expressed about unnecessarily creating anxiety among parents of normal but short children. Granted that anxiety may be unnecessarily created; however, the positives of identifying many children with unrecognized organic causes of growth retardation outweigh the negatives, in my opinion. It is particularly important to identify children who are <3 standard deviations below the mean in height, as 50% of these will have an organic cause for short stature, and to identify those growing less than 4.5 cm/y, as pointed out by Lindsay et al.

Robert M. Blizzard, MD

## Developmental Timing of Dynamic Mutations

Dynamic mutations differ from classic mutations in that the DNA change occurs once and is usually passed on exactly the same. Dynamic mutation is the term used to describe the change (increase or decrease) of DNA sequences resulting from the amplification of naturally occurring polymorphic trinucleotide repeats (Sutherland et al, 1992). In other words, this is a new and different type of mutation, and it is not stable. The mutation begins as small increases in the copy number, but the number is outside of the normal range. The increases may vary; therefore, they may be different sizes in different cells, and these may increase or decrease in size from one generation to the next. Some of the disorders associated with dynamic mutations are fragile X syndrome, myotonic dystrophy (MD), and Huntington disease (HD).

An excess number of repeats (CGG) in the Xq23.7 region has been reported in the fragile X syndrome. The normal number of repeats is 6 to 54. The premutations range from 52 to more than 200, and the mutation contains more than 200 repeats (Mandel, 1993). In fragile X syndrome, the person who carries the least number of trinucleotide repeats but who does not have the typical phenotype is said to be the carrier of a premutation. When passed from generation to generation, this premutation is unstable and may expand over a few generations, gradually increasing in size when transmitted by females but remaining the same when transmitted by males. When transmitted by a female and increased in size, it may become a full-blown mutation, which is clinically significant since it produces the typical fragile X syndrome phenotype with mental retardation.

HD is an adult-onset, progressively neurodegenerative disorder that presents with choreic movements, psychiatric changes, and intellectual deterioration. The mode of inheritance is autosomal dominant. Recent findings have shown that

the genetic defect in HD is related to an excess number of tandem GAC repeats in the chromosome 4p16.3 region due to allelic expansion. The number of tandem repeats in normal individuals is between 11 and 24. In contrast, individuals with HD have 42 to 86. The most elongated repeats are associated with individuals who acquired HD through a new mutation. It has also been shown that the length of repeats correlates with onset and severity of disease, and if the gene is paternally inherited, the phenotype appears earlier and is more severe.

In some disorders the number of copies is directly related to the age of onset of the disease. The congenital form of MD for example, is related to the largest number of repeats (Harley et al, 1992). The same situation occurs in HD, in which the juvenile-onset form is also associated with the largest number of repeats (Andrew et al, 1993). In Kennedy disease, the age of onset is inversely related to the increase in copy number. Another neurodegenerative disorder associated with triplet repeats is hereditary dentatorubral-pallidolysian atrophy (DRPLA) (Miwa, 1995).

A recent article by Sutherland et al (1993) reviews the latest findings regarding dynamic mutations. The authors suggest that their occurrence may be able to explain such concepts as incomplete penetrance, variable expression, and anticipation. The fact that all of the disorders described so far have been associated with triplet repeats and are neurodegenerative has led to the suggestion of a common mechanism for neuronal degeneration caused by unstable expansion of these repeats. The functions of the genes, however, remain unclear.

The mutation seen in fragile X syndrome has been shown (Wohrle et al, 1993) to vary in repeat length in different tissues (somatic mosaicism). The constancy of the repeat in HD was not known. In order to determine the degree of mosaicism and



the stability of the repeat, MacDonald et al (1994) studied 4 pairs of monozygous twins affected with HD and the sperm from HD gene carriers. The 4 pairs of monozygous twins had identical repeat lengths. Different repeat lengths (germline mosaicism) were detected in the sperm of HD carriers. When the repeat lengths of the HD carriers' sperm was compared with the repeat lengths in their lymphoblasts, it was found that the sperm DNA had longer repeat lengths and that the greatest degree of gametic mosaicism was found with the longest somatic repeats.

Because of the somatic variation seen in the repeats in fragile X syndrome it had been suggested that repeat expansion may occur during early embryogenesis. MacDonald et al concluded that even though allelic expansion may be a common underlying mechanism for HD and fragile X syndrome, the developmental timing of the instability of the repeats is different.

Andrew SE, et al. *Nat Genet* 1991;4:398-403.

Harley HG, et al. *Nature* 1992;355:547-548.

MacDonald ME, et al. *J Med Genet* 1993;30:982-986.

Mandel JL. *Nat Genet* 1993;4:8-9.

Miwa S. *Nat Genet* 1994;6:3-4.

Sutherland GR, Richards RL. *Nat Genet* 1994;6:44-46.

Wang Y-H, et al. *Science* 1994;265:669-671.

Wohrle D, et al. *Nat Genet* 1993;4:140-142.

**Editor's comment:** Using the term mutation for these disorders is not completely accurate. In the strictest sense of the word these mutations are not really mutations, but new and different

mechanisms that result in disease. Another important finding of the study of the association of trinucleotide repeats and neurodegenerative disorders has changed the methods of finding genes. As in the case of DRPLA, the gene for other neurodegenerative disorders that have been mapped to a specific chromosome may be found by looking for trinucleotide repeats.

Judith G. Hall, MD

**Second Editor's comment:** Evidence has been increasing that the presence of large blocks of trinucleotide repeats disrupts the transcription of genes in which they reside; however, the mechanisms involved have not been clear. The articles cited here provide insight not only into how this might happen but also why disorders associated with such blocks exhibit a threshold effect with regard to appearance of the clinical phenotypes. Indeed, as Wang et al point out, the presence of very strong nucleosome positioning signals where they do not normally reside may not only interfere with the expression of relevant genes but may also disrupt copying of the trinucleotide repeats by DNA polymerase, which could lead to further expansion of the repeats, another feature of these disorders. The article by Wang and colleagues also reminds us that we must keep our minds open and to look for new mechanisms to explain how mutations cause genetic disease.

William A. Horton, MD

## Sucralate Causes Malabsorption of L-Thyroxine

Following their experience with a hypothyroid woman in whom the dose requirement for L-thyroxine increased substantially after sucralate, a nonabsorbed aluminum salt of sucrose sulfate prescribed for treatment of dyspepsia, Sherman and colleagues studied the effect of this agent on absorption of L-thyroxine. In healthy adult volunteers, 80% of an ingested dose of 1,000 µg of L-thyroxine was absorbed. When L-thyroxine and sucralate were administered simultaneously, only 23% of ingested L-thyroxine was absorbed, and the rate of absorption slowed. When L-thyroxine was administered 8 hours after a dose of sucralate, 78% of administered L-thyroxine was absorbed. Thus, the authors concluded that sucralate impairs the absorption of L-thyroxine, probably by intraluminal binding of the hormone.

Sherman SI, et al. *Am J Med* 1994;96:531-535.

**Editor's comment:** Several medications inhibit intestinal absorption of L-thyroxine, including ferrous sulfate, aluminum hydroxide, and colestipol, as well as sucralate, an agent that is utilized for duodenal ulcers, gastritis, reflux esophagitis, and dyspepsia. Sucralate also impairs absorption of tetracycline, phenytoin, and digoxin. Although in children and adolescents poor compliance with medication intake is the most common cause of erratic dose requirements for L-thyroxine and other medications, it is important to remember when confronted with this problem that one drug may adversely affect the absorption, serum protein binding, excretion, or pharmacokinetics of another agent.

Allen W. Root, MD

## Snaring the Achondroplasia and Hypochondroplasia Gene

Achondroplasia (ACH) is the most common human chondrodysplasia. A milder form of disproportionate short stature that also has rhizomelic shortening and normal bone histology is hypochondroplasia (HCH).

The estimated incidence of these disorders is 1 in every 15,000 live births, and until now, their etiology has been unknown. Individuals affected with ACH have short (rhizomelic) limbs; macrocephaly; depressed nasal bridge; lordosis; and short, stubby trident hands. HCH presents with short stature but few clinical symptoms, and the radiologic findings are similar but milder than

those seen in ACH. Ninety percent of ACH and HCH cases are the first affected individual in the family. In the case of ACH, an association has been made with increased paternal age, as is often seen in de novo mutations. Affected individuals are fertile, and the disorder is passed on as an autosomal dominant trait.

In early 1994, the gene for ACH was localized to the tip of the short arm of chromosome 4 (4p16.3). Ironically, the locus mapped very near to another elusive disease gene locus, the Huntington disease (HD) gene locus. Three independent groups reported the localization.



Velinov et al studied 14 families with 40 DNA markers distributed randomly throughout the genome. When 1 of these showed a positive lod score, indicating possible linkage near the end of the short arm of chromosome 4 (4p), 10 other DNA markers were tested that mapped to the same region on chromosome 4. One marker showed a maximum lod score of 3.65. A lod score of more than 3.0 is generally accepted as demonstrating genetic linkage between a disorder and the chromosomal location identified by the marker. Multipoint linkage analysis, in which linkage of several markers is tested simultaneously, suggested that the gene most likely resided within a 4-cM genetic region distal to the HD locus at chromosome 4p16.3.

Le Merrer and colleagues investigated 6 families with ACH and 9 families with HCH. Because of the similar features, HCH has been suspected of being an allelic form of ACH. A lod score of 3.01 was established for the ACH families alone, and a lod score of 4.71 for the ACH plus HCH families combined. Importantly, when a computer program called HOMOG was applied to test the hypothesis that the 2 conditions are genetically homogeneous, the results suggested that they were, ie, they are allelic disorders. Francomano et al studied 18 families with ACH using 6 DNA markers that map to chromosome 4p. This analysis yielded maximum lod scores of 6.44 and 9.9, using 2-point and multipoint linkage analysis, respectively.

The 3 papers firmly placed the ACH locus within a region about 2.5 Mb of DNA at the tip of the short arm of chromosome 4. All 3 papers also pointed out that among known genes that map to this area, a fibroblast growth factor receptor gene (FGFR3) was a good candidate. Fibroblast growth factors (FGFs) transmit growth signals to cells in many tissues, and FGFR3 was known to be expressed in cartilage. However, despite this insight, most workers in the field did not expect the ACH locus to be identified quickly, perhaps biased by the many years it had taken to find the HD locus in the same region. Thus, it came as a surprise when Shiang et al reported 2 mutations in FGFR3 in several patients with ACH.

The authors (Shiang et al) used denaturing gradient gel electrophoresis (DGGE) to screen FGFR3 cDNA fragments derived from lymphoblasts and fibroblasts. When DGGE results from homozygous ACH patients and controls were compared, a sequence difference was suggested in a fragment corresponding to the transmembrane domain of the molecule. The investigators next sequenced this region in the ACH DNA, and found a consistent G to A base change at nucleotide 1138 of the FGFR3 coding sequence. This base change results in an arginine substitution for a glycine at position 380 of the mature protein.

The authors recognized that this base change also creates a cleavage site for the restriction enzyme *SfcI* that is not present in the sequence of the normal allele. This finding enabled them to easily distinguish the 2 alleles on the basis of the presence or absence of the restriction site. With this strategy, they analyzed genomic DNA from their patient material, which included 6 unrelated cases of homozygous ACH, the heterozygous ACH parents of 2 of these homozygotes, and 2 sporadic heterozygous ACH cases — for a total of 16 ACH chromosomes — and more than 50 controls.

The results showed that 5 of the 6 homozygous ACH patients were homozygous for the 1138 G to A base change and that all of the sporadic cases were heterozygous for this base change. It was not detected in any of the controls. Thus, 15 of 16 ACH chromosomes exhibited the same base, which was not found in controls. Further analysis of DNA from the homozygous ACH

infant in whom only a single copy of the 1138 G to A mutation was present revealed that the same 1138 G was changed to a C instead of an A. This change also results in an arginine substitution for glycine at position 380 in the mature gene product.

Thus, the same base, 1138 G, was changed in 16 of 16 ACH alleles examined and causes the same amino acid substitution in the transmembrane domain of FGFR3 in all cases.

The authors addressed several issues in their discussion. First, they pointed out that the G at nucleotide position 1138 must be extremely mutable. Second, they suggested that the glycine to arginine change at amino acid residue 380 of the mature receptor protein may be specific for the ACH phenotype. Third, they speculated on mechanisms by which such mutations might operate, including interference with FGF signal transduction through FGFR3 receptors and possibly through other FGF receptors. Finally, they stressed that the high frequency of this mutation should facilitate prenatal diagnosis in couples at risk for homozygous ACH.

Francomano CA, et al. *Hum Mol Genet* 1994;3(5):787-792.

Le Merrer M, et al. *Nat Genet* 1994;6:318-321.

Shiang R, et al. *Cell* 1994;78:335-342.

Velinov M, et al. *Nat Genet* 1994;6:314-317.

**Editor's comment:** After many years of pursuit and at least 1 false start, the search for the ACH gene appears to be over. Indeed, although yet to be published, the 1138 G to A mutation that causes a glycine to arginine substitution in the transmembrane domain of FGFR3 has been confirmed by others in an extremely high proportion of patients with typical ACH. This saga is of interest from many perspectives. For example, the relative merits of positional cloning versus candidate gene analysis strategies are often debated vigorously among gene hunters. The extremely rapid unfolding of this story demonstrates how the 2 strategies can be effectively coupled. It also underscores the value of characterizing the human genome to the extent possible so that when a given disease is linked to a particular chromosomal region, candidate genes can be readily identified and analyzed.

From a biologic perspective, the results raise as many questions as they answer. First, the reasons why the 1138 G should be so mutable are far from clear. The authors point out that the mutation occurs in the context of a CpG dinucleotide. C to T transitions are known to occur frequently in this setting, especially if the C is methylated, since the latter can become deaminated to a T, changing a G:C base pair to an A:T base pair. Even taking this phenomenon into account, the mutation rate for this nucleotide is extremely high. One wonders if factors not evident at this time are involved. It seems likely that further characterization of the human FGFR3 gene will provide insight into this question.

Similarly, despite a rapidly growing knowledge of FGF ligands, receptors, signaling pathways, and the relevance of FGF signaling to limb development, it is not at all evident how substituting an arginine for a glycine in the transmembrane domain of FGFR3 disrupts bone growth or how this specific change produces such a specific phenotype. For example, FGFR3 is expressed in many tissues, especially the brain. Why should abnormalities of FGFR3 signaling be restricted to skeletal development? Likewise, it is probably reasonable to assume that the FGF signaling defect responsible for ACH resides in the growth plate. However, could the defective signaling involve vascular cells that invade the growth plate or other cells, such

as perichondrial or bone marrow cells, that somehow influence growth plate function? What is clear is that much more work is needed to sort out the molecular pathogenesis of ACH.

Finally, it is somewhat comforting to discover that ACH mutations map to a growth factor receptor gene, since it has long been suspected that the basic defect in this condition involves growth plate regulation.

William A. Horton, MD

**Second Editor's comment:** The fact that deletions of the distal arm of chromosome 4 have been reported in Wolf-Hirschhorn syndrome and that the clinical findings are very different to those seen in ACH and HCH suggests that ACH and HCH may not occur due to dosage effect, but rather to the negative effect of the mutant gene. These findings suggest the possibility of prenatal diagnosis.

Judith G. Hall, MD

## Phenotype Specific RET Oncogene Mutations and Multiple Endocrine Neoplasia Syndromes

Multiple endocrine neoplasia (MEN) syndromes constitute a family of disorders characterized by neoplasias of 2 or more endocrine tissues. Medullary thyroid carcinoma (MTC) is a tumor of the thyroid C cells that can occur sporadically or as part of the inherited cancer syndromes MEN IIA, MEN IIB, and familial MTC (FMTC).

Individuals with MEN IIA are predisposed to C-cell hyperplasia or MTC, pheochromocytoma, and hyperparathyroidism. Individuals with MEN IIB are predisposed to mucosal neuromas and marfanoid habits.

The loci for MEN IIA, MEN IIB, and FMTC have been mapped to an interval on chromosome 10q11.2. The RET proto-oncogene is also located in this region. The RET proto-oncogene is a receptor tyrosine kinase gene expressed in MTC and pheochromocytoma and in normal thyroid and adrenal tissue.

Mulligan et al reviewed 118 unrelated families with inherited MTC for mutations of the RET proto-oncogene. They found mutations in 1 of the 5 cysteines of the proto-oncogene in 97% of patients with MEN IIA and in 86% of the patients with FMTC, but not in the MEN IIB patients. Eighty-four percent of the MEN IIA mutations affected codon 634, and patients with a Cys634 to

Arg substitution had a greater risk of developing parathyroid tumors than those with other codon 634 mutations.

They concluded that the precise location of the mutation corresponds with the clinical phenotype and that mutations in the 634 codon may be predictive in families predisposed to adrenal or parathyroid disease. The basis of the tissue specificity of these RET mutations is unclear, but the authors suggested the possibility of tissue-specific differences in RET expression or in RET protein interactions.

Mulligan LM, et al. *Nat Genet* 1994;6:70-75.

**Editor's comment:** It is not absolutely clear that there is just 1 mutation for MEN. The identification of a mutation for phenotype-specific RET mutations is important for early screening in individuals known to be at risk. Prenatal screening for the RET mutation, however, may prove to be controversial and cause serious ethical and moral dilemmas, since the decision to terminate a pregnancy is always difficult. This is especially true in adult-onset diseases.

Judith G. Hall, MD

## A Single Amino Acid Substitution in the Exoplasmic Domain of the Human Growth Hormone (GH) Receptor Confers Familial GH Resistance (Laron Syndrome) With Positive GH-Binding Activity by Abolishing Receptor Homodimerization

The absence of detectable growth hormone-binding protein (GHBP) was believed for some time to be a constant feature of the Laron syndrome. However, Aguirre et al (*Acad Sci Paris* 1990;311:315-319) and Buchanan et al (*Clin Endocrinol* 1991;35:179-185) described patients with classic Laron dwarfism except for the presence of high-affinity serum GHBP activity. Duquesnoy et al report in this article the following: (1) In 2 unrelated families, the same GH receptor (GHR) mutation was identified as the mutation resulting in the substitution of a highly conserved aspartate residue by histidine at position 152 of the exoplasmic domain. (2) The genetic analysis was consistent with a founder effect, ie, common origin, for this mutation. (3) The GHR mutant retains GH-binding capability and is correctly expressed at the plasma membrane. (4) The mutant GHR has lost reactivity of 1 mAb epitope, which is supposed to belong to the region where both receptor molecules contact each other, suggesting that the D152H substitution interferes

with the dimerization process and GHR activity. They conclude that these in vitro data, along with the phenotype observed in vivo in the proband patients, provide further support for the 3-dimensional model of the exoplasmic domain of GHR that has been produced in *Escherichia coli*.

Duquesnoy P, et al. *EMBO J* 1994;13:1386-1395.

**Editor's comment:** Geneticists and genetically oriented pediatricians will find this article to be of much technical and clinical interest. The authors apply multiple refined techniques to demonstrate and elucidate the conclusions reported. The original article is lengthy by necessity, but worth reading page by page for many scientific and clinical reasons. This journal is available in many university libraries.

Robert M. Blizzard, MD

## Growth After Renal Transplantation in Prepubertal Children: Impact of Various Treatment Modalities

The authors retrospectively evaluated growth in 47 prepubertal boys and 23 prepubertal girls following renal transplantation. The data were analyzed with respect to several variables, including initial growth retardation, type of immunosuppressive therapy (azathioprine versus cyclosporine), alternate-day versus daily prednisone, and total prednisone dose. Data were collected from the start of the first dialysis for up to 2 years after the first renal transplant. Height, weight, sexual maturation, serum creatinine, episodes and type of dialysis, and the number of renal transplants were also recorded. The primary renal disease of these children included glomerulopathies (50%), urinary tract abnormalities and/or renal hypoplasia (36%), and nephrotic syndrome (11%).

The mean height for all subjects was below the 3rd percentile at the start of the first dialysis and decreased significantly during the dialysis period. At the time of the first renal transplant, the mean height standard deviation score (SDS) for boys was -3.0 and -2.3 for girls. Following transplantation, height SDS increased by +0.3 in boys but decreased by -0.1 in girls. Catch-up growth did not occur over the next 2 years in 70% of these children. Gender, duration of initial dialysis, age at first renal transplantation, and the duration of a glomerular filtration rate (GFR) of  $<50 \text{ mL/min/1.73 m}^2$  were not associated with a change in SDS. But a significant positive association between height SDS and the percentage of time on alternate-day prednisone therapy was noted. Likewise, a positive association between the extent of urinary tract abnormalities and/or renal hypoplasia versus other types of renal disease was demonstrated. By using backward multiple regression analysis, the authors showed that percentage of time on alternate-day prednisone therapy, cumulative dose of prednisone, azathioprine versus cyclosporine, and duration of reduced GFR had a significant negative influence on height SDS 2 years after transplantation.

Hokken-Koelega A, et al. *Pediatr Res* 1994;35:367-371.

**Editor's comment:** Accurate knowledge of the possibility of spontaneous catch-up growth after kidney transplantation in children with chronic renal glomerular insufficiency is extremely important for interpreting the results of therapeutic trials with growth hormone (GH) and establishing appropriate indications. The homogeneity of the strictly prepubertal cohort followed for 2 years, the relevance of the auxologic data, and the quality of the statistical analysis make this paper valuable. One may regret that the endocrine data regarding GH secretion, insulin-like growth factor 1, and plasma binding proteins in these patients, if available, were not included in this work.

Jean-Claude Job, MD

**Second Editor's comment:** The authors summarize their data in some detail. They note that height SDS was already significantly decreased at the time dialysis was initiated and few children (30%) had catch-up growth after renal transplantation. Certainly these data suggest that alternative treatments are needed to stimulate growth in children with chronic renal disease post-transplantation. At the present time, biosynthetic GH has been approved for the treatment of growth retardation in children with chronic renal insufficiency, and trials are underway to demonstrate its effectiveness post-transplantation. Van Dop et al (*J Pediatr* 1992;120:244-250) have shown marked acceleration of growth rates (from 1.9 to 7.2 cm/y) in a group of post-transplant children treated with GH with a mean bone age of  $8.9 \pm 2.7$  years. Hopefully, studies such as these will change the outlook for children with renal transplants with regard to stature.

The readers may wish to read the next abstract dealing with growth post-transplant in adolescents treated with GH.

William L. Clarke, MD

## Growth Hormone Treatment in Growth-Retarded Adolescents After Renal Transplant

Administration of recombinant human growth hormone (rhGH) ( $10.8$  or  $21.6 \text{ mg/m}^2/\text{wk}$  in divided daily doses) to slowly growing, short adolescents with renal failure who had been recipients of a renal transplant for more than 1 year increased growth rate approximately 4-fold during the first year of treatment. During 2 years of therapy, the height increment of 15.7 cm realized by the rhGH-treated patients was 10 cm greater than that achieved by historical control subjects matched for chronologic age, sex, pubertal stage, time after renal transplantation, and immunosuppressive regimen. Bone age increased 0.8 years for each year of therapy with rhGH. The linear growth response was not related to the dose of rhGH administered, the pretreatment growth rate, or alternate-day as compared with daily prednisone administration ( $0.10$  to  $0.25 \text{ mg/kg/d}$ ), but was greater in subjects who were in early rather than late stages of adolescence at the initiation of therapy. Administration of rhGH had no adverse effect on glomerular filtration rate or effective renal plasma flow on the entire group beyond that experienced by the control subjects. However, there was an increased incidence of deteriorating renal function in patients receiving

alternate-day (6/11) rather than daily (1/7) prednisone therapy with rhGH, the reason for which was not clear. Serum concentrations of insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-3 increased. IGFBP-1 did not change, and IGF-2 declined during administration of rhGH. The investigators conclude that rhGH increases growth rates in adolescent recipients of renal transplants and may increase final height without untoward effects on graft function, particularly in patients receiving daily prednisone therapy.

Hokken-Koelega ACS, et al. *Lancet* 1994;343:1313-1317.

**Editor's comment:** GH has long-term stimulatory effects on the growth of children with chronic renal insufficiency and has received regulatory approval for use in these patients.<sup>1</sup> The present report indicates that it may also be useful in adolescents after renal transplantation. Hokken-Koelega et al (see previous abstract) also studied the growth of 70 prepubertal children with chronic renal failure during the first 2 years after renal transplantation and reported that in 70% the growth rate

did not accelerate after surgery. Factors that impacted adversely on growth after renal transplantation were daily prednisone therapy, a high cumulative dose of prednisone, use of azathioprine rather than cyclosporine as an immunosuppressive, and a glomerular filtration rate  $<50 \text{ mL/min/1.73 m}^2$ . However, as noted above, alternate-day prednisone administration together with rhGH was associated with an increased incidence

of declining renal function, implying that a daily dose of prednisone may be preferable when the 2 agents are administered concurrently.

Allen W. Root, MD

<sup>1</sup>Fine RN, et al. *J Pediatr Endocrinol* 1994;7:1-12.

## Diabetes Insipidus With Impaired Osmotic Regulation in Septo-optic Dysplasia and Agenesis of the Corpus Callosum

The authors reviewed the histories and presentations of 24 children with septo-optic dysplasia (SOD) and/or agenesis of the corpus callosum; 8 had the complete form of the syndrome (optic nerve hypoplasia, abnormal septum pellucidum, and pituitary deficiency); 21 had the incomplete form, having only 2 of the triad; and 3 had isolated agenesis of the corpus callosum with pituitary deficiency. Seven were completely blind; 8 had partial but significant visual impairment; although not stated in the article, the remaining 9 were presumably able to see without significant visual impairment. Thirteen were moderately or severely developmentally delayed. Twenty had growth hormone deficiency; 15 were documented to have corticotropin deficiency; and 10 of 14 were demonstrated to be luteinizing hormone/follicle-stimulating hormone deficient when tested with gonadotropin hormone-releasing hormone.

Particular attention in the article was given to the 9 patients with diabetes insipidus (DI). These 9 had a high incidence of mental retardation (7), blindness (7), and 1 or more episodes of hypoglycemia in infancy (4). Three presented with severe hyponatremia in the first 2 months of life, and a fourth before 1 year of age. Five manifested an impaired sense of thirst in the presence of hypernatremia. Treatment, even with antidiuretic hormone, was difficult in these 5. Five had 1 or more admissions

with seizures and impairment of consciousness associated with mild hyponatremia while on antidiuretic hormone therapy.

There was little correlation between the clinical features and the structural central nervous system lesions. Seventy-eight percent of the patients with DI had incomplete forms of SOD.

Masera M, et al. *Arch Dis Child* 1994;70:51-53.

**Editor's comment:** The authors make a contribution to our knowledge of SOD by reporting 21 patients seen with the entity over 21 years at the Institute of Child Health in London. The incidence of seeing 1 new patient per year in a large center reflects the fact that this entity is not common, but it is certainly one we all have been or will be exposed to. The data presented here permit us to make better prognoses and to provide better care for patients with the syndrome. This is a treacherous disease — particularly in those with DI and/or blindness. It may be worth mentioning that normal, delayed, and precocious sexual development occur in SOD patients in approximately equal numbers. (Hanna CE, et al. *AJDC* 1989;143:186-189).

Robert M. Blizzard, MD

## Changes in Body Composition of Children With Chronic Renal Failure During Growth Hormone Treatment

The success of growth hormone (GH) in increasing growth velocity (GV) in patients with chronic renal failure (CRF) has been reported previously. The present study was designed to investigate the changes in body composition during the first year of GH treatment in 8 CRF patients, 4.5 to 12.5 years of age, who had normal GH release to pharmacologic stimuli. The dose of GH was  $0.125 \text{ IU/kg/d}$ , which is the approximate equivalent of  $0.30$  to  $0.35 \text{ mg/kg/wk}$ .

Although the mean ( $\pm$  standard deviation [SD]) height increased from  $108.3 \pm 12.2 \text{ cm}$  to  $114.6 \pm 12.7 \text{ cm}$ , the mean GV increased from  $4.0 \pm 0.7 \text{ cm/y}$  to  $6.3 \pm 1.1 \text{ cm/y}$ , and the mean weight increased from  $20.1 \pm 5.8 \text{ kg}$  to  $22.9 \pm 5.9 \text{ kg}$ , data pertaining to bone mineral density (measured by dual photon absorptiometry), fat body mass, and lean body mass did not change. An unexplained but intriguing finding was a distinct fall in total body potassium.

Vaisman N, et al. *Pediatr Nephrol* 1994;8:201-204.

the basis of increasing GV. We now need to learn about its effect on body composition. These authors have made a start in this respect.

Robert M. Blizzard, MD

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**Editor's comment:** More studies regarding the effect of GH on the body composition of patients with CRF are needed. Food and Drug Administration approval has been given for its use on



## Idiopathic Prepubertal Short Stature Is Associated With Low Body Mass Index

Body mass index (BMI) was considered in 79 prepubertal children, 46 boys and 33 girls aged 3 to 12 years, followed for short stature below -1.4 standard deviations (SD). According to the results of BMI calculation compared with normal BMI values, the children were put into 2 groups: 1 below the mean for age ( $n = 53$ , mean BMI = -0.9 SD) and 1 above ( $n = 26$ , mean BMI = +0.6 SD). Age and target height of the 2 groups were not significantly different. Height and annual growth velocity were significantly less in the low BMI group, and bone age was more delayed. Growth hormone response to stimulation tests was normal in all the patients, and not significantly different between the 2 groups. In contrast, insulin-like growth factor 1 (IGF-1), measured in the plasma without extraction by a nonequilibrium technique, was very significantly lower in the low BMI group than in the high BMI group. Significant positive correlations were found between BMI and height, growth velocity, and plasma IGF-1.

The authors concluded that children with idiopathic short stature are leaner than the normal population, and that an inadequate or insufficient nutritional intake might be a contributing factor.

Thibault H, et al. *Horm Res* 1993;40:136-140.

**Editor's comment:** Much work had been done in the past to look for relationships between nutrition and growth, and many studies had found various quantitative and/or qualitative nutritional deficiencies associated with individual cases of height insufficiency. What is of interest in this study are the large number of children included; the use of BMI rather than less sensitive clinical indexes of nutrition; the positive correlation of BMI with annual growth velocity; and the contrast between the similar growth hormone response to stimulation in all children and the lower levels of plasma IGF-1 documented in the lean

children compared with the others. It is now well established that IGF-1 levels relate more to nutritional than to hormonal conditions. However, it is regrettable that certain important points, such as the psychosocial situation and the level of physical activity, have not been mentioned as having been investigated.

The practical consequence should be to carefully consider feeding habits and simple but relevant clinical and laboratory indexes of nutrition such as BMI and IGF-1 in so-called constitutionally short children. But this does not mean that any kind of nutritional supplementation would necessarily improve the growth of such children.

Jean-Claude Job, MD

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# GROWTH

## Genetics & Hormones

Vol. 11 No. 1

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### Osteochondrodysplasias With Mild Clinical Manifestations: A Guide for Endocrinologists and Others

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Increasingly, individuals with various osteochondrodysplasias are referred for endocrinologic assessment for consideration of growth hormone (GH) therapy. Experimental protocols for such treatment have become more common,<sup>1-3</sup> and thus the involvement of endocrinologists is more complicated than simply recognizing that a child has a chondrodystrophy in order to exclude him or her from further investigation or treatment. Nonetheless, whether hormonal treatment is considered appropriate,<sup>4</sup> rational intervention for genetic counseling and therapy requires accurate diagnosis.

Osteochondrodysplasias are inherited disorders of cartilage and bone, many of which result in short stature secondary to decreased growth of long bones and/or the spine. Hundreds of specific constitutional disorders of bone are recognized.<sup>5</sup> Of these, this review will cover only those disorders with short stature as the primary feature and subtle external features. It is these disorders for which the initial referral may be to a pediatric endocrinologist instead of a geneticist or other specialist with

expertise in the diagnosis and/or care of individuals with skeletal dysplasias. Those disorders to be considered here include achondroplasia, hypochondroplasia, pseudoachondroplasia, multiple epiphyseal dysplasia, spondyloepiphyseal dysplasia tarda, and certain mild metaphyseal dysplasias. Clinical characteristics that should lead one to suspect an osteochondrodysplasia and temporal points of referral, defined as when contact with an endocrinologist is most likely, will be pointed out and are compared in Table 1 (page 2). More detailed descriptions of these and other bone dysplasias are found elsewhere.<sup>6-9</sup>

#### ACHONDROPLASIA

Achondroplasia<sup>10-11</sup> was a general term applied to many forms of short-limbed dwarfism prior to the recognition that vast clinical heterogeneity of the osteochondrodysplasias occurs. It is now recognized that achondroplasia is a single, relatively homogeneous entity. Most often diagnosed in the newborn period, achondroplasia also is frequently diagnosed by prenatal ultrasonographic assessment. It is a useful paradigm with which to compare other, more subtle bone dysplasias.

Diminished linear growth is present from birth.<sup>12</sup> Therefore, diagnostic referral is most likely to occur in the first months of life. Short stature is disproportionate because of shortening of the limbs, particularly the rhizomelic or proximal segments. **Such disproportion and dyssynchronous growth are defining characteristics of most skeletal dysplasias.** In addition, infants and children with achondroplasia have skin redundancy of the rhizomelic segments (Figure 1, page 2), macrocephaly, midfacial hypoplasia (Figure 2, page 3), and a variety of other distinctive features, including hypotonia, which may arise secondary to dyssynchronous growth of the

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Table 1  
Key Clinical Features of Selected Osteochondrodysplasias

|                                    | <u>Achondroplasia</u>            | <u>Hypochondroplasia</u> | <u>Pseudo-achondroplasia</u>                | <u>Multiple Epiphyseal Dysplasia</u> | <u>Spondylo-epiphyseal Dysplasia Tarda</u> | <u>Metaphyseal Dysplasias</u> |
|------------------------------------|----------------------------------|--------------------------|---|--------------------------------------|--|-------------------------------|
| Short at Birth                     | Yes                              | Usually not              | No  | No                                   | No   | Usually not                   |
| Onset of Decreased Growth Velocity | Infancy                          | Early childhood          | Early childhood                             | Late childhood                       | Preadolescence                             | Early childhood               |
| Short Limbs                        | Yes; rhizomelic                  | Yes; rhizomelic          | Yes; later in onset                         | Mild                                 | No   | Subtle to marked              |
| Skin Redundancy                    | Yes                              | Variable                 | No  | No                                   | No   | No                            |
| Macrocephaly                       | Yes                              | Sometimes                | No  | No                                   | No   | No                            |
| Joint Hypermobility                | Yes; particularly hips and knees | Mild or absent           | Yes; particularly wrists and hands          | No                                   | No   | Variable                      |
| Upper:Lower Segment Ratio          | ↑                                | ↑                        | ↑   | Normal                               | ↓  | Variable                      |
| Inheritance                        | Autosomal dominant               | Autosomal dominant       | Autosomal dominant<br>(↑ gonadal mosaicism) | Autosomal dominant<br>(usually)      | X-linked recessive                         | Various                       |

foramen magnum and the spinal cord,<sup>13</sup> and joint hypermobility, another common feature of osteochondrodysplasias, which may be helpful in distinguishing such disorders from abnormalities of linear growth not intrinsically affecting cartilage. Although achondroplasia is considered easy to diagnose, severity of manifestations may not be obvious, as is often assumed. Two young children with relatively mild, although unequivocal, manifestations, whose features might not be readily recognized by casual observation, are pictured in Figure 1.

**As with all osteochondrodysplasias, specific diagnosis is ultimately dependent upon radiographic assessment.** While a complete skeletal survey is needed for definitive diagnosis in this and other bone dysplasias, a limited radiographic assessment will allow for recognition that a bone dysplasia exists. For this purpose, an anteroposterior (AP) view of the hand and wrist, an AP and lateral view of the thoracolumbar spine, an AP view of the pelvis and hips, and an AP view of 1 knee will suffice.

The importance of timely diagnosis is exemplified by the small but real risk of sudden unexpected death or profound neurologic sequelae occurring secondary to cord compression at the craniocervical junction. These risks can be avoided or minimized by appropriate anticipatory evaluation and/or with surgical intervention, which is desirable in a small proportion of babies with achondroplasia.<sup>14-17</sup>

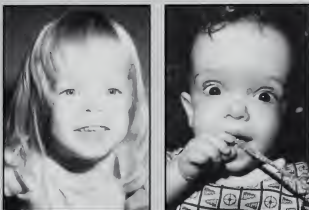
## HYPOCHONDROPLASIA

Hypochondroplasia<sup>18</sup> is another rhizomelic dwarfing disorder. Perhaps more than with any other osteochondrodysplasia, individuals with hypochondroplasia may be misdiagnosed as having familial short

Figure 1  
Achondroplasia: Two Children With Relatively Mild Phenotypic Features



**Figure 2**  
**Achondroplasia: Macrocephaly, Craniofacial Disproportion, and Midface Hypoplasia Are Always Present But Quite Variable in Severity**



stature, since the short stature often is relatively mild and body disproportion frequently is less obvious than in achondroplasia. Macrocephaly is not uniformly present and joint characteristics are not prominent (Figure 3).

Individuals with hypochondroplasia often have birth weights and lengths within the normal range, in contrast to achondroplastic infants. This and the subtlety of their disproportionate growth mean that the growth disorder is more likely to be recognized at 2 to 4 years of age. Recognition is further compromised by the considerable variability of growth abnormalities, with adult stature ranging from as little as 125 cm to as much as 160 cm. Indeed, in mildly affected individuals, clinical and radiographic features may not be clearly distinguished from variations of normal.

### **PSEUDOACHONDROPLASIA**

Like achondroplasia and hypochondroplasia, pseudoachondroplasia (or spondyloepiphyseal dysplasia of the pseudoachondroplastic type) is a single gene, dominant condition.<sup>19,20</sup> It is unusual among the dwarfing osteochondrodysplasias because clinical and radiographic manifestations may be delayed and often are not evident until after the first year of life. Early growth remains within the normal range and disproportionate growth is not immediately recognizable. Indeed, **typical growth in an individual with pseudoachondroplasia<sup>21</sup> mimics the growth failure that may be seen, for example, in postnatal endocrinologic disturbances** rather than the growth characteristics usually associated with intrinsic abnormalities of bone. It also serves to emphasize that **abnormal craniofacial characteristics are unreliable as indicators of the presence of an osteochondrodysplasia.** Indeed,

**Figure 3**  
**Hypochondroplasia: Rhizomelic, Disproportionate Shortening May Not Be Evident Without Complete Clinical Assessment**



people with pseudoachondroplasia have normal facial features and no craniofacial disproportion (Figure 4, page 4).

Nonetheless, certain clues should lead one to suspect this diagnosis early in life, including unusual gait (secondary to hip involvement); malalignment of the legs; and joint hypermobility, particularly of the hands and the wrists. Such features reflect general cartilaginous involvement. In a variety of conditions, clinical features of cartilaginous abnormality are important clues to the presence of an osteochondrodysplasia.

### **MULTIPLE EPIPHYSEAL DYSPLASIA**

Individuals with multiple epiphyseal dysplasia have often only very subtle growth disturbances.<sup>22,23</sup> Indeed, abnormalities of growth may remain unrecognized until late childhood or early adolescence. Body habitus is virtually normal and the face is unaffected. Most individuals will present because of either slowing growth rate or progressive joint involvement with pain, stiffness, and/or abnormality of gait. This entity should be considered when constitutional delay of growth is suspected. The epiphyses are the parts of bone that are affected, and X-ray films of these often raise the index of suspicion. Epiphyseal irregularity may be subtle and vary from site to site. The primary radiographic manifestation may be delayed epiphyseal development.



Figure 4

**Pseudoachondroplasia: These 3 Girls Exemplify the Completely Nondysmorphic Facial Features of This Osteochondrodysplasia**



This delay can confuse the diagnosis, particularly if only a hand and wrist film is obtained, and underscores the point that **delayed bone age is not necessarily the result of endocrinologic processes but can equally be secondary to an osteochondrodysplasia.**

#### **SPONDYLOEPIPHYSEAL DYSPLASIA TARDA**

Spondyloepiphyseal dysplasia (SED) tarda, unlike SED congenita, which is a severe disorder easily recognized in the newborn period, is usually not recognized until the immediate preadolescent period.<sup>24</sup> Even at that age, hip and back symptoms more often than small stature result in medical referral; however, the modest apparent slowing of growth may precipitate referral to an endocrinologist. The growth disturbance results almost exclusively from vertebral involvement, which causes a short trunk. **Additional clinical measurements, such as span:height and upper:lower segment ratios, are helpful** in raising the index of suspicion that a short-statured individual may have such a short trunk osteochondrodysplasia. Similar measurements are seen in boys and girls with congenital anomalies of the vertebrae. Since SED tarda is most often an X-linked recessive disorder, only males are affected by the classic form of this disorder.

#### **In Future Issues**

##### **The Neuroendocrine Landmarks of Puberty**

by Jean-Pierre Bourguignon, MD, PhD

##### **Imaging in Diagnosing Hypopituitarism**

by Raphael Rappaport, MD

#### **METAPHYSEAL DYSPLASIAS**

A variety of osteochondrodysplasias affect principally the metaphyses of the long bones. They range from quite subtle anomalies of growth, eg, Schmid type of metaphyseal dysplasia, to potentially lethal conditions, eg, cartilage-hair hypoplasia. The Schmid type<sup>25</sup> is the most common and best delineated form of metaphyseal dysplasia. Usually, individuals with this disorder are not recognized as being in any way abnormal until around 2 years of age. Often leg bowing and/or joint prominence are the first signs, rather than slowing growth. Nonetheless, slowing of growth ultimately results in adult heights ranging from about 130 cm to 160 cm.

#### **SUMMARY AND RECOMMENDATIONS**

A series of concepts and caveats that might be helpful to clinicians have been generated (Table 1). Suspicion that an osteochondrodysplasia may be present should be raised when:

- Disproportionate and dyssynchronous growth are present, since these are defining characteristics of most skeletal dysplasias. Such disproportion may result in a short trunk as in SED tarda, in primarily short limbs as in hypochondroplasia, or in disproportionate limb segments as in achondroplasia. Additional segment measurements and additional calculations, such as span and sitting height measurements and calculations of the upper:lower segment ratio, are important in clinically differentiating the milder osteochondrodysplasias. Growth patterns themselves may *not* allow differentiation of endocrine from osteochondrodysplastic causes since some bone dysplasias show normal or near-normal growth early in life and subsequent evidence for growth failure, eg, pseudoachondroplasia, SED tarda, metaphyseal dysplasias.

Figure 5  
Hand and Wrist X-ray Films



**A)** 15-month-old achondroplastic female. Note minimal metacarpal deformity, short proximal and middle phalanges, and brachydactyly.

**B)** 48-month-old pseudoachondroplastic female. Note marked delay in carpal development, shortening of all long bones, and widened and unusually configured metaphyses.

- Craniofacial disproportion is recognized, as occurs in achondroplasia. Nonetheless, abnormal craniofacial characteristics are not uniformly present in osteochondrodysplasias, eg, pseudoachondroplasia and metaphyseal dysplasias.
- Features suggesting generalized cartilaginous abnormality are found. Often this will result in such joint characteristics as ligamentous laxity, as in pseudoachondroplasia; joint hypermobility, as in achondroplasia; joint pain, as in multiple epiphyseal dysplasia; or joint prominence, as in metaphyseal dysplasias.
- Specific radiographic features are discovered. Specific diagnoses of all osteochondrodysplasias ultimately rest almost exclusively with radiographic identification. Nonetheless, as with clinical features, radiographic manifestations may not be evident from birth, eg, pseudoachondroplasia. Hand and wrist films usually are obtained in children referred for endocrinologic assessment of small stature. Since they often will demonstrate specific features of a bone growth disorder, these should always be reviewed not only for establishing bone age but also for discerning subtle and not so subtle features of osteochondrodysplasias (Figure 5). It should be stressed that when radiographic assessment is limited to the hand and wrist, some osteochondrodysplasias may show only delayed bone age, eg, multiple epiphyseal dysplasia. A delay in skeletal maturation should not be misinterpreted as unequivocally reflecting an endocrinologic process. X-ray films of other joint sites may reveal that chondrodystrophy is

responsible for the short stature. While a skeletal survey of all bones and joints is needed for definitive diagnosis of specific osteochondrodysplasias, the endocrinologist's role might be viewed as more limited: determining that some osteochondrodysplasia is present in order to initiate appropriate referral. For this purpose, an AP view of the hand and wrist, an AP and lateral view of the thoracolumbar spine, an AP view of the pelvis and hips, and an AP view of 1 knee will suffice.

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## Letter From the Editor

### To Our Readers:

Welcome to the **10th birthday of *GROWTH, Genetics, & Hormones (GGH)***! Now that 10 years of publication have been completed, a historical accounting of our goals and accomplishments is in order. On this occasion, an expression of sincere gratitude and appreciation from us, the Editorial Board, and from you, the 12,000-plus readers, is extended to Genentech, Inc., which has been particularly insightful and/or responsive to the need for this newsletter. *GGH* has been generously supported by Genentech, Inc. through an educational grant, and published by an Editorial Board that operates totally independently from Genentech.

Historically, *GGH* was created with the vision of providing an objective and unfettered opportunity for teachers and scientists in the areas of growth, endocrinology, genetics, and nutrition. We believe that those goals continue to be met. Forty-one issues of *GGH* have been published, comprised of 91 lead articles and 313 abstracts of journal articles — each with at least 1 editorial comment; 20 reviews of important medical conferences and/or meetings; a glossary of genetic terms; a tabulation from the genome project of the chromosomal sites of most of the genes related to growth and hormones; and a significant supplement about the ethics of growth hormone use. Over 12,000 physicians, nurses, and medical libraries receive *GGH* every quarter.

Verification that the goals are being met and that the readers of *GGH* hold it in high esteem was obtained recently through replies to a reader survey, distributed with the September 1994 issue. The response to the survey overwhelmingly confirmed the goals continue to be met and the readers very much appreciate that which was created by the donor and the recipient of the educational grant. As of the first of January,

2,210 responses were received from the 12,000-plus recipients of *GGH*. By November 9, within 6 weeks of receipt of the survey, 1,290 readers had responded. Of these, more than 800 replies came from endocrinologists and geneticists, and the remainder were from nephrologists, practicing and academic pediatricians, nurses, and others. Eighty-four percent of the 1,290 placed *GGH* as a significant priority on their reading list each quarter, and 50% of these rated it as a *very high* or *high priority*. As of November 9, 64% of respondents stated that they publish, and 20% of these cite *GGH* in their references — a phenomenal reflection of the respect that *GGH* has among contributors to the literature. The current Editorial Board members — Dr. William Clarke, Jr; Dr. Judith Hall; Dr. William Horton; Dr. Fima Lifshitz; Dr. Allen Root; and myself — express our thanks most sincerely to you our readers for having replied promptly. Again we thank Genentech Corporation for the opportunity for *GGH* to have met, and to continue to meet, the goals established 10 years ago. The Editorial Board also recognizes and thanks the distinguished previous members of the Board who contributed in a laudatory manner. These include Dr. Jurgen Bierich of Germany (now deceased); Dr. Alan Rogol and Dr. David Rimoin of the United States; Dr. James Tanner of England; and Dr. Jean-Claude Job of France.

We conclude by noting that *GGH* is requested and read by individuals of many disciplines in many countries (more than 500 readers in Europe), and is made available in many medical school and hospital libraries in the western hemisphere.

HAPPY BIRTHDAY, *GGH* !!!

Thank you,

Robert M. Blizzard, MD  
Editor-in-Chief  
For the Editorial Board

## Abstracts From the Literature

### Catch-up Growth After Glucocorticoid Excess: A Mechanism Intrinsic to the Growth Plate

In an attempt to better understand catch-up growth and determine whether the mechanism governing such growth resides in the central nervous system or in the growth plate, the authors devised a series of novel experiments using rabbits. Stainless-steel needles (27 gauge) were inserted through the proximal tibial growth plate and attached to an osmotic pump, which administered dexamethasone continuously for 4 weeks. Similar incisions and needle placements were made on the contralateral tibia. Three metal pins were placed: 1 in the bony

metaphysis 2 to 3 mm distal to the growth plate; 1 that was 5 mm distal to the first pin; and 1 that was 5 mm proximal to the first pin. Radiographs of the tibiae were examined under a dissecting microscope and the distances between the proximal and middle pin, the middle pin and the distal pin, and the distal pin and the distal tibial epiphysis were measured. All measurements were done in duplicate. The distance between the middle and distal pins did not span a growth plate and was therefore expected to remain constant. Measurement of

this distance was used as a determinant of the variability of measurement.

After 4 weeks of dexamethasone administration, the treated proximal tibia grew  $37\% \pm 8\%$  less than the contralateral control proximal tibia. Growth inhibition resolved once dexamethasone was stopped, and the growth rates on the 2 sides were equalized by week 6 (2 weeks after the end of the infusion). The observed growth velocity in the treated growth plate surpassed that of the contralateral growth plate and corrected  $54\% \pm 13\%$  of the growth deficit. The distal tibial growth plates did not change during the period of catch-up growth, ie, there was no significant difference in cumulative growth at the distal growth plate. In addition, femoral length showed no significant discrepancies at the end of the experiment, suggesting that the increased growth was observed only in the growth plate in which growth inhibition had occurred.

Baron J, et al. *Endocrinology* 1994;135:1367-1371.

**Editor's comment:** Although it is not usually the policy of GGH to abstract animal studies, this particular study should be of sufficient interest to be included here. These data suggest that catch-up growth is intrinsic to the growth plate and not the result of a systemic hormonal mechanism. The authors also have demonstrated that catch-up growth may not be 100% complete. They suggest that this may be due to the number of stem cells or chondrocytes present in the affected growth plate.

This study is important for what it tells us about the mechanisms of growth retardation and catch-up growth. In humans, as in the animal model, catch-up growth is presumably intrinsic to the growth plate and the removal of the growth inhibitor rather than a response to changes in hormonal secretion. It would be interesting to see similar studies performed in models of other disorders associated with decreased growth velocity and subsequent catch-up growth.

William L. Clarke, MD

## Sex, SOX, and the Skeleton

It is well established that the *SRY* (sex-determining region Y) gene is critical for testicular development in mammals. Since its protein product contains a DNA-binding motif that is found in many transcription factors, it is assumed that it functions to regulate the expression of relevant genes. Recently, a family of structurally related genes, the *SRY*-related genes, so-called *SOX* genes, has been identified. In the paper by Foster and colleagues, mutations of the *SOX9* gene were discovered to cause campomelic dysplasia (CD) with sex reversal. This disorder has always been an enigma because it was hard to understand the connection between abnormal bone growth and sex reversal.

Previous reports of chromosomal rearrangements in CD with sex reversal had localized the gene(s) responsible to the long arm of chromosome 17, specifically 17q24.1-q25.1. Foster et al carried out high-resolution mapping of this region to position a translocation breakpoint in one patient with this disorder close to the *SOX9* locus, which had been previously mapped. They next characterized the structure of the *SOX9* gene, showing that the predicted polypeptide would be 509 amino acids in length and contain the DNA-binding motif. Fluorescence in situ hybridization (FISH) confirmed its localization to chromosome 17q24; and northern blot analysis demonstrated the presence of mRNA transcripts in adult testes as well as in adult heart and fetal brain.

Next, they utilized single-strand confirmation polymorphism (SSCP) analysis to find 6 mutations in DNA from 9 patients with CD. Three would be expected to abolish gene function by shifting the reading frame of the mRNA transcripts so that translation would be prematurely terminated. In 2 cases, about one third of the protein would be lost, and in 1 case about 60% would be lost. The missing part contains the putative activation domain of the protein. Another mutation predicted that splicing of *SOX9* transcripts would be altered, and 2 others predicted that amino acids would be substituted in the protein. The mutations were not present in parental DNA, indicating that they arose de novo. Also, they were not found in surveys of normal individuals. Importantly, the mutations were heterozygous, denoting that the disorder behaves in an autosomal dominant manner.

The authors speculated about how mutations in the *SOX9* gene might cause CD with sex reversal. Since the mutations were predicted to destroy the function of the gene product and since the patients were found to have both a mutant and a normal *SOX9* allele, they proposed that the mutations operate through a loss of function mechanism, ie, haploinsufficiency, rather than through gain of function or dominant negative mechanisms. They noted that dosage sensitivity is a feature of many regulatory genes and has been reported for several sex determination systems. They further pointed out that the sex-determining function of *SRY* is believed to be expressed in pre-Sertoli cells in the developing gonadal ridge. They raised the possibility that interactions between *SRY* and *SOX9* gene products may be necessary for normal testicular development, perhaps by influencing the behavior of mesenchymal cells in the ridge.

Wright and coworkers shed light on the situation by demonstrating that the mouse *SOX9* gene is expressed abundantly in regions of the developing skeleton just before cartilage forms. The normal developmental sequence is that mesenchymal cells in areas destined to become skeleton "condense" shortly after which they begin to generate cartilaginous molecules that assemble into templates of future bones. The observations of Wright et al suggest that *SOX9* expression activates the chondrocytic developmental program and, as such, acts as a master gene for chondrogenesis, thereby performing a role somewhat analogous to MyoD in myogenesis.

The authors also provided strong linkage evidence that a mouse skeletal mutant called Tailshort (Ts) maps to the *SOX9* locus and may be the mouse equivalent of CD in humans. Interestingly, Ts mice do not exhibit abnormalities of sexual development.

Foster JW, et al. *Nature* 1994;372:525-530.

Wright E, et al. *Nat Genet* 1995;9:15-20.

**Editor's comment:** Envisioning the relationship between defective testicular and skeletal development in CD has always been difficult. These papers provide considerable insight into this matter. Given that its expression coincides spatially and temporally with early skeletal development, one can postulate



many ways in which SOX9 mutations could disrupt skeletogenesis. Indeed, it is surprising that the clinical features are not more severe than are typically found. The connection between SOX9 mutations and defective testicular development is less obvious. However, the similar structure of SOX9 and SRY provides a good basis for speculation. The suggestion of Foster et al that the transcription factor products of the 2 genes may need to interact to carry out their normal function during testicular differentiation seems tenable and is supported by another recent paper by Wagner et al, which demonstrated SOX9

expression in the fetal testes. Although unproven, the idea that SOX9 is a master gene for chondrocytic differentiation is very enticing, since many researchers have been looking very hard for such a gene with little success. It will be very interesting to see how this story plays out.

William A. Horton, MD

Wagner T, et al. *Cell* 1994;79:1111-1120.

## The Genes for Crouzon Craniofacial Dysostosis and Pfeiffer Syndrome Are Fibroblast Growth Factor Receptor Genes

Crouzon craniofacial dysostosis (CFD) is an autosomal dominant inherited disorder characterized by premature closure of the cranial sutures (craniosynostosis), shallow orbits, and hypoplastic maxillae. The incidence of Crouzon syndrome has been estimated to be 1/25,000. It has been associated with advanced paternal age, and at least 50% of reported cases are thought to be due to *de novo* mutations.

A recent paper by Preston et al studied 2 very large kindreds affected with CFD in which they successfully mapped the gene for CFD to the long arm of chromosome 10. They used a candidate locus approach because no consistent cytogenetic abnormalities have been found in CFD. They first tried mapping the CFD gene to chromosome 7p, because of the similarities found between CFD and Greig cephalopolysyndactyly, which has been localized to 7p13 (Brueton et al [1988a]; Brueton et al [1992]) and Saethre-Chotzen syndrome, which has also been mapped to the 7p21 region (Brueton et al [1988b]). Their initial mapping results, however, did not support linkage to chromosome 7p. Based on the evidence that mutations in developmental control genes in mice cause abnormal morphogenic phenotypes similar to CFD, they continued their search in regions known to contain developmental regulatory genes (*HOX*, *PAX*, *POU*, and zinc finger genes). Their results showed that both families had linkage to chromosome 10q25-q26.

Preston et al's mapping work was rapidly followed by a paper by Reardon et al in which they presented evidence that mutations in the fibroblast growth factor receptor 2 gene (*FGFR2*) cause Crouzon syndrome. Fibroblast growth factor (FGF) receptors act by binding activating-specific cell-surface receptors. *FGFR2* has been shown to map to human chromosome 10q25.3-26 and has 2 alternative gene products, KGFR (keratinocyte growth factor receptor) and BEK (bacterially expressed kinase). These 2 gene products have different patterns of expression in murine embryogenesis, with the BEK gene transcripts concentrated in the frontal bones, maxillae, mandibulae, and ossicles in the middle ear.

This association and the recent report of linkage of CFD to 10q25-q26 led Reardon et al to study 20 CFD cases and 89 unaffected controls by amplifying the coding sequence and splice junctions of *FGFR2*. The product was analyzed by single-stranded conformational polymorphism (SSCP) analysis. They found alterations of SSCP migration proteins in 9 of 20 CFD cases and a variety of shift bands in the other patients, indicating a heterogeneous range of mutations. Further study of these 9 cases showed that 3 had a G→A transition at nucleotide 1037 resulting in a Cys342Tyr substitution within the third

immunoglobulin domain. The other patients had a variety of different transitions in nucleotides 1036, 1030, 1073, and 1044. No SSCP variations were found in unaffected individuals.

Jabs et al analyzed the same region of the *FGFR2* gene in additional families with CFD and in a family with the related syndrome, the Jackson-Weiss syndrome. The latter syndrome has many of the cranial features of the former but differs in that they are more variable and also that patients have foot deformities, including broad great toes with medial deviation and tarsal-metatarsal coalescence, and occasionally hand malformations. In the CFD patients, they confirmed 2 of the previously described heterozygous mutations and identified 2 others, both of which would be predicted to introduce additional cysteines into the third Ig domain of the protein. In the Jackson-Weiss family they found a mutation predicted to substitute a glycine for an alanine at residue 344, only 2 amino acids from the cysteine 342 mentioned above.

Given these observations and the fact that they had recently mapped a similar autosomal dominant syndrome, the Pfeiffer syndrome, to chromosome 8p11.2-p12, where another *FGFR* gene, *FGFR1*, resides, Muenke and coworkers did the obvious. They analyzed the structure of *FGFR1* in a large family with Pfeiffer syndrome. This syndrome is characterized by premature fusion of several sutures of the skull, broad thumbs and great toes, short fingers and toes, and variable degrees of syndactyly. They began their analysis by PCR amplifying exons 3 through 7 of the *FGFR1* gene. These exons code for the second and third Ig domains of the receptor. The products were analyzed by SSCP. When an anomaly was detected in a fragment from exon 5 in affected but not in unaffected family members, they sequenced the fragment. A heterozygous single base pair change predicting a proline to arginine substitution at amino acid residue 252 was found. The same heterozygous mutation was subsequently detected in affected members from 4 other Pfeiffer syndrome families. Proline 252 is a highly conserved amino acid that is located between the second and third Ig domains. The authors acknowledge that the specific way in which the mutation causes the clinical phenotype is not known.

Preston RA, et al. *Nat Genet* 1994;7:149-153.  
Brueton J, et al. *Am J Med Genet* 1988;31:799-804.  
Brueton LA, et al. *J Med Genet* 1992;29:681-685.  
Reardon W, et al. *Nat Genet* 1994;8:98-103.  
Jabs EW, et al. *Nat Genet* 1994;8:275-279.  
Muenke M, et al. *Nat Genet* 1994;8:269-274.

**Editor's comment:** It is clear that growth factor receptors play an important role in embryologic development. The previous report of mutations in FGFR3 as a cause for achondroplasia and this Crouzon syndrome report suggest cartilage growth is influenced by certain FGFs and their receptors. Further studies will be helpful in establishing the roles of the various FGFRs and their differential expression in different tissues and body areas. It seems likely these expression patterns will be time-in-development specific as well.

Readers may wish to review an abstract in GGH Vol. 10, No. 4 entitled "Snaring the Achondroplasia and Hypochondroplasia Gene." The discussion is related because in these entities the FGFR3 and FGFR3 are involved.

Judith G. Hall, MD

**2nd Editor's comment:** These papers clearly establish an important link between FGF signaling and skeletal development both in the skull and in the distal limbs. When achondroplasia is taken into account, this link is expanded to include development of the spine and proximal limbs as well. On the basis of the disease phenotypes, it is tempting to speculate about how mutations of these genes operate and about the roles that the receptor proteins play during normal skeletal development.

However, one must be very cautious considering the extreme complexity of FGF signaling as its story unfolds. For instance, consider that there are at least 9 FGF ligand and at least 4 FGF receptor genes. Alternative splicing is known to generate

different forms of the receptor proteins. Although both ligands and receptors are thought to dimerize in order for signals to be transmitted, it is not known which ligands bind to which receptors. Moreover, it is suspected that heterodimers may form between different ligands and between different receptors. The downstream signaling pathways are not well defined. To make matters worse, extracellular matrix constituents, such as heparin sulfate, appear to influence diffusion of ligands to target cells and binding of ligands to receptors. Thus, the number and types of signals transmitted by FGFs are potentially extremely large and diverse, as are the ways in which FGF signaling might be disturbed by mutations.

Also keep in mind that virtually all of the work on the receptor mutations to date has been carried out on DNA, ie, the amino acid substitutions, and functional sequelae are predicted rather than observed. There may still be surprises as the venture extends to the protein level. Despite these cautions, the tracing of 4 human genetic disorders of skeletal development to 3 receptor genes in such a short period is a very significant accomplishment.

Finally, it is intriguing that, as with achondroplasia mutations in the FGFR3 gene, mutations of the FGFR1 and FGFR2 genes tend to cluster at particular sites. As the biology of FGF signaling becomes better understood, these observations should provide valuable clues to elucidating the molecular pathogenesis of these disorders.

William A. Horton, MD

## Long-Term GH Therapy in GHD and Non-GHD Boys: Effect on Bone Age and Pubertal Maturation

Zadik and colleagues report the effects of 4 years of therapy with recombinant human growth hormone (rhGH) (0.1 mg/kg 3 times/wk) on the growth of full-term, short, slowly growing males with substantial delay in skeletal maturation ( $>2$  standard deviations [SD] for age) and subnormal 24-hour mean integrated serum GH concentrations ( $<3.2$  ng/mL [double antibody polyclonal radioimmunoassay]). These children were subdivided into those with classic GH deficiency (peak GH secretory response to 2 provocative stimuli  $<10$  ng/mL,  $n=40$ ) and those whose peak GH responses were  $>10$  ng/mL and were said to have GH neurosecretory dysfunction ( $n=43$ ). Both groups of children grew more rapidly while receiving rhGH than in the pretreatment period. However, after completing 4 years of treatment, those with classic GH deficiency had a somewhat greater cumulative gain in height than did those with GH neurosecretory dysfunction ( $+1.6$  vs  $+1.2$  SD score), and the predicted adult height increased to a greater extent in the former group ( $+9.3$  vs  $+5.4$  cm).

Loche and coworkers treated 15 short, slowly growing, otherwise normal children (10 males) with delayed skeletal maturation and normal spontaneous and stimulated GH secretion with rhGH. Doses of 0.19 or 0.38 mg/kg/wk were administered in 4 to 7 weekly subcutaneous injections until final height (growth rate  $<2$  cm/yr and/or skeletal epiphyseal fusion) was achieved after 5 or more years of therapy. Although children receiving the larger dose of rhGH initially grew more rapidly, both groups of subjects ultimately achieved similar increments in height during therapy. Final heights did not differ significantly from pretreatment predicted adult heights, or from mean target heights (calculated from midparental heights).

Zadik Z, et al. *J Pediatr* 1994;125:189-195.

Loche S, et al. *J Pediatr* 1994;125:196-200.

**Editor's comment:** The report of Loche et al demonstrates that rhGH at the 2 dosages tested did not increase final height of normal short children beyond their predicted or target heights. However, the therapeutic programs were not uniform, and whether larger doses or more frequent administration of rhGH will increase final height is as yet unresolved. It is likely that any such effects will be much more subtle and difficult to demonstrate than the obvious growth-promoting effect of rhGH in children with the somatic and radiographic characteristics of the truly GH-deficient patient. These investigators also demonstrated that growth rate often declined after 5 years of rhGH

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administration. Thus, the report of Zadik et al, in which 4 years of observations are recorded, must still be considered preliminary until this group reports their final height data.

The diagnosis of permanent GH deficiency, particularly when it is an isolated defect, remains difficult. Adan et al<sup>1</sup> reevaluated children in whom the diagnosis of hyposomatotropism had been established and treatment with rhGH administered. They observed that compared with children with transient deficiency of GH (those in whom GH secretion at an older age was normal), patients with permanent GH deficiency were much more likely to have onset of growth failure before 5 years of age; neonatal hypoglycemia; micropenis (males); radiographic evidence of interruption of the pituitary stalk; lower plasma concentrations of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor-binding protein 3 (IGFBP-3) but not stimulated GH secretion at the point of diagnosis; and often several pituitary hormone deficiencies. Cohen and Berg<sup>2</sup> recently noted that 29/34 patients with suspected isolated deficiency of GH had normal GH secretion and/or IGF-1 concentrations when retested at an older age. Since the state of thyroid function may fluctuate in patients with autoimmune thyroid disease, depending on the biology of the autoantibody generated at any one point in the disease, the concept of a transient deficiency of

hGH secretion that requires therapy with GH and then remits spontaneously is not necessarily unique. However, there are no data supporting an autoimmune mechanism in the latter disorder, nor are there long-term follow-up reports to document the natural history of this phenomenon in adulthood. Currently, this writer has difficulty with the diagnosis of transient GH deficiency and has attributed most such diagnoses to our inability to identify the truly GH-deficient subject in the absence of findings listed by Adan et al. Marin et al<sup>3</sup> point out just how little GH may be secreted in response to provocative stimulation in children of normal stature, particularly if they are prepubertal. Even with sex hormone priming, the data of these authors indicate that a peak GH concentration of 7 ng/mL (double antibody polyclonal radioimmunoassay) is normal. Thus, the selection of a minimum normal GH value of 10 ng/mL for the diagnosis of GH deficiency is quite arbitrary and not substantiated.

Allen W. Root, MD

1. Adan L, et al. *J Clin Endocrinol Metab* 1994;78:353-358.
2. Cohen AJ, Berg L. Proceedings of the Eighth Annual Investigators Meeting of the National Cooperative Growth Study; October 27-29, 1994; Orlando, Fla.
3. Marin G, et al. *J Clin Endocrinol Metab* 1994;79:537-541.

## Longitudinal Analysis of Somatic Development in Paediatric Patients With IDDM, Part 1: Genetic Influences on Height and Weight

Holl et al evaluated height and weight in 389 insulin-dependent diabetes mellitus (IDDM) patients (188 males, 201 females) between the years of 1980 and 1992. All were treated with 2 to 4 daily injections of regular and NPH insulin. All medical care was provided by the same group of health-care professionals. Height was measured with a Harpenden stadiometer until 1989, and then with an electronic stadiometer with automatic recalibration. Bone ages were determined according to the method of Gruelich and Pyle. Families were included only if both parents were nondiabetic and available for measurements. Complete data were available for 177 pairs of parents and 186 unaffected siblings. Height, weight, and body mass index (BMI) standard deviation (SD) scores were calculated yearly. Nonparametric tests were applied.

At the onset of IDDM, the patients were significantly taller compared with normative data (SD score +0.37,  $P < 0.001$ ). During the course of the disease, the median Z score for height progressively decreased. But after 10 years, the height decrement was reversed and scores returned to above zero.

Even during the first year of diabetes, the children were heavier than the normative sample, (BMI Z score +0.26,  $P < 0.001$ ). The weight Z score increased while the height Z score decreased.

Seventy-six patients, assumed to be at adult height (chronologic age, 18 years) had a mean height SD score of +0.30, nearly identical to that of their siblings (+0.22). The Z score for weight at age 18 was +1.06 and for BMI was +1.23, with no significant differences between boys and girls. Both midparental height and midparental weight were significantly related to the respective SD scores for their diabetic children ( $r = +0.43$ ,  $P < 0.0001$  for height, and  $r = +0.23$ ,  $P < 0.002$  for weight). At the onset of diabetes, bone ages were not retarded, but they were progressively delayed during the course of diabetes.

The authors conclude that age- and sex-standardized height in diabetic children is not significantly different from the respective measurements in their unaffected siblings and that adult height is not compromised in diabetic individuals.

Holl R, et al. *Diabetologia* 1994;37:925-929.

## Part 2: Final Height Attainment in Girls and Boys With Insulin-Dependent Diabetes Mellitus

d'Annunzio et al studied 37 Italian insulin-dependent diabetes mellitus (IDDM) patients (15 males, 22 females) who, at last evaluation, had a mean age of  $20.6 \pm 3.3$  years and an average disease duration of  $11.8 \pm 3.7$  years. Patients were divided into 2 groups on the basis of the presence of pubertal development at diagnosis (20 patients prepubertal, 17 pubertal). All subjects were treated with 2 or more daily injections of a mixture of short- and long-acting insulin. Height at diagnosis and final height were assessed with a Harpenden stadiometer and height was converted to standard deviation (SD) scores. Bone age at diagnosis was determined by the method of Gruelich and Pyle. Predicted adult height was determined by the Bailey and Pinneau method. Target genetic height was assumed to be the mean

parental height plus 6.5 cm for males, and the mean parental height less 6.5 cm for females.

Height at diagnosis was variable for boys and girls. It was above the 50th percentile in 10 of 22 females, at the 50th percentile in 6, and between the 3rd and 50th in another 6. Final height in girls was above the 50th percentile in 9, at the 50th percentile in 6, and between the 3rd and 50th in 7. Final height in both males and females was higher than their target genetic height, although not significantly. No difference was observed in final height between patients diagnosed in the prepubertal or pubertal stages. In boys, height at diagnosis was above the 50th percentile in 8, at the 50th percentile in 4, and between the 25th and 50th in 3. Final height was above the 50th percentile



in 7, and between the 10th and 50th in 8. No correlation was observed between final height and glycosylated hemoglobin concentrations, early microangiopathic complications, or thyroiditis.

The authors conclude that there was no growth retardation in their patients, that final height exceeded target genetic height, and that diabetes by itself did not impair final height.

d'Annunzio G, et al. *Diabetes Res Clin Pract* 1994;24:187-193.

**Editor's comment:** These 2 papers from Germany and Italy present similar findings. The data are both welcome and reassuring. Although the literature is replete with descriptions of height at diagnosis of children with diabetes, and examples of poor growth associated with extremely poor glucose control, there have been few data regarding final height in these children. The findings, however, are probably not surprising to those who care for young adults with IDDM, for short stature is not a term frequently used to characterize the adult who has had IDDM as a child.

What both of these papers fail to clarify is how one is to interpret the array of data currently published with regard to growth parameters in the diabetic child, ie, growth velocity, integrated growth hormone concentration, pulse amplitudes and frequencies, insulin-like growth factor (IGF)-1, IGF-binding protein 3, growth hormone-binding protein, and IGF-binding protein 1. What is the clinical and pathophysiologic significance of these data if the majority of diabetic children reach or exceed their predicted final adult height? Perhaps this question is best posed to the researchers currently publishing these data. Are their patients destined to have short stature, or like the patients of Holl et al, will they eventually regain their growth potential despite early decreased linear growth velocity? Long-term studies of the patients reported in previous studies are very much needed.

William L. Clarke, MD

## Preliminary Localization of a Gene for Autosomal Dominant Hypoparathyroidism to Chromosome 3q13

In a kindred in which 7/15 members over 3 generations had mild, generally asymptomatic hypocalcemia associated with hyperphosphatemia and low or inappropriately normal concentrations of parathyroid hormone, linkage to chromosome 3q13 was established. This region of chromosome 3q is near that for the parathyroid cell  $\text{Ca}^{++}$ -sensing membrane receptor mapped to chromosome 3q2 (Brown et al and Pollak et al). The investigators suggest that a mechanism opposite to that identified in patients with familial hypocalciuric hypercalcemia, in which the sensitivity of the receptor for  $\text{Ca}^{++}$  is decreased or downregulated, is operative in the present family. This would mean that receptor sensitivity is upregulated and, therefore, lower concentrations of  $\text{Ca}^{++}$  are required to depress the secretion of parathyroid hormone.

Finegold DN, et al. *Pediatr Res* 1994;36:414-417.

Brown EM, et al. *Nature* 1993;366:575-580.

Pollak MR, et al. *Cell* 1993;75:1297-1303.

**Editor's comment:** This report illustrates yet another possible mechanism for familial hypoparathyroidism, in addition to abnormalities within the gene for parathyroid hormone itself (chromosome 1p15), which is transmitted as an autosomal recessive characteristic, and embryonic dysgenesis of the parathyroid gland, which is inherited as an X-linked recessive trait. One awaits analysis of the gene for the  $\text{Ca}^{++}$ -sensing receptor in this family and its expressed characteristics.

Allen W. Root, MD

## Growth Hormone Releasing Activity by Intranasal Administration of a Synthetic Hexapeptide (Hexarelin)

This study was designed to compare the effects of intranasal versus intravenous growth hormone (GH)-releasing peptide (the hexapeptide, His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>, known as hexarelin). Ten children with familial short stature and 2 young adults with GH deficiency were tested. The children with familial short stature had normal GH responses to clonidine and insulin, whereas the GH-deficient subjects failed to show responses to either. The GH-deficient subjects were studied after they had been off human GH for at least several years.

Each subject was given hexarelin twice within 1 week, either intravenously (IV) (1 µg/kg) or intranasally (IN) (20 µg/kg) initially. Blood samples for GH, thyrotropin (TSH), free thyroxine (T<sub>4</sub>), and triiodothyronine (T<sub>3</sub>) concentrations were obtained at 0, 15, 30, 60, 90, and 120 minutes.

There were no differences in the mean peak GH response to hexarelin administration depending on its route of administration (79.6 ± 53.1 mU/l IV versus 72.2 ± 35.5 mU/l IN). However, the peak GH concentration occurred approximately 15 to 30 minutes after IV administration, while the peak GH concentration after IN hexarelin occurred 30 to 60 minutes after administration. TSH concentrations fell significantly by 120 minutes, but remained within the normal range. This fall in plasma TSH

following hexarelin administration may be the result of partial action on the hypothalamus. There were no significant changes in plasma T<sub>4</sub> or T<sub>3</sub>. The authors conclude that this particular hexapeptide is effective as a provocative test for GH secretion.

Laron Z, et al. *Clin Endocrinol* 1994;41:539-541.

**Editor's comment:** This is a short but important report. GH-releasing peptides (GHRPs) are now being studied for their activity in human subjects. Although the authors of this report suggest that IN hexapeptide would be a good provocative test for GH secretion, the obvious inference is that this compound or a similar synthesized GHRP may someday be useful in treating individuals with defects in GH secretion. Demonstrating that the IN route of administration induces similar GH release as does IV administration strengthens the practicality of these compounds for use in children with GHRP-treatable disorders. The effects of chronic GHRP administration on thyroid function, however, would need to be carefully monitored based on the TSH-lowering effects of hexarelin in the present study.

William L. Clarke, MD



## Estrogen Resistance Caused by a Mutation in the Estrogen-Receptor Gene in a Man

The authors describe a fully virilized, 28-year-old adult male with absence of a functional estrogen receptor. This disorder was characterized by: (a) tall stature and continuous linear growth throughout adult life (during childhood height pursued the National Center for Health Statistics 75th percentile while at the age of the report height was 204 cm, or +4.2 standard deviations (SD)); (b) unfused epiphyseal growth plates of the long bones and a wrist bone age of 15 years; (c) osteopenia; (d) increased serum concentrations of luteinizing hormone and follicle-stimulating hormone, and estradiol and estrone with normal levels of testosterone; (e) normal sperm number but decreased sperm viability; (f) mild glucose intolerance, hyperinsulinism and acanthosis nigricans; and (g) lack of effect of high-dose estradiol delivered transcutaneously on sexual characteristics, breast growth, or bone mineral density. The parents of this patient were second cousins. This autosomal recessive trait was associated with substitution of thymine for cytosine at codon 157 in exon 2 of the estrogen receptor gene, resulting in substitution of a stop codon (TGA) for arginine (CGA) at this position and a highly truncated estrogen receptor with no DNA- or hormone-binding domains.

This patient, when compared with his normal siblings and parents, demonstrates that: (1) estrogen activity is not essential for life, fetal development, postnatal growth, or virilization in the male; (2) heterozygous males and females with one defective estrogen receptor allele are normal; (3) estrogen is essential for complete epiphyseal maturation and fusion and for normal skeletal mineralization in the male; (4) estrogen is essential for regulation of gonadotropin secretion in the male;

and (5) estrogen may be necessary for normal insulin sensitivity and sperm viability.

Smith EP, et al. *N Engl J Med* 1994;331:1056-1061.

**Editor's comment:** This report clarifies earlier reports in which the importance of aromatization of androgen to estrogen in the regulation of gonadotropin secretion in the male had been questioned. Since the level of insulin-like growth factor 1 was normal in this subject, it is possible that the secretion of growth hormone is not dependent on estrogen action. Studies of endogenous and stimulated secretion of somatotropin in this subject would be of interest.

The phenotype of a homozygous female deficient for the estrogen receptor is unknown, but one might speculate that such an individual may be virilized in utero and during adolescence. Shozu et al<sup>1</sup> and Conte et al<sup>2</sup> report the occurrence of female pseudohermaphroditism and pubertal virilization in females with an abnormality in the gene encoding the P450 enzyme aromatase, leading to decreased estrogen production in utero and unopposed androgen activity. In these respects, females with aromatase deficiency resemble female spotted hyenas who have aromatase deficiency; these females are virilized and quite aggressive.<sup>3</sup>

Allen W. Root, MD

1. Shozu M, et al. *J Clin Endocrinol Metab* 1991;72:560-566.

2. Conte FA, et al. *J Clin Endocrinol Metab* 1994;78:1287-1292.

3. Yalcinkaya TM, et al. *Science* 1993;260:1929-1931.

## The Small Nuclear Ribonucleoprotein-Associated Polypeptide N (SNRPN) Gene in Prader-Willi and Angelman Syndromes

Imprinting is the process by which differences in the phenotype of a specific disorder are expressed depending on whether the allele was paternally or maternally derived. Imprinting occurs during gametogenesis; it is heritable and reversible.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) map to chromosome 15q11-q13, and they represent 2 of the best examples of imprinting in humans. PWS is characterized by infantile hypotonia, mental retardation, hyperphagia, and small hands and feet. AS is characterized by severe mental retardation, absent speech, seizures, ataxic gait, and bouts of uncontrollable laughter.

In PWS, approximately 70% of patients have a deletion involving the paternally derived chromosome 15; almost all of the rest of PWS patients have maternal uniparental disomy (UPD) of chromosome 15. In contrast to PWS, AS is associated with a similar area of chromosome 15 deletion but on the maternally derived chromosome 15 and with paternal UPD. The study of the molecular similarities and clinical differences between these 2 syndromes has provided valuable information regarding the gene control mechanisms involved in imprinting.

The small nuclear ribonucleoprotein-associated polypeptide N (SNRPN) gene has been mapped to the 15q11-q13 region. It is known to display paternal allele-specific expression in mouse and to be expressed exclusively from the father's allele in human fetal brain (Reed et al). Following the localization of the SNRPN gene, Sutcliffe et al constructed a complete yeast artificial chromosome (YAC) containing the region commonly

deleted in PWS and AS (Lalande) in order to determine the molecular basis for PWS and AS.

Two genes, PAR-1 and PAR-5, were isolated and mapped distal to SNRPN. Both PAR-1 and PAR-5 were detected in cultured cells of AS deletion individuals but not in cells of PWS patients, suggesting that these 2 genes are expressed only from the paternal chromosome.

The fact that PAR-1, PAR-5, and the SNRPN gene are in close proximity led them to the suggestion that these genes lie in a domain, ie, a group of genes with similar genetic control, of imprinted transcription. The highest levels of expression of SNRPN were in brain. PAR-5 expression also was highest in brain, while PAR-1 expression was highest in skeletal muscle. This suggests tissue specificity of gene expression.

Reed ML, et al. *Nat Genet* 1994;6:163-167.

Sutcliffe JS, et al. *Nat Genet* 1994;8:52-58.

Lalande M. *Nat Genet* 1994;8:5-6.

**Editor's comment:** Imprinting is increasingly being recognized as a very important molecular mechanism. It appears to be involved in genetic control of growth and behavior, and in early development. Intensive investigation of the 15q12 region is showing important differences in gene expression between the maternally and paternally derived chromosomes. The expression is tissue specific, time-in-development specific, and strain specific.

Judith G. Hall, MD

## Predicting Adult Stature Without Using Skeletal Age: The Khamis-Roche Method

Khamis and Roche developed a modification of the Roche-Wainer-Thissen (RWT) stature prediction model in which the skeletal age was not used to calculate the predicted height. The parameters considered for the calculation of predicted adult height were current height and weight and midparental height, ie, the mean of the parents' heights. They obtained these data from a group of white American children (223 males and 210 females) residing in southwest Ohio; they were participants of the Fels Longitudinal Study and were followed with measurements of height and weight every 6 months from the age of 3 years until 18 years. The stature of each parent also was measured. Linear regressions of adult stature (considered for their purpose as the stature attained at age 18 years) were calculated using the 3 variables. The following equation was used: predicted adult stature =  $\beta_0 + \beta_1$  stature +  $\beta_2$  weight +  $\beta_3$  midparental stature. The tables for males and females list the intercepts ( $\beta_0$ ) and the coefficients of the 3 variables ( $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ , respectively) for each chronologic age, expressed in 6- and 12-month intervals. The accuracy of the prediction method was measured using the median absolute deviation (MAD), which is the median of the absolute differences, regardless of the signs, between actual and predicted stature at age 18. The smaller the MAD, the better the accuracy. There was only a slight deterioration of the accuracy with this method as compared with RWT, which uses estimations of skeletal age.

Khamis HJ, Roche AF. *Pediatrics* 1994;94:504-507.

**Editor's comment:** The authors present an ingenious method of predicting final adult stature in children without using skeletal age. This method might be a useful adjunct in the clinic and allows comparisons of predicted adult height by anthropometric determinations. Large discrepancies between the 2 methods may indicate inaccurate measurements and/or inaccurate bone

age estimation. Two problems may still preclude its use in a pediatric endocrine setting: first, its accuracy seems to be worse in the peripubertal years, especially in males, where it overestimates predicted heights. Second, the predictability is good only in the absence of pathologic conditions that alter the potential for linear growth. Thus, caution must be exercised if it is used as an adjunct diagnostic tool in children with short or tall stature. However, it may be of a great value as a descriptive instrument for prediction of adult stature in normal children, and in epidemiologic studies of population when adult height predictors without bone age estimates may be an important index of health status.

Fima Lifshitz, MD

**2nd Editor's comment:** After publication of this article, the authors noted an error in Tables 1 and 2 presenting the weight coefficients necessary for calculating adult stature with the above equation. The decimal point was displaced one space to the left. The weight coefficients may be corrected by shifting the decimal points one space to the right of their present locations. This error is to be corrected in a "Letter to the Editor" of *Pediatrics*. Readers may wish to correct the tables in the original article for their own use or watch for publication of the corrected tables in *Pediatrics*.

As the authors point out, the described method for prediction of adult height is based on measurements of healthy white children who are growing normally and, therefore, strictly applicable only to this group. This reviewer seldom predicts stature in normal children, because if the prediction is below that which the parents desire, pressure for intervention—no matter how futile—may be increased.

Allen W. Root, MD

## Growth of Short Normal Children in Puberty Treated for 3 Years With Growth Hormone Alone or in Association With Gonadotropin-Releasing Hormone Agonist

Thirty early pubertal short normal subjects received growth hormone (GH) at 0.1 IU/kg/d, 6 d/wk ( $-0.2$  mg/kg/wk) for 3 years. These included 16 males, aged  $14.4 \pm 0.8$  years, and 14 females, aged  $12.2 \pm 1.2$  years. All were at pubertal stage 2 or 3, with slow pubertal growth ( $4.2 \pm 1.2$  cm/y) and a mean bone age delay of 2 years. There was no detected GH deficiency or other cause for short stature. Their mean birth length was  $48.6$  to  $49.5$  cm at term; the mean of midparental heights was  $-0.6$  to  $-0.8$  standard deviations (SD) below the mean of the general adult population. They were randomized in 2 groups: group A received GH alone; group B received gonadotropin hormone-releasing hormone agonist (GnRHa) plus daily GH injections for 2 years, and for year 3.

The annual growth velocity (GV) increased during the first year in both groups and sexes, the increase being significant ( $P < 0.01$ ) in group A only. The patients of group A maintained an improved GV in the second year, and then returned to pretreatment GV in the third year, while completing their sexual development and bone maturation. Their height, expressed as SD score (SDS) for bone age, improved in the first 2 years but decreased thereafter. Group B patients returned to pretreatment GV in the second year, and demonstrated no significant

improvement when treated with GH alone during the third year of the study. They had no significant progress of height for age at any time. Their bone maturation, slow when they were receiving GnRHa, accelerated when sexual development resumed.

At the end of the 3 years, height, expressed as SDS for age, improved in group A from  $-2.5 \pm 0.6$  SD to  $-1.5 \pm 0.4$  SD in males ( $P < 0.05$ ) and from  $-2.8 \pm 0.5$  SD to  $-2.1 \pm 0.9$  SD in females (NS). Expressed as SDS for bone age, mean height slightly improved in males (NS) but not in females. In both groups and sexes, the mean predicted height according to Bayley and Pinneau was only slightly increased at the end of 3 years on GH, with a gain of 2 to 5 cm on the average. There was a wide interindividual variability in these results within each group. Pretreatment characteristics of the patients did not account for individual differences. Annual measurement of plasma insulin-like growth factor 1 showed different degrees of increase, not correlated with any parameter of the patients' growth.

The authors drew the following conclusions: (1) Inhibiting sexual development in short early pubertal subjects has no advantage. This was previously demonstrated with GnRHa alone (see GGH 1993;9[4]:13), and now is confirmed for GnRHa plus GH. (2) GH alone, at the dose used, can accelerate for

2 years the growth of such slow-growing normal short adolescents, and slightly improve their predicted height in relation to the result of the first year of treatment. However, the expected results should not be overestimated, nor should this be considered as an indication for any routine use of GH in endocrinologically normal and constitutionally short pubertal individuals.

Job JC, et al. *Horm Res* 1994;41:177-184.

**Editor's comment:** This report will discourage only the most desperately short children from trying to achieve normal height by using GnRHa plus GH.

Robert M. Blizzard, MD

## Susceptibility Gene Loci for Insulin-Dependent Diabetes Mellitus (IDDM): A Review

Insulin-dependent diabetes mellitus (IDDM) is a polygenic multifactorial disease, ie, it is caused by different susceptibility genes and environmental factors in different people. Other disorders thought to be polygenic multifactorial include ischemic heart disease, asthma, and schizophrenia.

In mice, IDDM has been shown to be a polygenic trait, with the major locus encoded in the major histocompatibility complex (MHC) with at least 10 other loci contributing to the development of the disease. In humans, the MHC HLA region on chromosome 6p21 and the insulin gene region on chromosome 11q23 have been associated with IDDM. However, in families with multiple affected individuals, these 2 loci have been suggested to account for less than 50% of the genetic risk of the disease.

Two recent papers by Hashimoto et al and Davies et al reported genome-wide linkage studies for the localization of IDDM susceptibility loci. Hashimoto et al applied highly informative markers to a panel of 314 white IDDM-affected sibling pairs and found evidence for the localization of a previously undetected susceptibility locus for IDDM in the region of the fibroblast growth factor 3 (FGF3) gene on chromosome 11q.

These results were confirmed by Davies et al, who also used the same method of genome-wide searches to study 96 sibpair

families and a linkage map of 290. This group also found linkages between IDDM and chromosomes 11q and 6q, and suggested that there may be a fifth susceptibility locus on chromosome 18. Davies et al point out, however, that the genome linkage map had an average spacing of 11 centimorgans (cM) and that gaps still exist in this map. They suggest that in order to detect all the susceptibility loci for IDDM, it may be necessary to test with markers that are only 3 cM apart.

Hashimoto L, et al. *Nature* 1994;371:161-163.

Davies JL, et al. *Nature* 1994;371:130-136.

**Editor's comment:** The genome-wide search method has been used for other multifactorial disorders, especially psychiatric disorders (Lander and Botstein. *Genetics* 1989;121:185), but has not provided any linkage data so far, much less specific genes. These new methods are very powerful and should hasten the progress of the Human Genome Project effort to identify all 100,000 human genes.

Judith G. Hall, MD

## Prenatal Treatment of Congenital Adrenal Hyperplasia: A Review

Congenital adrenal hyperplasia (CAH), an autosomal recessive disorder, is the most common cause of ambiguous genitalia in females. Ninety percent of CAH cases are caused by 21-hydroxylase deficiency. In order to prevent virilization in utero, maternal glucocorticoid therapy (specifically, dexamethasone) is started immediately after detection and continued throughout pregnancy; this suppresses fetal androgen production.<sup>1-3</sup> Most of the reported cases that were treated early and adequately were born with normal female genitalia.

A recent paper by Wudy et al<sup>4</sup> documents another successful prenatal treatment of CAH. They report a newborn girl born with normal female genitalia after prenatal dexamethasone treatment (the index case in the family was a boy suffering from 21-hydroxylase deficiency). Molecular genetic diagnosis was not available, and prenatal diagnosis relied on amniocentesis with karyotyping and 17  $\alpha$ -hydroxyprogesterone determination. In order to get an accurate amniotic fluid steroid analysis, dexamethasone treatment was suspended for 5 days prior to amniocentesis.

Previous reports have shown that prenatal dexamethasone treatment must begin as early as the 5th to 9th week of gestation, which may be before the diagnosis of a female fetus is made by amniocentesis or chorionic villus sampling (CVS). Both the dosage and temporary suspension of dexamethasone have been controversial issues in prenatal CAH treatment.

Some authors suggested that excessive virilization may occur due to a rebound effect.<sup>3</sup> However, Wudy et al's paper has shown that this is not necessarily true. Furthermore, with the advent of molecular diagnosis, interruption of maternal glucocorticoid therapy may not even be necessary. The potential maternal side effects of dexamethasone therapy include development of a cushingoid face, massive weight gain, and marked striae. Hypertension and increased urinary glucose have also been reported.<sup>3</sup> Nevertheless, many families will opt for prenatal therapy for affected female fetuses.

1. Pang S, et al. *Trends Endocrinol Metab* 1990;1:300-307.

2. Forest MG, et al. *Horm Res* 1990;33:43.

3. Loeuille GA. *Eur J Pediatr* 1990;149:237-240.

4. Wudy S, et al. *Eur J Pediatr* 1994;153:556-559.

**Editor's comment:** Major progress has been made in diagnostic and therapeutic modalities for this common disorder. With the identification of the gene structure and common mutations, more accurate diagnosis is possible both prenatally and at birth. However, therapy must begin before accurate diagnosis of the fetus is possible, since CVS and amniocentesis are contraindicated before the 10th to 11th week of pregnancy.

Judith G. Hall, MD

## Volume 10, Number 1

"Overgrowth Syndromes and Disorders: Definition, Classification, and Discussion" David D. Weaver, MS, MD

"The Etiology and Diagnosis of Overgrowth Syndromes" Kenneth Lyons Jones, MD

### Clinical Pearls:

The Differentiation of Constitutional Growth Delay From Nutritional Dwarfism (ND) Fima Lifshitz, MD

### Abstracts:

Effect of Weight Loss by Obese Children on Long-Term Growth

Pharmacologic, Biologic, and Clinical Effects of Recombinant Human Insulin-Like Growth Factor 1 in Growth Hormone Insensitivity Syndromes

A Constitutively Activating Mutation of the Luteinizing Hormone Receptor in Familial Male Precocious Puberty

Reduction of Bone Density: An Effect of Gonadotropin Releasing Hormone Analogue Treatment in Central Precocious Puberty

Leprechaunism and the Insulin Receptor Gene

Mild to Moderate Zinc Deficiency in Short Children: Effect of Zinc Supplementation on Linear Growth Velocity Effects of Calcitriol and Phosphorus Therapy on the Growth of Patients With X-Linked Hypophosphatemia

Effects of Human Growth Hormone Therapy on Melanocytic Naevi

Growth Hormone (GH) Receptors, GH Binding Protein and GH: An Autoregulatory System?

Failure to Improve Height Prediction in Short-Stature Pubertal Adolescents by Inhibiting Puberty With Luteinizing Hormone-Releasing Hormone Analogue

Evolution of the Sex-Determining Gene

The Role of Estrogens in Disorders of the Male Reproductive Tract

## Volume 10, Number 2

"Neuroendocrinology of Growth Hormone Secretion" Jesús Argente, MD, PhD, and Julie Ann Chowen, PhD

"Serum Polypeptide Hormone-Binding Proteins Part 1: Growth Hormone-Binding Proteins" Allen W. Root, MD

"Serum Polypeptide Hormone-Binding Proteins Part 2: Insulin-Like Growth Factor-Binding Proteins" Allen W. Root, MD

### Abstracts:

Influence of the High-Affinity Growth Hormone (GH)-Binding Protein on Plasma Profiles of Free and Bound GH and on the Apparent Half-Life of GH: Modeling Analysis and Clinical Applications

A Linkage Between DNA Markers on the X Chromosome and Male Sexual Orientation

Exclusively Paternal X Chromosomes in a Girl With Short Stature

In Vivo Gene Therapy of Hemophilia B: Sustained Partial Correction in Factor IX Deficient Dogs

Molecular Basis of the *Little Mouse* Phenotype and Implications for Cell Type-Specific Growth

Overexpression of Dystrophin in Transgenic *mdx* Mice Eliminates Dystrophic Symptoms Without Toxicity

The Y Chromosome in Turner Syndrome

A Nonpeptidyl Growth Hormone Secretagogue and Stimulation of Growth Hormone Release From Rat Pituitary Cells by L-692,429, a Novel Nonpeptidyl GH Secretagogue

Influence of Spontaneous or Induced Puberty on the Growth Promoting Effect of Treatment With Growth Hormone in Girls With Turner's Syndrome

Hazards of Pharmacological Tests of Growth Hormone Secretion in Childhood

Life With Turner's Syndrome: A Psychosocial Report From 22 Middle-Aged Women

Final Height in Patients Treated for Childhood Acute Lymphoblastic Leukemia

Growth and Growth Hormone Secretion After Bone Marrow Transplantation

Growth and Growth Hormone in Children During and After Therapy for Acute Lymphoblastic Leukemia

## Volume 10, Number 3

"Insulin-Like Growth Factor 2 and Growth" Helene Schneid, PhD, and Yves Le Bouc, MD

"Placental Growth Hormone Variant: A Specific Marker of Pregnancy With Still Unknown Functions" Danièle Evain-Brion, MD, PhD

"The Diagnosis and Management of Craniopharyngioma" Edward R. Laws, Jr, MD, FACS, and Kamal Thapar, MD

### Meeting Reviews:

Third International Symposium on Insulin-Like Growth Factors February 6-10, 1994; Sydney, Australia Paul Saenger, MD

The First International Meeting of the Growth Hormone Research Society June 1-4, 1994; Århus, Denmark Paul Saenger, MD

### Abstracts:

Chromosomal Localization of the Human Renal Sodium-Phosphate Transporter to Chromosome 5: Implications for X-Linked Hypophosphatemia

Turner Syndrome: Natural History, Ethnic and Genetic Influences, Methods for Evaluation of Growth

Perspectives of Longitudinal Growth in Cystic Fibrosis From Birth to Adult Age

Calcium-Sensing Receptor Genes Mutate and Produce Metabolic Disease

Genetic Mapping of Quantitative Trait Loci for Growth Fatness in Pigs

## Volume 10, Number 4

"Noonan Syndrome: A Review" Michael A. Patton, MA, MSc, MB, FRCP

"Prader-Willi Syndrome: Chromosomal and Gene Aberrations" Uta Francke, MD

"Rationale for Dosing Recombinant Human Growth Hormone by Weight Rather Than Units" Margaret H. MacGillivray, MD, and Robert M. Blizzard, MD

### Abstracts:

Utah Growth Study: Growth Standards and the Prevalence of Growth Hormone Deficiency

Developmental Timing of Dynamic Mutations

Succalfate Causes Malabsorption of L-Thyroxine

Snaring the Achondroplasia and Hypochondroplasia Gene

Phenotype Specific RET Oncogene Mutations and Multiple Endocrine Neoplasia Syndromes

A Single Amino Acid Substitution in the Exoplasmic Domain of the Human Growth Hormone (GH) Receptor Confers Familial GH Resistance (Laron Syndrome) With Positive GH-Binding Activity by Abolishing Receptor Homodimerization

Growth After Renal Transplantation in Prepubertal Children: Impact of Various Treatment Modalities

Growth Hormone Treatment in Growth-Retarded Adolescents After Renal Transplant

Diabetes Insipidus With Impaired Osmotic Regulation in Septo-optic Dysplasia and Agenesis of the Corpus Callosum

Changes in Body Composition of Children With Chronic Renal Failure During Growth Hormone Treatment

Idiopathic Prepubertal Short Stature Is Associated With Low Body Mass Index



## Meetings Calendar

**March 30-April 1, 1995** 13th Testis Wkshp, Raleigh, NC. Info: C Desjardins. Tel: 804-982-4310; Fax: 804-924-8785; E-mail: REPROD@VIRGINIA.EDU.

**May 12-13, 1995** 2nd Intl Wkshp on Thyroid Hormone Resistance, Padua, Italy. Info: Dr P Beck-Peccoz. Tel: 39-2-546-4063; Fax: 39-2-5519-5438.

**May 24-28, 1995** Eur Soc for Human Genet Mtg, Berlin, Germany. Info: Amer Soc of Human Genet. Tel: 301-571-1825; Fax: 301-571-1895.

**June 14-17, 1995** Endocrine Soc: 77th Ann Mtg, Washington, DC. Info: Endocrine Soc. Tel: 301-941-0200; Fax: 301-941-0259.

**June 22-23, 1995** 2nd Mtg of Bone Dysplasia Soc, Versailles, France. Info: Organizing Committee, Dr P Maroteaux. Tel: 011-33-1-44-49-44-82; Fax: 011-33-1-45-66-02-86.

**June 25-28, 1995** 34th Ann Mtg of the Eur Soc for Paediatr Endocrinol, Edinburgh, Scot. Info: ESPE. Tel: 44-41-553-1930; Fax: 44-41-552-0511.

**July 17-28, 1995** Mendelian Genet, Bar Harbor, ME. Info: The Jackson Laboratory. Tel: 207-288-3371; Fax: 207-288-5079.

**July 29-August 2, 1995** Recent Progress in Hormone Research 51st Conf, Stevenson, WA. Info: Endocrine Soc Mtgs Dept. Tel: 301-941-0200 or 1-800-HORMONE; Fax: 301-941-0259.

**July 29-August 3, 1995** The David Smith Morphogenesis Mtg, Big Sky, MT. Info: Dr KL Jones. Tel: 619-294-6217; Fax: 619-291-8938.

**August 2-5, 1995** Portland Bone Symp, Portland, OR. Info: OHSU, Continuing Education. Tel: 800-452-1048; Fax: 503-494-3400.

**August 19-25, 1995** Intl Human Genet Mtg, Rio de Janeiro, Brazil. Info: Amer Soc of Human Genet. Tel: 301-571-1825; Fax: 301-571-1895.

**September 10-15, 1995** 11th Intl Thyroid Cong, Toronto, Can. Info: Amer Thyroid Assoc. Fax: 718-882-6085.

**September 13-15, 1995** Intl Symp on DHEA Transformation Into Androgens and Estrogens in Target Tissues: Intracrinology, Quebec City, Quebec, Can. Info: Intl Symp on DHEA.

**September 17-20, 1995** 5th Intl Cong on Hormones and Cancer, Quebec City, Quebec, Can. Info: 5th Intl Cong.

**September 27-30, 1995** Molecular and Developmental Biol of Cartilage, Bethesda, MD. Info: Conf Dept. Tel: 800-843-6927 x-324; Fax: 212-838-5640.

**October 13-15, 1995** Symp on Advances in Clin Nutrition, Washington, DC. Info: Amer Coll of Nutrition. Tel: 212-777-1037; Fax: 212-777-1103.

**October 18-20, 1995** Intl Symp on Growth, Santiago de Compostela, Spain. Info: Profs F Casanueva, C Dieguez, or M Pombo. Fax: 34-81-572121.

**October 24-28, 1995** 45th Ann Mtg of the Amer Soc of Human Genet, Minneapolis, MN. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

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# GROWTH

## Genetics & Hormones

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June 1995

### Magnetic Resonance Imaging in Pituitary Disease

**Raphael Rappaport, MD**

*Chairman, Pediatric Endocrinology Unit  
Department of Pediatrics  
Hôpital des Enfants Malades  
Paris, France*

Magnetic resonance imaging (MRI) permits visualization of the anterior and posterior pituitary glands and the pituitary stalk, which connects the pituitary gland to the median eminence. This noninvasive technique has greatly improved our understanding of dysfunction of the anterior pituitary and neurohypophysis in children with growth hormone (GH) deficiency (GHD), idiopathic diabetes insipidus, and central precocious puberty (CPP). This review presents the latest considerations regarding the use of MRI in pituitary disease.<sup>1</sup>

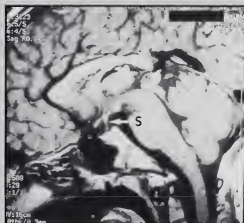
#### **NORMAL MRI OF THE HYPOTHALAMIC-PITUITARY AXIS**

The anterior pituitary lobe develops from an upward diverticulum of the primitive buccal cavity. The posterior pituitary lobe, or neurohypophysis, originates as a downward extension from the hypothalamus. The pituitary stalk consists primarily of the neural connection between the median eminence and the posterior pituitary lobe. This neural connection consists of axonal processes down which vasopressin and oxytocin travel to the posterior lobe, from which they are then released by exocytosis.

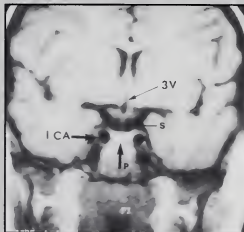
The pituitary stalk consists secondarily of a delicate layer of tissue that is permeated by numerous capillary loops of the hypophyseal-portal blood system. This vascular structure also provides the principal blood supply to the anterior pituitary lobe as there is no direct arterial supply to this organ. In contrast, the posterior pituitary lobe has a direct

vascular supply. Therefore, the posterior lobe can be more rapidly visualized in a dynamic mode after administration of gadolinium (gadopentetate dimeglumine) as contrast material during MRI (Figure 1). The use of gadolinium is necessary for optimal study of the pituitary stalk since it contains abundant vascular structures that are not obscured by the blood-brain barrier.

**Figure 1**  
**Pituitary Anatomy and**  
**Normal MRI Findings**



Sagittal  
View



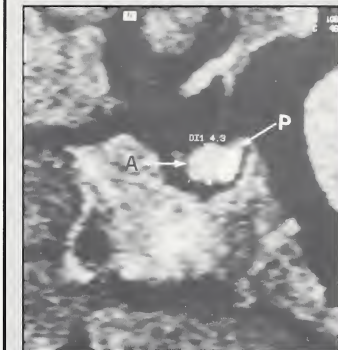
Coronal  
View

S: pituitary stalk; P: posterior pituitary bright spot;  
3V: 3rd ventricle; CA: carotid artery

#### **In This Issue**

**Abstracts ..... pg 5**

Figure 2  
Anterior Pituitary Height  
Measurement (A) and  
Posterior Pituitary Bright Spot (P)



Standards for proper imaging have emerged, eg, obtaining sagittal and coronal images that are less than 5 mm in thickness, in order to visualize the pituitary stalk and the posterior pituitary lobe. The maximal height of the anterior pituitary gland is measured in a perpendicular plane to the floor of the sella turcica (Figure 2). Additional axial images using precise midline positioning are necessary to visualize any structural defect of the stalk. The mean anterior pituitary height, when measured using strict midline sagittal T1-weighted sections that are 3 to 5 mm thick, varies according to age. In neonates, the mean height is 4.5 mm, as the pituitary is typically convex.<sup>2,3</sup> After 2 months of age, the superior face becomes flatter and the height decreases to 3 mm. One may consider the lower limit of normal height prepubertally to be 3 mm, as the mean height increases progressively to 5.3 mm. A further increase of pituitary height occurs during puberty.<sup>4</sup> The pituitary stalk also increases in diameter.

The posterior pituitary lobe can be easily distinguished by a round, high-intensity signal in the posterior part of the sella turcica on T1-weighted images (Figure 2). Provided the appropriate MRI sections have been performed, all normal children and adolescents have a bright spot in the posterior pituitary, which serves as a marker of normal neurohypophyseal function. In adults, an age-related decline of 1% per year in detecting this bright spot was reported.<sup>5</sup> Phospholipid components in the neurohypophysis are believed to account for the signal.<sup>6</sup> The

location and shape of the posterior pituitary signal may vary slightly by having a ring appearance or an extension along the inferior part of the pituitary stalk. Any process that disturbs the neurosecretory transport or function may lead to an accumulation of high signal intensity material and an obstruction in ectopic positions, such as in the median eminence or along the pituitary stalk cephalad (Figure 3). The ectopia depicted in this dense image, which is associated with pituitary stalk defects, is generally observed in pathologic conditions such as GHD or destruction of the posterior pituitary. However, ectopia has been found on rare occasion in patients with otherwise normal pituitary function and normal imaging findings of the pituitary stalk.<sup>7</sup>

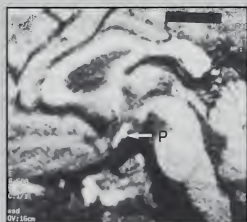
### MRI IN ASSOCIATION WITH GHD

Several reports<sup>8-13</sup> have compared MRI and endocrine evaluations of patients with GHD. Pituitary stalk interruption (PSI) with resultant ectopic position of the neurohypophyseal bright spot has been one of the main MRI findings. Hypoplasia of the pituitary gland, with or without ectopic position of the neurohypophyseal bright spot, also occurs. Hypoplasia may occur with a sella turcica of normal or diminished size.

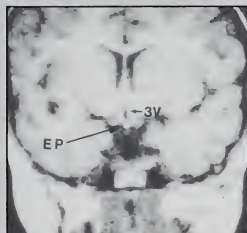
Findings associated with the PSI syndrome are presented in Table 1. Multiple anterior pituitary hormone deficiencies were recently reported in 18 of 29 GHD patients with PSI syndrome, even though diabetes insipidus was absent.<sup>13</sup> Severe hypoplasia of the anterior pituitary with reduced sellar size was present in most. However, a minority of patients with PSI syndrome had apparent isolated GHD. These patients are probably at risk of developing additional pituitary hormone deficiencies in the future, principally at the time of puberty. Additional follow-up data are needed to assess the functional prognostic value of PSI. GHD definitely is more severe in patients with PSI.<sup>14</sup> There is no report of reversal of an abnormal stalk to a normal stalk image.

The pathology of an ectopic neurohypophysis, which is associated with PSI, was first described at autopsy: the tissue of the posterior pituitary lobe was present in a nodule at different levels of the hypothalamohypophyseal tract. The stalk was reduced to a filament, and the intrasellar pituitary lobe was hypoplastic.<sup>15</sup> This condition is likely caused at times by a pituitary stalk transection, as produced experimentally in animals and observed after pituitary surgery in humans.<sup>16</sup> After pituitary stalk transection, neuroendocrine fibers may regenerate from the hypothalamus. This may explain the fact that diabetes insipidus is usually absent. Pituitary infarction may cause the GHD or multiple pituitary hormone deficiencies. In several studies,

**Figure 3**  
**Posterior Pituitary Stalk Interruption**



Sagittal View



Coronal View

P: ectopic posterior tissue  
EP: ectopic posterior pituitary tissue

authors reported the occurrence of significant head trauma in the histories of their patients. This correlated with late onset of growth retardation.<sup>17</sup> Injury also may be of vascular origin because of the structure of the pituitary stalk. Therefore it was appropriate to correlate PSI with the high frequency of perinatal adverse events observed in hypopituitary patients. Inconsistent histories have been obtained and range from significant trauma to perinatal anoxia.<sup>9-13</sup> Events occurring prior to birth also can be considered as a cause of such lesions. For instance, some data suggest that PSI may be associated with midline malformations, a developmental defect occurring before the pituitary is fully formed in patients, such as those with septo-optic dysplasia or a single incisor (Table 1).

Anterior pituitary hypoplasia in the absence of other MRI abnormalities has been reported with GHD, including isolated GHD. Therefore, measurement of pituitary heights is an important method of evaluating pituitary function. The age-related pediatric reference data cited above<sup>3</sup> provides important normal data for pituitary height and, hence, volume.

Patients with only hypoplasia of the pituitary, demonstrated by MRI, tend to have less severe GHD than those with PSI. In a study of 21 children with isolated GHD, the pituitary stalk was intact in all.<sup>18</sup> The anterior pituitary lobe was hypoplastic in 17, and the sella was partially empty in 13. None of these children had a history of perinatal asphyxia. The authors postulated that an embryonic defect was the most likely cause.

Because there are multiple different appearances of the hypothalamic-pituitary axis in association with GHD, the results of MRI may assist in classifying the type of GHD. A prospective comparison of functional and anatomic data in GHD patients remains to be undertaken. In Table 2 (page 4) the etiologies of idiopathic GHD are listed. MRI can assist in differentiating the 4 major groups of GHD considered in the table. In addition, imaging of the pituitary and pituitary stalk can be a valid diagnostic tool when infantile GHD is suspected but not proven by the usual testing techniques.

#### **MRI OF PATIENTS WITH CENTRAL DIABETES INSIPIDUS**

The round, high-intensity signal seen in the normal posterior pituitary lobe is usually absent in patients with central diabetes insipidus. However, this signal is present in children with dipsogenic polyuria or nephrogenic diabetes insipidus, or in those with autosomal dominant familial cases of central diabetes insipidus.<sup>19</sup> MRI remains the best technique with

**Table 1**  
**Pituitary Stalk Interruption Syndrome (PSIS)**

- PSIS is strongly associated with multiple pituitary hormone deficiency, which is associated with organic hypopituitarism. When associated with isolated GHD, it is a valuable diagnostic sign of a permanent defect and may be predictive of later occurring anterior pituitary hormone deficiencies
- PSIS is frequently associated with injury at birth
- Several likely causes of PSIS are:
  - Prenatal developmental defect
  - Perinatal insult
  - Postnatal trauma
- The variable clinical expression of PSIS may reflect a progressively diminishing hypothalamic trophic control with a predominant effect on GH secretion



Table 2  
**Etiologies of Idiopathic Growth Hormone Deficiency**

- Molecular defects of growth hormone or growth receptor genes
- Developmental defects
  - Somatotrophic cell differentiation (pituitary factors)
  - Anterior pituitary development (midline defects)
- Pituitary stalk interruption
  - Prenatal vascular compromise or trauma
  - Perinatal asphyxia or trauma
  - Postnatal trauma or infection
- Defective hypothalamic control with isolated anterior pituitary hypoplasia

which to evaluate the pituitary stalk and infundibulum in patients with idiopathic polyuria. A finding such as thickening of the pituitary stalk may be helpful in determining the etiology of apparent diabetes insipidus.<sup>19</sup> Such thickening may be isolated. The degree of thickening is best evaluated after administration of gadolinium. When thickening and/or enlargement is marked, the question of an infiltrative process destroying the neurohypophyseal tract must be considered. Malignant tumors such as germinomas of the hypothalamus must be considered. Our experience is that if such a germinoma is not detected at the time of presentation, it may be recognized within a few months or years by repeat MRI. Measurement of circulating tumor markers such as the beta unit of human chorionic gonadotropin (hCG- $\beta$ ) and  $\alpha$ -fetoprotein also is indicated.<sup>20</sup> Surprisingly, some patients present with an isolated thickening of the pituitary stalk that may remain stable over years and eventually regress without either any change in MRI findings or the appearance of a tumor. In most, diabetes insipidus remains isolated and normal anterior pituitary function persists. The absence of associated systemic abnormalities (cutaneous, skeletal, pulmonary) and/or hypercalcemia rules out organic infiltration due to histiocytosis, sarcoidosis, or tuberculosis.

Diabetes insipidus may be isolated or associated with GHD (Figure 4). The association of diabetes insipidus with GHD is usually considered as a result of invasion by a craniopharyngioma or by histiocytosis X.<sup>21</sup> However, diabetes insipidus and GHD may be present without evidence of infiltration of any type. In such cases GHD may be variable and, if prolonged, produce growth retardation.<sup>22</sup> Indirect evidence for autoimmune neurohypophysitis, based on the presence of autoantibodies to vasopressin

cells, was suggested,<sup>23</sup> but no follow-up of these patients has been reported. More recently, lymphocytic infundibuloneurohypophysitis was described as a cause of central diabetes insipidus. Histologic evidence was obtained in biopsy specimens from adults in this series. Impairment of GH secretion was often associated. The natural course of this condition was unique with possible regression of the stalk width. There was a preponderance of females, and the authors considered this compatible with the autoimmune hypothesis of idiopathic diabetes insipidus with the presence of T-cell infiltration.<sup>24</sup> Whether these findings apply to diabetes insipidus in the pediatric population remains to be demonstrated. For practical purposes, it may be recommended to rigorously follow up patients with combined diabetes insipidus and GHD with repeat MRI every 6 to 12 months for a period of 2 to 4 years, as the appearance of a dysgerminoma should not be missed. After 2 to 4 years, a repeat MRI every 2 to 3 years is adequate.

## MRI OF PATIENTS WITH CPP

MRI should be pursued in patients with CPP. Invasive tumors like optic and hypothalamic gliomas and congenital malformations such as hydrocephalus and hamartomas may be easily identified. The latter often produce luteinizing hormone-releasing hormone (LHRH), which accounts for the sexual precocity. Except for extensive hCG- $\beta$ -secreting dysgerminomas in boys extending into the pituitary sella, which are invariably associated with diabetes insipidus, there are no invasive intrasellar lesions causing CPP. However, the pituitary gland undergoes transient hypertrophy during normal puberty. The height of the anterior pituitary lobe increases with convexity of its upper surface; this should not be mistaken for a tumor. Similar changes occur in patients with CPP.<sup>4</sup> However, small pituitary glands with markedly concave upper borders were observed in patients presenting with associated GHD and sexual precocity.<sup>25</sup>

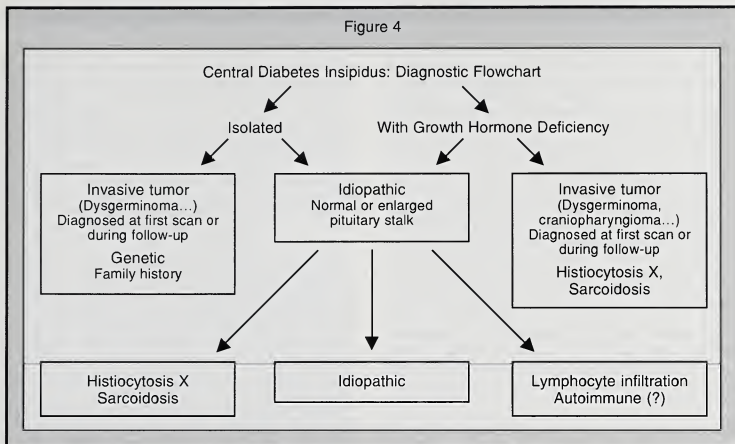
## SUMMARY

MRI is essential in the evaluation of any patient with suspected pathology of the hypothalamic-pituitary axis. Physicians involved in the care of such children should not hesitate to refer such patients to the appropriate specialists. The morbidity and mortality statistics will be improved significantly.

## ACKNOWLEDGMENTS

The author is grateful to Drs. R. Brauner, F. Brunelle, and M. Argyropoulou for their critical contribution and Mrs. C. Castanera for her skillful assistance.

Figure 4



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## Abstracts From the Literature

## Is the Etiology of Insulin-Dependent Diabetes Mellitus Related to Superantigen Involvement?

The etiology of insulin-dependent diabetes mellitus (IDDM) remains obscure, although most accept that this is usually a T cell-mediated autoimmune disease, the onset and/or progression of which is very possibly triggered by unknown environmental factors (possibly viruses) acting on a predisposing genetic background. Conrad et al studied the islet-infiltrating T (IIT) cells from 2 IDDM patients who were dying at the onset of disease. Their results can be interpreted as providing evidence for the involvement of a pancreatic islet cell membrane-bound superantigen (SAg) as an etiologic factor.

Conrad et al report a correlation between the presence of insulitis and the presence of an abundance of CD4<sup>+</sup> (helper cells). CD8<sup>+</sup> (killer cells) were also present in the areas of insulitis. In another article in the same issue of *Nature*,

MacDonald and Acha-Orbea comment upon the definition and function of SAgS. The foundation for their speculation is based primarily upon a unique property of SAgS—ie, their ability to activate a large population of T cells in a given person by interacting specifically with amino acid sequences on the "variable" domain (V) of the  $\beta$  chain ( $\beta$ ) of the T-cell receptors (TCRs), and, therefore, the TCR V $\beta$  and, specifically in the instance of insulin producing cells, the TCR V $\beta$ 7 receptor. Conrad et al demonstrated a strong overexpression of the V $\beta$ 7 family of cells in the IIT cells from the 2 patients studied, suggesting the presence of a SAg that triggers preferentially V $\beta$ 7 T cells, rather than conventional Ags, in the etiology of IDDM.

SAgs are unique products of ubiquitous bacteria and viruses. They postulated that if the first exposure to the SAg is at a very

early age, potentially autoreactive T-cell clones are inactivated. The silencing of such "dangerous" T-cell clones may then protect against the development of IDDM. If instead the first exposure is several years after birth, many different T-cell clones expressing the same TCR V $\beta$  will become simultaneously activated; and in a genetically predisposed individual, some of these T cells are able to initiate the process that eventually results in the destruction of the  $\beta$  cells of the pancreas.

MacDonald and Acha-Orbea discuss 2 possible theses for how the polyclonal T cell-activating property of SAg could be responsible for the onset of a specific autoimmune disease such as IDDM. Readers who are interested in these alternatives are referred to the excellent presentation by MacDonald and Acha-Orbea, who point out that all SAGs identified to date are the products of either bacteria or viruses. This raises the obvious possibility that the IDDM-associated SAg, if it exists, is of infectious origin. These commentators further state that although the arguments advanced by Conrad et al in favor of SAg involvement in IDDM are thought provoking, they should be hedged with caveats. First, the authors have data on only 2 rather unusual IDDM patients who died rapidly after the onset of disease; and second, neither the SAg nor its putative causative agent has been identified.

Conrad B, et al. Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology. *Nature* 1994; 371:351-355.

MacDonald HR, Acha-Orbea H. Superantigen as suspect. *Nature* 1994;371:283-284.

**Editor's comment:** The concepts presented above are exciting to consider. Thirty-four years ago in 1961, my collaborators and I postulated that IDDM was an autoimmune disease in many instances. This theory, although slow in being accepted, has now been accepted for approximately 20 years. However, the causative factors in the autoimmune process remain unclear. Pursuit of the SAg hypothesis is essential. Recently, some of us received a letter from Dr. Dorothy Becker, in which she requested your potential collaboration in helping elucidate further the possible role of SAGs in the etiology of IDDM. Because your assistance in this elucidation is important, I have invited Dr. Becker to add her own comment below.

Robert M. Blizzard, MD

**2nd Editor's comment:** It is now clear that the development of IDDM in animal models and the majority of humans with the disorder is an autoimmune process that develops in genetically susceptible individuals. Our group has sought an environmental trigger for the induction of this process for the past 15 years. Work from Pittsburgh, as well as that from many groups around the world, has shown epidemiologic associations with a variety of viruses and food components. However, rigorous examination has continually failed to elicit a clear association of IDDM with any one environmental agent. The relatively recent explosion in the application of immunologic techniques to IDDM research and the availability of pancreatic tissue from new-onset IDDM children have allowed the proposal of the SAg theory in the etiology of IDDM described above. As SAGs have been invoked as causative agents in other autoimmune diseases, this theory has some precedent. If a SAg could be proved to be an initial trigger or a subsequent "hit" that either induces or allows the continued progression of the autoimmune process, IDDM theoretically could be prevented by antibiotic treatment of the agent (such as streptococcal disease) or vaccination against the agent. We therefore feel that this avenue of research has to be pursued as we continue our efforts to ultimately prevent the onset of IDDM in children. Fortunately, any given center in the United States does not experience frequent mortality in children with IDDM, which, unfortunately, leads to a major lack of availability of material with which to work. Therefore, Dr. Massimo Trucco and I have requested the assistance of pediatric endocrinologists and pediatricians around the country in obtaining fresh pancreatic material from any individual who might die at the onset of IDDM. In addition, it is important to investigate individuals from different regions of the country to ensure that Dr. Trucco's findings in 2 children who came from the same area are applicable over a wider geographic region. We feel that Dr. Trucco's work in the immunogenetics division of the Children's Hospital and University of Pittsburgh is extremely exciting, and we hope we can get the assistance and support of pediatricians around the country, which would allow its rapid continuation and progress. Dr. Trucco can be reached at (412)692-6570, or one of our colleagues can be reached at any time through the operator at Children's Hospital of Pittsburgh, (412)692-5325.

Dorothy Becker, MD

## Cognitive Abilities Associated With the Silver-Russell Syndrome

The developmental status of 25 children between 6.0 and 11.8 years of age (20 males, 5 females) with the Silver-Russell syndrome was evaluated. Based on assessment of the father's occupation, more than half of the children were from middle class and upper socioeconomic groups and the ascertainment bias of sample would, if anything, be expected to have identified children with above average abilities. Of the 25 children, only 3 (12%) had full-scale IQ scores above average (IQ >116 to 130); 9 (36%) scored within average range of abilities (IQ 85 to 115); 5 (20%) had scores in the range of borderline mental retardation (IQ 70 to 84); and 8 (32%) had scores in the range associated with mild to moderate learning disability (IQ <70). There was little variation between the mean full-scale IQ

of  $85.9 \pm 23.7$  (using the Wechsler Intelligence Scale for Children [WISC]), the mean verbal IQ of  $89.3 \pm 22.6$ , and the mean performance IQ of  $84.3 \pm 23.5$ . The authors reported that mean performance IQ scores were lower in girls than in boys; however, the number of females (n=5) studied was small.

The IQ scores correlated best with head circumference measured at the time of the test. The 3 children with superior IQ scores all had normal head circumferences for chronologic age. Utilizing the Neale analysis of reading ability, the mean reading comprehension was delayed relative to chronologic age by 15.4 months, accuracy by 14.4 months, and rate of reading by 13.8 months. Twelve of the 25 children required special education or remedial assistance.

The study indicates that as a group, children with the Silver-Russell syndrome have an average IQ that is 1 standard deviation (SD) below that of the general population; one third of these have a developmental ability classified within the learning disability range.

Fifteen of the children were treated with growth hormone (GH). The mean change in height SD score between diagnosis and testing was +1.97 in GH-treated children and 0.32 in untreated children, suggesting that GH increased the growth of the treated children. Data concerning bone age advancement and similar parameters were not reported as part of the study.

Lai KYC, et al. *Arch Dis Child* 1994;71:490-496.

**Editors' comment:** These data will be useful in counseling the parents of children with this syndrome and in planning early educational intervention for those children who require remediation. Surprising is the fact that there is as much mental retardation and learning disability as is reported here. Other investigators have alluded that mental retardation might be part of the syndrome upon occasion, but the data have been exceedingly limited. This, of course, was why the study was undertaken by the authors and why it is of significant value. Differences found between the males and females, however, must be interpreted cautiously because of the very small number (5) of females in the study.

Surprisingly, 4 of 18 of the children were found to have concomitant GH deficiency (GHD). GHD has been recognized in

the past as occurring very occasionally in the Silver-Russell syndrome, but there are probably not more than 6 to 10 cases in the world's literature of patients with Silver-Russell syndrome having GHD. At least one of those had an associated craniopharyngioma. The authors' reports concerning the results of GH therapy are very limited in this article. The patients reportedly did have an increase in the mean change in height SD score of +1.97. No reference is made as to whether the patients treated included any or all 4 of the patients with GHD. There is no discussion of the therapeutic regimen, nor of the advancement or delay in bone age, or, therefore, any potential change in predicted height. In our opinion, this portion of the presentation should have been omitted because it suggests that GH treatment may be effective in patients with Silver-Russell syndrome when no data are given to permit evaluation of that possibility. We have repeatedly observed that patients with Silver-Russell syndrome treated with GH grow at an increased rate for the first 1, 2, or 3 years; frequently, however, the patients have marked slowing of growth while on GH and proceed to grow at a rate less than the pretreatment rate—even though GH is given at progressively increased doses. We rush ahead to say that our impressions are exactly that, impressions, and an inadequate number of patients have been given treatment over a period of 7 to 10 years to permit evaluation of the true response to GH treatment. Such studies do need to be done.

Allen W. Root, MD, and Robert M. Blizzard, MD

## Molecular Basis of Mammalian Sexual Determination: Activation of Müllerian Inhibiting Substance Gene Expression by SRY

The pathway of male sexual development in mammals is initiated by SRY, a gene on the short arm of the Y chromosome. Its expression early in the differentiating gonadal ridge directs testicular morphogenesis from the bipotential gonadal anlagen. The testis then produces testosterone from the Leydig cells and müllerian inhibiting substance (MIS) from the Sertoli cells. The latter prevents the müllerian system from developing into a uterus and fallopian tubes. There is a gene for SRY and a gene for MIS. Functional studies of SRY in a cell line taken from the embryonic gonadal ridge revealed that its activation leads to expression of MIS. SRY molecules containing mutations producing human sex reversal have altered structural interaction with DNA and fail to induce transcription of MIS.

The molecular mechanisms of interaction between SRY protein, DNA, and the target gene or genes regulated by SRY have not been identified. The investigators did demonstrate that there is a connection between SRY and MIS, and they provide evidence for an intervening factor or factors, which are designated SRYIFs and which are interposed in the action between SRY and the MIS promoter. Their studies permitted them to conclude that SRY induces expression of the human MIS promoter in vitro. Mutation at SRY-168 abolishes the transcriptional response of the MIS promoter to SRY. Although mutation of the MIS promoter region itself diminished binding of SRY to the promoter, there was no diminished transcriptional response of the MIS promoter to SRY in the cell line used in vitro. This suggests that SRY-dependent transcriptional activation of the

MIS promoter is indirect, and perhaps occurs through postulated SRYIFs, which may be the primary target genes of SRY.

Haqq CM, et al. *Science* 1994;266:1494-1500.

**Editor's comment:** Readers who are intrigued with sexual differentiation will find this article most elucidating. The data presented are important for their concepts. The reviewer suggests that this article be read in conjunction with a review by Bogan and Page, entitled "Ovary? Testis? — A Mammalian Dilemma" (*Cell* 1994;76:603-607).

Dr. Alfred Jost started the story rolling in 1953 when he discovered that the presence of testes during mammalian embryogenesis results in male differentiation of both the internal sex organs and external genitalia. The story is obviously still rolling. Today's findings are as exciting in explaining the mechanisms of sexual differentiation as were Dr. Jost's.

Allen W. Root, MD

### In a Future Issue

#### The Neuroendocrine Landmarks of Puberty

by Jean-Pierre Bourguignon, MD, PhD



## Family History of Alzheimer's Disease May Increase Risk of Birth of Children With Down Syndrome

A progressive neuropathy is seen in adult individuals over the age of 40 with Down syndrome (DS) that is similar to that seen in Alzheimer's disease (AD).<sup>1</sup> The brain pathology seen in both disorders has led to the suggestion that DS and AD may be genetically related. Heston et al<sup>2</sup> discussed an association between the incidence of DS and AD in some families. Van Duijn et al<sup>3</sup> reported an increased frequency of DS births in the families of individuals with AD, as well as an increased incidence of AD in relatives of individuals with DS.

In a recent report by Schupf et al,<sup>4</sup> the parents' history of dementia was examined in families of 96 adults with DS and 80 adults with other forms of mental retardation. Schupf et al postulated that since most of the nondisjunction events leading to trisomy 21 in DS are maternal, there would be an associated increased frequency of AD among mothers but not fathers of individuals with DS.

They studied the families of 96 adults with DS and separated the groups of mothers into those who gave birth at over the age of 35 and those under the age of 35. An increase in dementia was found among the mothers of DS probands in both groups when compared with the mothers of individuals with other types of mental retardation. This increase was more significant in the younger group (<35 years) than in the older group of mothers. Schupf and colleagues suggest that familial aggregation of dementia among mothers of adults with DS supports the hypothesis of genetic susceptibility to both disorders.

After the age of 35, the risk for having a child with DS increases. In mothers under the age of 35, the incidence of DS births is much lower. Schupf et al suggest that a susceptibility factor probably related to accelerated aging may be playing a

role in the birth of individuals with DS in the study cohort of mothers under the age of 35. They also suggest that a family history of AD should be considered as a risk factor for DS birth, especially in women under the age of 35.

1. Wisniewski KE, et al. Alzheimer's disease in Down syndrome: clinicopathological studies. *Neurology* 1985;35:957-961.
2. Heston LL, et al. Dementia of the Alzheimer type: clinical genetics, natural history and associated conditions. *Arch Gen Psychiatr* 1981;38:1085-1091.
3. Van Duijn CM, et al. Familial aggregation of Alzheimer's disease and related disorders: a collaborative reanalysis of case-control studies. *Int J Epidemiol* 1991;20(suppl 2):S13-20.
4. Schupf N, et al. Increased risk of Alzheimer's disease in mothers of adults with Down's syndrome. *Lancet* 1994;344:353-356.

**Editor's comment:** Over the last few years, research has led to the recognition of different susceptibility factors for a number of genetic disorders. The report by Schupf et al is interesting in that an association between a chromosomal disorder and a neurologic disorder has been made. These 2 conditions are related by the histopathologic findings of the brain. However, with an increased risk for DS in the AD families, other causative factors may be found. This may be just a scratch on the surface of other possible associations. The next question to explore is what causes the susceptibility and whether it can be modified.

Judith G. Hall, MD

## Neurofibromatosis Type 1 Due to Germ-Line Mosaicism in a Clinically Normal Father

Two siblings with neurofibromatosis type 1 (NF1) were born to normal parents. There was no family history of this disease. Both had a 12-kb deletion of the *NF1* gene, which is located on chromosome 17; *NF1* genes in the lymphocytes of both parents were normal. Nevertheless, the intragenic deletion of *NF1* in the patients was of parental origin. Analysis of DNA from the father's spermatozoa revealed that 10% of the sperm had the typical *NF1* deletion that was present in his affected offspring. This father had an isolated mutation in *NF1* confined to a small number of germ cells.

Lázaro C et al. *N Engl J Med* 1994;331:1403-1407.

**Editor's comment:** The *NF1* gene encodes a peptide known as neurofibromin that is homologous to ras guanine triphosphatase. Approximately 50% of patients with *NF1* arise from fresh mutations; in 90% of the patients with a sporadic mutation in *NF1* the mutation has occurred in the paternally derived *NF1* allele. The data presented in this abstract illustrate the role of postzygotic mutation, which may lead to somatic and/or germ-line mosaicism in the cells programmed for gamete formation (Bernards A, Gusella JF. *N Engl J Med* 1994;331:1447-1449). Since spermatogenesis is an ongoing and active

process, errors might be expected to occur more commonly in genes of paternal than of maternal origin. One wonders if the incidence of male germ-line mosaicism may be greater than suspected.

Allen W. Root, MD

### Editorial Board

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## The Mouse Obese Gene and Its Human Homologue

The structure of the *ob* gene in mice, which is associated with obesity and type II diabetes mellitus, was determined. The mouse *ob* gene codes for a 167 amino acid peptide. Amino acids 1 to 21 are likely to be a signal sequence, suggesting that the mature protein has 146 amino acids. In accord with the thesis that adipose tissue secretes a substance acting upon the hypothalamus to suppress appetite, messenger RNA of the *ob* gene is expressed only in white adipose tissue in the mouse. In the homozygous *ob/ob* mouse, a mutation in codon 143 prevents translation of this gene product. The *ob* gene homologue is found in the rat, rabbit, vole, eel, sheep, pig, and cow and in humans. The human homologue of *ob* gene product also has 167 amino acids and is 84% homologous with the mouse gene product. The hypothesis examined by Zhang et al<sup>1</sup> is that this peptide is a product of the fat cell and that it is secreted and serves as a negative feedback signal to the ventromedial nucleus of the hypothalamus, thereby establishing a homeostatic mechanism for caloric intake and energy utilization.

Rink,<sup>2</sup> in an editorial appearing in the same issue of *Nature*, provides an excellent review of the current concepts pertaining to the possibility that such a protein exists. He states that localized damage to the hypothalamus, which is the main control center for satiety and energy expenditure, produces obesity similar to that observed in the genetic *ob/ob* mouse. He also recounts that rats forced to overeat lay down excessive fat, but when offered a normal diet, they eat less until their normal body weight is restored; and removal of a substantial mass of fat is followed by extra eating, which increases the remaining fat stores. Rink also states that the data of Zhang et al support the concept of a fat-derived satiety factor, which is the most promising hypothesis of several that have been proposed. The postulated *ob* protein is likely to be a fat-derived molecule with a long half-life that acts on the hypothalamus to exert long-term overriding control of appetite and, most likely, fuel storage and energy expenditure.

Some cases of morbid obesity in humans may reflect a homozygous condition analogous to that in the *ob/ob* mouse where the protein is not produced. The more common forms of obesity might reflect subnormal production of the protein.

1. Zhang Y, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425-432.
2. Rink TJ. In search of a satiety factor. *Nature* 1994;372:406-407.

**Editor's comment:** The theories concerning the regulation of obesity include: (1) lipostasis, which is the synthesis and secretion by fat of an agent that inhibits appetite at the level of the hypothalamus; (2) glucostasis, which is a theory that blood glucose values regulate the body energy stores by acting on the hypothalamus; (3) body temperature control of energy utilization and fat storage; and (4) dilution of a hypothetical fat-soluble factor that inhibits feeding. Under this thesis, the greater the body fat mass, the greater the storage of this lipophilic agent, which theoretically lowers its circulating levels and lessens its inhibitory influence on feeding. The reported data do lend support to the lipostasis theory, although the secretion, biologic activity, and mechanism of action of this agent have not been determined.

There are 6 genes that have been associated with obesity in the mouse, which means that much more work is necessary to determine the role of all of these, and possibly other, genes. The biologic activity of the *ob/ob* protein and its pattern of regulation of secretion also need to be determined before therapeutic approaches become evident in the clinic. If this gene product proves to be a satiety factor, an exciting period of experimental observations and therapeutic effort is beginning.

There is much need in this area. Zhang et al have opened the door for this research.

Allen W. Root, MD

## Estrogen Levels in Childhood Determined by an Ultrasensitive Recombinant Cell Bioassay

An extraordinarily sensitive (0.02 pg/mL estradiol equivalence) bioassay for the measurement of serum/plasma concentrations of estrogen was developed by the investigators. The unknown plasma and standard samples of estradiol are incubated with transformed yeast. An increase in activity of  $\beta$ -galactosidase is determined to measure the response of estrogen in a sample. Overexpression of the estrogen receptor accounts for the extreme sensitivity. Other factors contribute. Specificity for estradiol is surprisingly great, and variants such as ethinyl estradiol, estradiol sulfate, estradiol glucuronide, estrone, estradiol, and diethylstilbestrol are recognized only to a slight extent (<3%). At an estradiol concentration of 2 pg/mL, the intra-assay and interassay coefficients of variation were 15% and 13%, respectively.

In 21 prepubertal girls, aged 5.5 to 10.5 years, serum estradiol concentrations measured by the assay were  $0.6 \pm 0.6$  pg/mL estradiol equivalents, with a range of <0.02 to 2.2 pg/mL. In 23 prepubertal boys, aged 4.5 to 13.0 years, the concentrations were measured at  $0.08 \pm 0.2$  pg/mL, with a range of <0.02 to

0.7 pg/mL. Thus, bioactive estradiol levels were substantially greater in prepubertal females than males ( $P < 0.05$ ).

Klein KO, et al. *J Clin Invest* 1994;94:2475-2480.

**Editor's comment:** This bioassay is 100-fold more sensitive than the most sensitive of established radioimmunoassays for estradiol. Its specificity for estradiol was unexpected but is exceedingly useful since estradiol is the principal endogenous estrogen in children and adolescents. The higher levels of estrogen in prepubertal girls than in boys, as determined by this assay, may explain some of the variations in the growth patterns of the 2 sexes, such as earlier onset of the growth spurt in girls, earlier pubertal maturation of the hypothalamic-pituitary axis, and more rapid advancement of skeletal maturation.

The assay is technically demanding and tedious, but offers promise for the evaluation of the dynamics and regulatory controls of estrogen secretion in infancy, childhood, and early adolescence. An assay with this sensitivity has long been needed.

Intriguingly, previously unanswered questions in all probability will now be answered. Application of this methodology to measurement of other substances present in small amounts in other body fluids is anticipated. The importance of this article is 2-fold: (1) it sets the precedent for a new type of assay for measuring exceedingly small quantities of substances in fluids; and

(2) estrogen is measured in serum at levels never before attainable. Readers may wish to refer to the original article for details concerning the methodology. Congratulations Dr. Klein and collaborators.

Allen W. Root, MD

## Identical Mutations in the *FGFR2* Gene Cause Both Pfeiffer and Crouzon Syndrome Phenotypes

Pfeiffer and Crouzon syndromes are 2 well-characterized autosomal dominant malformation syndromes. Both exhibit craniosynostosis, or premature fusion of the skull bones. Patients with Pfeiffer syndrome also manifest digital abnormalities, including broad, medially deviated great toes and thumbs and variable degrees of syndactyly or brachydactyly of other digits. Although subtle differences exist in the craniofacial features, it is the presence or absence of digital abnormalities, which typically breeds true in families, that distinguishes the 2 syndromes.

Very recently, mutations in the fibroblast growth factor receptor 1 (*FGFR1*) gene have been found in Pfeiffer syndrome and mutations in the *FGFR2* gene have been identified in Crouzon syndrome. The mutations map to similar locations in the respective genes. Surprisingly, Rutland et al now have demonstrated that both Pfeiffer and Crouzon syndromes can result from *FGFR2* mutations. Five patients with Pfeiffer syndrome had a mutation identical to one found previously in a patient with Crouzon syndrome, and 1 patient with Pfeiffer syndrome had the same mutation detected in 3 cases of Crouzon syndrome. Furthermore, the amino acid substitutions that resulted involved residues immediately adjacent to one another: Cys 342 and Pro 341.

These observations raise interesting questions about how the same mutations can give rise to 2 syndromes that have been considered distinct. This article and an accompanying editorial discuss several possibilities. One is genetic variation in other loci whose gene products interact functionally with the

mutant *FGFR2* gene product. The interaction between fibroblast growth factors and FGFRs is known to be very complex. Another possibility is a genetic variation in the second "normal" *FGFR2* allele or a variation occurring at another location in the same "mutant" *FGFR2* allele.

Rutland P, et al. *Nat Genet* 1995;9:173-176.

**Editor's comment:** Ignorance is a wonderful thing. In the absence of facts it is easy, even fun, to speculate about how diseases come about. Indeed, as pointed out in Mulvihill's editorial, the classic genetic concepts of variable expressivity and pleiotropy have served us well to explain phenomena such as reported here. Differences in genetic background also were commonly invoked to explain such observations. However, as knowledge chips away at ignorance, our explanations must be revised accordingly. The findings reported here provide an excellent opportunity to explore specific mechanisms by which identical mutations can produce seemingly different clinical syndromes. In any event, it is now clear that signaling through the *FGFR2* receptor is very important to both craniofacial and limb development and that alterations in the extracellular domains of this receptor protein can lead to well-defined malformation syndromes.

William A. Horton, MD

Mulvihill JJ. Craniofacial syndromes: no such thing as a single gene disease. *Nat Genet* 1995;9:101-102. Editorial.

## Deconvolution Analysis of Spontaneous Nocturnal Growth Hormone Secretion in Prepubertal Children With Preterminal Chronic Renal Failure and With End-Stage Renal Disease

Tönshoff et al studied spontaneous nocturnal growth hormone (GH) secretion in 12 children with end-stage renal disease (ESRD) and in 11 children with preterminal chronic renal failure (CRF), and in a control group of 12 matched children with idiopathic short stature (ISS). All subjects were prepubertal. The children with ISS were defined by normal plasma GH responses to pharmacologic stimulation and height  $\leq 2$  standard deviations (SD) below age- and sex-matched normative values and the exclusion of any endocrine or other metabolic disorders. The children with preterminal CRF were defined as those with a glomerular filtration rate (GFR) of  $< 70$  mL/min/1.73m<sup>2</sup>. Children with ESRD were on regular continuous ambulatory peritoneal dialysis. All had growth retardation defined

as a height SD score for chronologic age  $\leq 2$  and a height velocity SD score for chronologic age  $\leq 0$ . The primary renal disease in these children was renal dysplasia/hypoplasia (n=8) and chronic glomerulopathy (n=6). Patients with renal disease were receiving vitamin D, water-soluble vitamins, oral phosphate binders, and oral sodium bicarbonate; they did not receive glucocorticoids, immunosuppressants, or clonidine.

All subjects underwent blood sampling (0.5 mL) every 20 minutes for 10 hours, beginning at 2000. Multiparameter deconvolution analysis was used to determine the number, duration, amplitude, and mass of GH secretory bursts, and to estimate the subject-specific GH half-life in children with preterminal CRF and ESRD. Data were reported as means

± standard error of the mean (SEM) and nonparametric tests were used to determine statistical significance.

Age, weight, height, height velocity, and body mass index did not differ between ISS controls, children with preterminal CRF, and those with ESRD. Bone age was delayed to a comparable degree in all 3 groups. The mean endogenous GH half-life in patients with ESRD was significantly higher than in ISS controls, while that in preterminal CRF patients was shorter than the value in ESRD patients but significantly higher than in ISS controls. A significant inverse linear correlation was observed between GFR and GH half-life. The mean number of detectable GH secretory bursts in ESRD children was greater than in ISS controls and in patients with preterminal CRF, while the interburst interval, the half-duration of the GH secretory burst, and the mean burst amplitude were not significantly different among groups. GH production rate (product of the mean mass of GH secreted per burst and the number of bursts/10 h) was significantly greater in ESRD patients than in CRF patients and tended to be higher than in ISS controls. Both mean and integrated plasma GH concentrations were significantly elevated in the ESRD group compared with the other 2 groups. However, plasma insulin-like growth factor 1 (IGF-1) levels did not vary among groups.

The authors state that the mechanism for increased GH secretion in uremia is unknown, but that the increased frequency of GH bursts is consistent with reduced somatostatinergic inhibitory tone. They theorize that such a decrease in tone could be due to either reduced hypothalamic or pituitary feedback action of IGF-1 or GH itself. IGF bioactivity is known to be reduced in uremic plasma due to the accumulation of binding proteins that are normally removed by renal filtration. This reduced

bioactivity presumably leads to reduced feedback potency of IGF-1. Thus, the increased number of GH bursts, despite the increased GH concentrations, suggests tissue resistance to the actions of GH at the level of the hypothalamus. The authors speculate that the increased number of GH secretory bursts results from attenuated bioactive IGF-1 or GH feedback of the somatotrophic axis and that this suggests an insensitivity to the action of GH in uremia.

Tönshoff B, et al. *Pediatr Res* 1995;37:86-93.

**Editor's comment:** This is a very sophisticated analysis of the abnormalities in the GH/IGF-1 axis in children with CRF and ESRD. The authors point out that deconvolution analysis of spontaneous nocturnal GH secretion is exceedingly important to understanding the mechanism of GH secretion and its physiology and pathophysiology in a variety of disorders as well as in normal individuals.

This particular study answers previous questions concerning the observation of normal IGF-1 and elevated GH levels in children with CRF and ESRD. It also aids in understanding the feedback mechanisms regarding GH and IGF-1 at the level of the hypothalamus and pituitary. However, what remains uncertain is why exogenous GH is so useful in stimulating linear growth in children with CRF. It would be interesting to perform studies similar to those reported in this paper on children before and after exogenous GH administration. Such studies might delineate how exogenous GH affects the GH/IGF-1 axis and its effect on tissue sensitivity to the actions of GH at the level of the hypothalamus.

William L. Clarke, MD

## The Detection of Subtelomeric Chromosomal Rearrangements in Idiopathic Mental Retardation

About 3% of the population has an IQ <70; a cause is known in less than half. Chromosomal abnormalities identified by routine cytogenetic analyses account for an estimated 40% of severe mental retardation (MR) and an estimated 10% to 20% of mild MR. Subtle chromosomal defects that are not evident by routine testing could be responsible for a substantial portion of patients in whom a cause for idiopathic MR is not evident. Recent advances in molecular genetic techniques that allow detection of extremely small portions of chromosomes based on analysis of DNA polymorphisms (variable number of tandem repeats, or VNTRs) make it possible to detect such "cryptic" chromosomal defects.

Flint and colleagues used this approach to study 99 patients with idiopathic MR. Their attention focused on the subtelomeric portions of chromosomes because several known MR syndromes have been mapped to these regions and also because lesions in these regions might be repaired by telomeric repetitive DNA, masking the abnormalities from routine cytogenetic detection.

Using highly informative DNA markers that mapped to 28 chromosome tips (normal male karyotype has 48 short and long arm chromosome tips), they found cryptic rearrangements in 3 patients. One had a de novo deletion on the long arm of chromosome 13 and 2 others had de novo deletions of different sizes on the long arm of chromosome 22.

Thus, they found cryptic deletions in about 3% of the patients with MR whom they studied. The authors pointed out that 20 subtelomeric regions were not analyzed. If this was taken into account, together with the facts that the probes were not completely informative and in some instances mapped to regions not as close to the telomeres as they had wished, they estimated that the true frequency of cryptic subtelomeric deletions in MR is at least 6% and probably higher.

Flint J, et al. *Nat Genet* 1995;9:132-139.

**Editor's comment:** What do mental and growth deficiency have in common? A lot more than sharing the term "deficiency." Both are multifactorial in their causation. The cause is not known in a high percentage of cases in both instances. They are associated with each other in many instances. Easily detectable chromosomal abnormalities are known to cause short stature and mental retardation, as in Down syndrome. Thus, it is not at all unreasonable to speculate that a substantial portion of "idiopathic" short stature might be caused by cryptic subtelomeric rearrangements as with MR. One has to assume that someone is already looking into this matter, and we look forward to the results.

William A. Horton, MD



## Normal Final Height After Treatment for Acute Lymphoblastic Leukemia Without Irradiation

Twenty-eight Dutch children (16 males, 12 females) with acute lymphoblastic leukemia (ALL) who did not have involvement of the central nervous system and who had achieved long-term remission after 1 course of treatment were followed. Median age at diagnosis was 4.4 years, with a range of 2.2 to 12.7 years. A variety of chemotherapeutic (CT) regimens were used, with a mean duration of treatment of 3.1 years and a range of 3.0 to 5.2 years. Treatment was administered between 1970 and 1986, with reevaluation 5 or more years after the completed therapy. Their heights at time of diagnosis were normal, declined significantly ( $P < 0.006$ ) during treatment, and then accelerated during the first 2 years after completion of therapy (Table 1).

Twenty-two children achieved final height. In 18, final height was greater than midparental target height, which was very encouraging. Body proportions, including sitting measurements, were normal. The therapeutic regimens employed for the management of ALL did intensify over the 16 years of treatment covered in this study, but no relationship between the treatment program and final height was demonstrable. The data support the concepts that: (1) CT influences growth negatively during treatment; (2) catch-up growth occurs; (3) final height is normal and sometimes even better than expected based on target height; and (4) body proportions are normal in those who attain normal final height. The authors did emphasize that further investigations are required to evaluate the influence of newer and more intensive CT regimens on linear growth and final height.

Holm K, et al. *Acta Paediatr* 1994;83:1287-1290.

Table 1  
Height Standard Deviation Scores  
(n=28)

|        | Diagnosis      | Completion<br>of<br>Treatment | Year           |                |                |
|--------|----------------|-------------------------------|----------------|----------------|----------------|
|        |                |                               | 1              | 2              | 5              |
| Median | -0.01          | -0.17                         | 0.11           | 0.24           | 0.29           |
| Range  | -2.1<br>to 2.8 | -1.0<br>to 2.6                | -0.9<br>to 2.8 | -1.3<br>to 2.9 | -1.3<br>to 2.7 |

**Editor's comment:** Cranial irradiation unequivocally has adverse short- and long-term effects on the growth of children with ALL. The present data strongly suggest that CT alone does not permanently impair the growth of children who achieved a sustained remission after 1 course of treatment. The authors cite several other studies that support these findings. They also refer to an article by Sklar et al (*J Pediatr* 1993;123:59-64), who reported that CT alone led to a significant reduction in final height (-0.49 standard deviation score). Katz et al (*J Pediatr* 1991;118:575-578), among others, observed no significant effect of CT alone on final height of children with ALL. The reason for the discrepant observations of the various groups is not obvious; however, the consensus of multiple studies is in accord with that of Holm et al.

Allen W. Root, MD

## Delayed Adolescent Growth in Homozygous Sickle Cell Disease

This paper reports longitudinal observations of height in a Jamaican cohort of children with homozygous sickle cell (SS) disease, sickle cell hemoglobin C (SC) disease, and normal (AA) hemoglobin. The subjects were identified by neonatal screening of 100,000 consecutive deliveries at a major maternity hospital in Jamaica and included 315 children with SS disease, 201 with SC disease, and 250 AA controls (aged 11 to 18 years at the time of the study). All were followed prospectively every 3 months at the sickle cell clinic. The analysis was confined to postpubertal children with observations available to the age of 16 years. Parenthetically, 3 SS males with extreme retardation of sexual maturation who were growing normally for their bone age were excluded from the analysis as they were prepubertal and 29 of the SS group were excluded for various reasons. The final analysis included 44 SS patients (mean age,  $17.9 \pm 0.6$  years; 21 males, 23 females), 44 SC individuals ( $17.3 \pm 0.8$  years), and 44 AA control children ( $17.9 \pm 0.5$  years).

Height was measured at 3-month intervals in SS and SC children and every 6 months in AA controls using a Harpenden stadiometer. In addition, sitting height was measured for SS and AA, but not SC children, at 6-month intervals using a sitting height table (Holtain Instruments). Tanner staging of the SS and AA subjects was assessed at 6-month intervals from the age of 8 years. The data was fitted by computer to the

Preece-Baines model 1 (described in the article), which has been used previously to fit longitudinal height data. This particular mathematical model has the advantage of not requiring subjects' final height. It resolves complex growth curves into a variety of biologically meaningful parameters, including age of onset of the adolescent growth spurt and the age of peak height velocity. Multivariate analysis of variance was used to compare the growth parameters of the different groups. The significance levels were adjusted using Bonferroni's method of correction.

The data demonstrate that SS patients have a 1.4-year delay in mean age of initiation of the adolescent growth spurt, a 1.6-year delay in mean age at peak height velocity, and a lower height velocity at the time of the onset of the growth spurt compared with AA controls. The first pubertal changes (Tanner stage 2) occurred at  $12.8 \pm 1.6$  years in SS males and  $12.0 \pm 1.8$  years in SS females, compared with  $11.1 \pm 1.2$  years in AA males and  $10.1 \pm 1.2$  years in AA females. Adjusting for the age of onset of the growth spurt or peak height velocity reduced the genotype difference from 1.8 to 1.2 years. The delay in onset of puberty in SS patients compared with AA controls correlated with their delay in peak height velocity. The age of menarche in SS girls was significantly later than in AA girls ( $15.4 \pm 1.3$  vs  $13.1 \pm 1.3$  years;  $P < 0.001$ ). After adjusting for the delay in the adolescent growth spurt, the genotype difference in age of menarche was no longer significant.

The authors suggest that error could be introduced into their data by studying children as early as 1 year of age, inaccuracy in measuring young children, measurement error due to observer variation over the 18-year span of the study, and technical variation arising from changing hairstyles. They further suggest that the etiology of the delays observed may be multifactorial, with contributions from abnormal endocrine function (including hypogonadism), suboptimal nutrition, and increases in metabolism as the result of a high rate of erythropoiesis. Interestingly, these changes do not affect the final heights of these children.

Singhal A, et al. *Arch Dis Child* 1994;71:404-408.

**Editor's comment:** This is a very carefully performed study. The authors are to be congratulated for identifying individuals in the neonatal period and following them through their adolescent growth spurt. It is unfortunate, however, that more information

either was not collected or provided regarding the etiology of the retarded growth or the delay in onset of puberty in these children. The pattern observed seems consistent with constitutional delay of growth and adolescence. It is unclear how abnormal endocrine function, suboptimal nutrition, or increased metabolism could have a transient effect on the onset of puberty and yet not affect final height. Interestingly, there is no mention of rates of infection, number of hospitalizations, or numbers of crises, all of which may have temporarily affected growth in enough children to produce the observed delay in growth. Since the SS children achieve a normal final height, one might question the desirability of continuing research into the etiology of their delay. However, these individuals appear to provide a model of constitutional delay of growth and adolescence that might prove useful in better understanding the physiology of the timing of pubertal events in healthy children.

William L. Clarke, MD

## Gene Therapy for Familial Hypercholesterolemia

In theory, diseases caused by genetic deficiency can be treated by the introduction and expression of a normal gene into the affected tissue. Because of the possibility of a noninvasive and accurate monitoring method for familial hypercholesterolemia (FH), and based on previous promising results of gene therapy in animal models (Chowdhury et al<sup>1</sup>), Grossman et al<sup>2</sup> recently reported the first successful ex vivo gene therapy treatment for FH in a human, specifically, in a 29-year-old woman.

FH is an autosomal dominant disorder caused by a deficiency of low density lipoprotein (LDL) receptors. Patients with FH have very high blood levels of cholesterol that deposits in the coronary arteries and leads to premature coronary artery disease (Brown and Goldstein<sup>3</sup>). The homozygous form of FH is a lethal disorder. It is very hard to treat; however, the progress and response to treatment can be easily monitored by measuring serum lipid profiles.

The protocol reported by Grossman and colleagues<sup>2</sup> was as follows: a partial liver resection was performed on the patient (15% of the total mass) and the liver section was perfused with collagenase to obtain hepatocytes, which were then cultured. The cells were exposed to recombinant retroviruses that had a new gene recombined into their DNA that contained the LDL receptor. The genetically-corrected hepatocytes were harvested and infused back into the patient via the inferior mesenteric vein, leading to their deposit in the liver. The patient's serum lipid profile was measured before and after treatment. Two

weeks after the procedure, the ratio of LDL to high density lipoprotein (HDL) was noted to drop from 10:13 to 5:8. The patient remained stable for 18 months without further complications. The authors concluded that hepatic reconstitution of LDL receptor expression is sufficient for metabolic correction.

1. Chowdhury JR, et al. Long-term improvement of hypercholesterolemia after ex vivo gene therapy in LDLR deficient rabbits. *Science* 1991;254:1802-1805.
2. Grossman M, et al. Successful ex vivo gene therapy directed to liver in a patient with a familial hypercholesterolemia. *Nat Genet* 1994;6:325-341.
3. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986;232:34-37.

**Editor's comment:** The use of gene therapy such as that reported here is encouraging. In FH, the liver is an easy organ to target. Successful gene therapy in disorders primarily involving a specific organ may be easier to achieve than gene therapy for disorders that affect many systems. The possibility of removing cells from the affected individual, treating them, and then reinserting them avoids the possibility of rejection and further complications related to immunosuppression. Unfortunately, the same type of approach cannot be easily used in cystic fibrosis.

Judith G. Hall, MD

## Gene Therapy for Cystic Fibrosis

Cystic fibrosis (CF) is a common autosomal recessive disorder. It is characterized by gastrointestinal and respiratory symptoms. The pulmonary complications of CF include mucus plugging and chronic bacterial infections. Ninety percent of CF patients die of respiratory complications. Because the high mortality of CF is related to respiratory symptoms, the lungs have been the logical target for gene therapy, as reported by Cutting.<sup>1</sup>

CF is caused by a mutation of the CF transmembrane conductance regulator (CFTR) gene on chromosome 7. The CFTR gene codes for a transmembrane channel on the surface of the epithelial cells that affects electrolyte transport and balance. CFTR mutations result in the mislocalization of the protein or in reduced function at the membrane that leads to an abnormal electrolyte exchange and, consequently, very thick pulmonary and intestinal secretions.

Earlier work on gene therapy for CF was directed at the respiratory epithelial cells of mice. Human epithelial airway tissue is one site of the expression of the disorder. However, targeting respiratory epithelial cells has been difficult, mainly because the epithelial tissue is composed of a number of different cells at different stages of differentiation, and it is unclear which are the cells that express the defective *CFTR* gene.

Recently, Crystal et al<sup>1</sup> reported their results of gene therapy with a recombinant adenovirus vector (AdCFTR) containing the human *CFTR* cDNA, administered to the respiratory tracts of 4 individuals with CF. All individuals had baseline evaluation of the respiratory epithelium before and after the administration of AdCFTR. The AdCFTR was then inhaled by the patients. The number of "corrected cells" was difficult to assess, but the epithelial cells did express the corrected *CFTR*. The authors concluded that it is feasible to use an adenovirus vector to introduce the gene and to achieve expression of the normal human *CFTR* in the epithelial tissue in a living patient. They point out, however, that their study does not establish whether this therapy will be successful in treating the common respiratory symptoms of CF or whether incorporation will be stable for long periods. No change in respiratory function was noted.

1. Cutting GR. Two steps closer to gene therapy for cystic fibrosis. *Nat Genet* 1992;2:4-5.
2. Crystal RG, et al. Administration of an adenovirus containing the human *CFTR* cDNA to the respiratory tract of individuals with cystic fibrosis. *Nat Genet* 1994;8:42-51.

**Editor's comment:** Gene therapy for CF has proven "tricky." Animal studies have been encouraging in that the gene can be incorporated, so the next step was to try gene therapy in humans. However, since CF involves the lung, pancreas, and other organs in humans, the incorporation of the gene is harder to assess. It is unclear whether the respiratory symptoms accessible for monitoring improve after gene therapy, and it may be that the improvement is only temporary. The dosage of gene therapy has been problematic as well. Perhaps the "trick" is to concentrate on targeting only one organ for treatment and involve the stem cells. The question then becomes what organ or which tissue in that one organ should be targeted?

Judith G. Hall, MD

## Body Composition and Spontaneous GH Secretion in Normal Short Stature Children

In adults, the effect of obesity in suppressing growth hormone (GH) release has been well studied over 30 years. In children, only a few studies to determine the effect of obesity on GH release have been published. Abdenur et al attempt to fill that void in the study reported in this article.

Fifteen pubertal and 22 prepubertal short normal children were studied in relation to auxologic parameters, with emphasis on the measurements of body fat (BF) composition and the relationship of BF with spontaneous GH secretion (SGHS) over 12 hours at night.

A significant negative correlation between the degree of adiposity and mean SGHS was reported. A strong negative correlation was demonstrated with %BF as determined by bioelectrical impedance and BF mass index (BFMI), which is calculated as BF in kilograms/height in meters squared (Figure 1). Females required greater adiposity levels than males to decrease SGHS in pubertal subjects. Correlation between SGHS and BF was best with the mean pulse amplitude in pubertal subjects and the number of pulses and the sum of pulse amplitudes in prepubertal subjects.

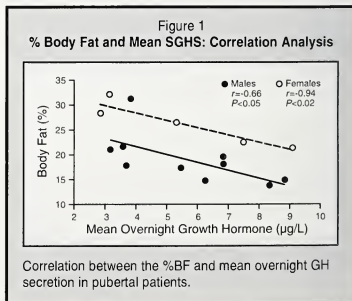
The authors conclude that in normal short-statured children, body composition greatly influences SGHS. Consequently, SGHS levels that appear low may actually be normal for a short child with mildly increased BF, and SGHS values that appear normal may be abnormally low for a lean individual who would be expected to have high SGHS levels because of leanness. This is an extremely important concept for a patient being evaluated for possible relative alterations in GH secretion. These results suggest also that normal values for SGHS must take into account not only pubertal status but also gender and body composition. The use of a mathematical formula that considers SGHS and IGF-1 values has been proposed (Oerter KE et al. *J Clin Endocrinol Metab* 1992;75:1413-1420). However, with a sufficient number of male and female patients, a more practical approach would be the use of confidence intervals to

define normal values of SGHS according to body composition and gender.

Abdenur JE, et al. *J Clin Endocrinol Metab* 1994;78:277-282.

**Editor's comment:** The authors are to be commended on performing and presenting a much needed study pertaining to the correlations of SGHS and various measurements of BF. Space did not permit an elaboration of the different important ways that BF was assessed. GGH readers are encouraged to read this article in its entirety, particularly to attain better comprehension of the various ways that BF can be measured and what those parameters really mean and reflect.

Robert M. Blizzard, MD



## Growth of Short Normal Children in Puberty Treated for 3 Years With Growth Hormone (GH) Alone or in Association With Gonadotropin-Releasing Hormone Agonist (GnRHa)

GH at 0.1 IU/kg/d, 6 days per week ( $\approx 0.2$  mg/kg/wk) was given to 30 early pubertal short normal subjects for 3 years; 16 males, aged  $14.4 \pm 0.8$  yrs; 14 females, aged  $12.2 \pm 1.2$  yrs, all at Tanner stage 2 or 3, with slow pubertal growth ( $4.2 \pm 1.2$  cm/yr), a mean BA delay of 2 years, and no detected GH deficiency or other cause for short stature. Their mean birth length was 48.6 to 49.5 cm at term; the mean of midparental heights was  $-0.6$  to  $-0.8$  SD below the mean of the general adult population. They were randomized in two groups: group A received GH alone; group B received GnRHa for 2 years plus daily GH injections, and on the third year GH alone.

The annual growth velocity (GV) increased during the first year in both groups and sexes, the increase being significant ( $P < 0.01$ ) in group A only. The patients of group A kept an improved GV in the 2nd year, then returned to pretreatment GV in the 3rd year, while completing their sexual development and bone maturation. Their height, expressed as SDS for bone age, improved in the first two years but decreased thereafter. Group B patients returned to pretreatment GV in the 2nd year, and had no significant improvement when treated with GH alone during the 3rd year of the study. They had no significant progress of height for age at any time. Their bone maturation, slow when on the GnRHa accelerated when sexual development resumed.

At the end of the 3 years, height expressed as SDS for age improved in group A from  $-2.5 \pm 0.6$  to  $-1.5 \pm 0.4$  SD in males ( $P < 0.05$ ) and from  $-2.8 \pm 0.5$  to  $-2.1 \pm 0.9$  SD in females (NS). Expressed as SDS for bone age, mean height slightly improved

in males (NS) but not in females. In both groups and sexes, the mean predicted height according to Bayley and Pinneau was only slightly increased at the end of 3 years on GH, with a gain of 2 to 5 cm on the average. There was a wide interindividual variability in these results within each group. Pretreatment characteristics of the patients did not account for individual differences. Annual measurement of plasma insulin-like growth factor 1 (IGF-1) showed different degrees of increase, not correlated with any parameter of the patients' growth.

The authors reached 2 conclusions. First, inhibiting sexual development in short early pubertal subjects has no advantage. This was previously demonstrated with GnRHa alone (see *GGH* 1993;9[4]:13), and now is confirmed for GnRHa plus GH. Second, GH alone, at the dose used, can accelerate for 2 years the growth of such slow-growing normal short adolescents and slightly improve their predicted height in relation to the result of the first year of treatment, but the expected results cannot be overestimated or considered as an indication for any routine use of GH in endocrinologically normal and constitutionally short pubertal individuals.

Job JC, et al. *Horm. Res* 1994;41:177-184.

**Editor's comment:** *The contents of this report will discourage only the most desperately short children from trying to achieve normal height by using GnRHa plus GH.*

Robert M. Blizzard, MD

## Catch-up Growth, Persisting Short Stature, and Adult Height of Children Born Small for Gestational Age

Two recent papers give large-scale data on the natural history of statural growth in children born with intrauterine growth retardation (IUGR), defined as  $-2$  standard deviations below the mean reference values of Usher and MacLean.

One is a longitudinal retrospective study<sup>1</sup> of a cohort of 3,650 healthy individuals born at full term in Sweden who have reached their adult height when arriving in the final grade of school. The growth data of those who were born with weight and length in the normal range were used as reference values. The values in IUGR children ( $n=198$ ) were calculated separately for those born with a subnormal length ( $n=141$ ) or a subnormal weight ( $n=111$ ), and for those born both short and light ( $n=54$ ).

A spontaneous catch-up growth occurred before age 2 years in 87% of the total IUGR group. The 13% whose height remained  $\leq 2$  SD at age 2 years were all from the short-at-birth group. They remained in the subnormal range of height throughout childhood. Their puberty started at a normal time, somewhat early. Their mean final height was  $-1.7$  SD. It is to be noted that midparental height was approximately  $-1$  SD in this non-catch-up group.

The other study<sup>2</sup> reports the final heights of 47 healthy subjects (23 males, 24 females) followed in pediatric endocrine clinics for severe height retardation of prenatal onset. Their mean

birth length at term was  $< -2$  SD. They were referred after the age of 4 years for persistence of a height deficit of  $> 2$  SD. Patients with malformation syndromes and those with subnormal GH responses to usual stimulation tests were not included in the series studied. Puberty started late for chronological age but early for bone age: in males at  $14.2 \pm 0.8$  years with bone age of  $11.9 \pm 0.7$  years and a mean height of  $139.2 \pm 4.5$  cm; in females at  $12.4 \pm 0.7$  years with a bone age of  $10.1 \pm 0.7$  years and a mean height of  $130.9 \pm 6.2$  cm. The mean pubertal growth was  $23.0 \pm 4.0$  cm in boys and  $15.5 \pm 4.6$  cm in girls.

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The 23 males thus reached an adult height of  $161.9 \pm 8.0$  cm, and the 24 females  $147.6 \pm 7.2$  cm. Bone age before puberty or at the onset of puberty was not a valuable individual predictor of final height in these IUGR children. The adult heights correlated significantly with birth length ( $r=0.45$ ), with height at age 2 years ( $r=0.50$ ) and closely with height at the onset of puberty ( $r=0.81$ ), but not with birth weight or with midparental heights.

1. Albertsson-Wikland K, Karlberg J. *Acta Paediatr Scand* 1994; 399(suppl):64-70.
2. Chaussain JL, et al. *Acta Paediatr Scand* 1994;399(suppl): 72-73.

**Editor's comment:** Very few data had been previously reported regarding the statural growth of small-for-date newborns beyond childhood and were somewhat discrepant, probably since they were based on either birth length or weight. This situation was reflected in a study previously abstracted in GGH. In spite of their very different protocols, the two studies summarized here (the second one having been abstracted in GGH previously [1993;9(4):10] agree on the same main facts; (1) in long-term growth studies, birth length, not birth weight, is the criterion to take into consideration for definition of IUGR; (2) in more than 85% of infants born short at term, a spontaneous catch-up growth occurs before the age of 2 years; and (3) those IUGR children who remain short at the onset of

puberty do not experience pubertal catch-up growth. These points seem important for the design of clinical trials using GH or growth-related peptides in IUGR children. The data collected will be useful as historical references when the final heights of IUGR children presently involved in trials with GH will be known.

Jean-Claude Job, MD

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# GROWTH

## Genetics & Hormones

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### Letter From the Editor

#### To Our Readers:

The December 1995 issue (Vol. 11, No. 4) of *GROWTH, Genetics, & Hormones (GGH)* will be an index issue. This cumulative index will list all of the lead articles and each of the literature abstracts published during the last 11 years. The special index issue is in response to multiple requests for such a comprehensive listing—a testament to *GGH's* extensive use as a reference

source. Watch for the December issue—it will be of considerable value to you.

If *GGH* has been a significant resource for you, please be encouraged to write to me, in care of SynerMed, expressing your appreciation to Genentech, Inc. for its support in funding this publication over the years.

For the Editorial Board,

Robert M. Blizzard, MD  
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## The Neuroendocrinology of Puberty

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Central nervous system (CNS) control of the reproductive hormonal axis was well recognized 75 years ago by clinicians caring for patients with hypothalamic lesions and associated disorders of puberty. During the past 20 years, a vast body of information has accumulated about the neuroendocrinology of the reproductive axis. However, the complex changes that combine to trigger the onset of puberty remain a puzzle that is not yet solved. It is hoped that the facts and presumptions presented in this review will provide a framework for the further development and testing of hypotheses to help unravel the mysteries of puberty. The functional, anatomic, and biochemical aspects of the neuroendocrinology of pubertal development will be

discussed sequentially, as well as how these intrinsic hypothalamic processes are modulated by gonadal steroids and extrahypothalamic signals to influence the onset of puberty.

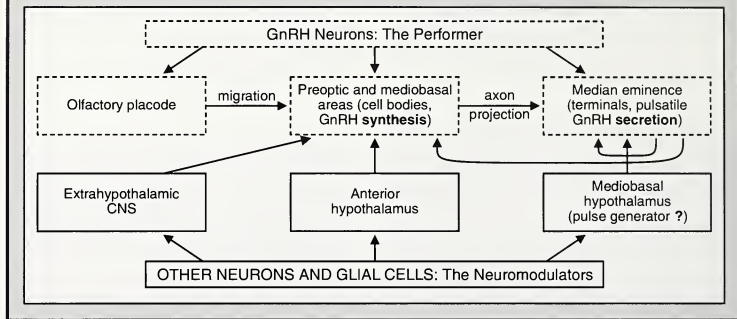
### FUNCTIONAL MANIFESTATIONS OF THE NEUROENDOCRINOLOGY OF PUBERTY

Direct gonadotropin hormone-releasing hormone (GnRH) measurements in vitro from rat hypothalamic and in vivo from the hypophyseal portal circulation of the rhesus monkey support the concept that the onset of puberty is marked by an increase in frequency and amplitude of GnRH secretion.<sup>1,2</sup> In humans, the same conclusion is supported indirectly by sensitive gonadotropin measurements, which have been used to track hypothalamic GnRH secretion in the peripheral circulation. Serum luteinizing hormone (LH) concentrations reflect the secretion of detectable pulses in prepubertal children, the frequency of which increases by 2-fold, particularly at night, during the late prepubertal period.<sup>3-5</sup> In addition, LH pulse amplitude increases as a result of changes in hypothalamic function and pituitary sensitivity to GnRH. As shown by Knobil and coworkers in primates, the increased frequency of intermittent stimulation of the pituitary gland by

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Figure 1  
Anatomic Basis of the Neuroendocrinology of Puberty



GnRH is critical for the appropriate increase in gonadotropin secretion at the onset of puberty.<sup>6,7</sup> Others have confirmed that the receptivity of the gonadotropes to GnRH is frequency-modulated. Thus, studies on the hypothalamic mechanism in the onset of puberty have focused on the control of pulsatile GnRH secretion and its ontogeny.

### THE ANATOMIC BASIS OF THE NEUROENDOCRINOLOGY OF PUBERTY

The hypothalamic involvement in the neuroendocrinology of puberty can be viewed as a 2-compartment model consisting of the GnRH neurons and the neuromodulators released from other neurons and/or glial cells (Figure 1). The GnRH neurons represent the performer, the ultimate structure where most neural factors converge to regulate pituitary-gonadal activity, although some factors might act at the pituitary to moderate or to prime the action of GnRH. GnRH neurons have a fascinating developmental history. They arise outside the brain in the olfactory placode and subsequently migrate during fetal life to the preoptic area in rodents—and even farther in primates—to the anterior hypothalamus.<sup>8</sup> In humans and primates this process takes place during the first trimester of pregnancy, whereas it occurs during the second half of gestation in rodents. This migratory process is complex and likely depends on several factors, one of which has homology with the neural cell adhesion molecule, a member of a family of adhesion factors interacting to attach neurons to axons to defined brain structures during migration or growth processes; it is encoded by a gene on the short arm of

the X chromosome (Xp22.3). When this *Kal-1* gene is deleted or mutated, there is a defect in the migration of both GnRH and olfactory neurons, which gives rise to the hypogonadotropic hypogonadism and anosmia of Kallmann syndrome.<sup>9</sup> From their normal position in the anterior hypothalamus, GnRH cell bodies project a majority of their axons caudally to the median eminence, where they terminate on the hypophyseal portal vessels. GnRH is initially synthesized as pro-GnRH (a precursor), which is prominently present in the GnRH cell bodies. In the male rat, GnRH mRNA and the content of pro-GnRH increase markedly at 20 to 24 days of age, immediately preceding the onset of puberty.<sup>10</sup> During axonal transport, the GnRH precursor is processed into the mature decapeptide form, which is primarily located and stored in the arcuate nucleus-median eminence area.

While there is general agreement that GnRH pulsatility is crucial to stimulate physiologic gonadotropin secretion, there is no consensus as to what constitutes the "pulse generator." An interesting model is provided by GnRH neurons immortalized through targeted tumorigenesis using an oncogene coexpressed with the GnRH gene.<sup>11,12</sup> The intermittent secretion by immortalized GnRH cells in vivo provides evidence that pulsatility could be an intrinsic property of the GnRH neurons.<sup>11,12</sup> The requirement for synchronous secretion to yield the GnRH pulses measured in vitro and in vivo suggests that there is cross talk between GnRH neurons. The concept of such a mechanism is further supported by ultrastructure data revealing interconnections between GnRH neurons in vitro and in vivo. The potential role of GnRH itself as the coordinator of

synchronous activity of a population of GnRH neurons is supported by experiments demonstrating inhibitory autorefeedback.<sup>13,14</sup>

Alternatively, it is possible that a pulse generator distinct from GnRH neurons exists. There is evidence that there is in the rat mediobasal hypothalamus a pacemaker distinct from the GnRH neuron, since LH and GnRH pulsatility continue following disconnection of GnRH axons from their cell bodies in vivo<sup>15,16</sup> and in vitro.<sup>2</sup> This hypothesis is consistent with electrophysiologic recordings in vivo. In primates, Knobil and colleagues have localized the GnRH pulse generator electrophysiologically in the mediobasal hypothalamus.<sup>6,7</sup> However, the nature of the pacemaker has not been elucidated.

The neuromodulators (Figure 1, lower section) signaling to GnRH neurons may originate from neurons or glial cells in extrahypothalamic areas of the CNS, as well as in the anterior and mediobasal hypothalamus, and may exert their effect by impinging on GnRH neuronal cell bodies in the preoptic area and, presynaptically, on GnRH nerve terminals in the arcuate nucleus-median eminence. Anatomic changes in these interactions during development may involve particular processes such as synaptic plasticity or programmed cell death. The role played by glial cells can be pivotal through the production of potentially active peptides such as transforming growth factor- $\alpha$  (TGF- $\alpha$ )<sup>17</sup> or enzymes controlling the biosynthesis and degradation of neuropeptides or neurotransmitters. As an example, we showed very recently that glutaminase, an enzyme produced by astroglial cells and involved in the biosynthesis of glutamate, played a critical role in pulsatile GnRH secretion.<sup>18</sup> In summary, the anatomic basis for the regulation of GnRH synthesis and secretion is complex and includes an array of neuronal-glial elements in the hypothalamus.

## THE BIOCHEMICAL MECHANISMS INVOLVED IN THE NEUROENDOCRINOLOGY OF PUBERTY

Conceptually, puberty may result from either *decreasing inhibition* or *increasing facilitation* of GnRH secretion. Progressive dissipation of inhibition has been considered as the most likely hypothesis since the hypothalamic pulse generator is active during human fetal life, subsequently becomes restrained, and then reactivates.<sup>19</sup> However, in the rat, early activation of GnRH and, consequently, LH secretion preceding the restrained, or so-called juvenile pause, period has not been observed so far, which is in contrast to the observed early activity in primates and humans.

Numerous neuromodulators are known to affect GnRH secretion as neurotransmitters or neuropeptides.<sup>20</sup> Some directly inhibit and others directly

stimulate GnRH secretion. A number of neurotransmitters and peptides are dual regulators, since they show both inhibitory and facilitatory effects in different experimental conditions (Figure 2). Specific anatomic sites probably come into play, as a single neuromodulator may exhibit different effects in the preoptic area than in the mediobasal hypothalamus. Puberty can be viewed as resulting from either a change in the activity of neuromodulators, such as a reduction of inhibition or an increase of stimulation, or, alternatively, a change in sensitivity of GnRH neurons to the effect of neuromodulators—or even as a result of both mechanisms (Figure 2). It is important to remember that most data have been

Figure 2  
Possible Biochemical Regulators and Mechanisms Involved in the Neuroendocrinology of Puberty

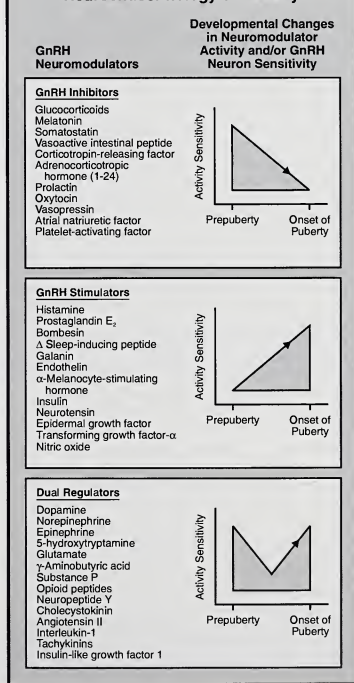
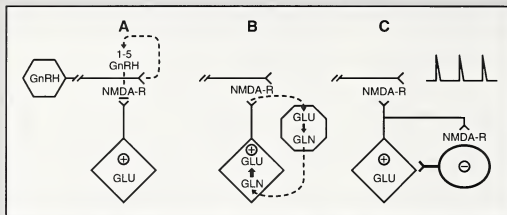




Figure 3



Three different mechanisms could account for loop circuits controlling the interval between episodes of stimulation of GnRH secretion by glutamatergic neurons in the male rat hypothalamus. At onset of puberty, the increase in frequency of pulsatile gonadotropin hormone-releasing hormone (GnRH) release may involve (A) reduction in potency of competitive antagonism at N-methyl-D-aspartate recep-

tors (NMDA-R) by 1-5GnRH, a physiologic degradation product of GnRH; (B) increased rate of restoration of the releasable pool of glutamate (GLU) by glutaminase, the enzyme controlling GLU biosynthesis from glutamine (GLN); and (C) disappearance of inhibitory GABAergic interneurons activated through NMDA receptors.

obtained in the rat, and that the effects of neuro-modulators may be different in rodents than in primates or humans.

Since the GnRH neurons attain full functional capacity early in fetal life, the prepubertal pause of secretory activity of those neurons is likely to involve a superimposed brake driven by distinct neurons, presumably in the pulse generator. Our work in the male rat has highlighted the possible role of glutamatergic neurons in such a mechanism (Figure 3). The developed concept relies on intermittent presynaptic stimulation of GnRH axons by glutamatergic neurons through facilitatory N-methyl-D-aspartate receptors (NMDA-R) so that GnRH secretory pulses are generated. Three different mechanisms theoretically can account for loop circuits controlling the interval between episodes of stimulation of GnRH secretion by glutamatergic neurons (Figure 3). The 1-5GnRH fragment, a physiologic breakdown product of the secreted decapeptide, can act as a competitive antagonist at NMDA receptors and so contribute to the inhibitory autotfeedback of GnRH (Figure 3A).<sup>14</sup> If physiologically relevant, the onset of puberty could involve reduction in potency of, or sensitivity to, that autotfeedback.<sup>13</sup> A second possible mechanism is one based on the increase in glutaminase activity that is observed after the onset of puberty (Figure 3B).<sup>18</sup> Glutaminase controls the biosynthesis of glutamate from glutamine, which is synthesized by astroglial cells from glutamate after reuptake of the excitatory amino acid from the synaptic cleft. It is not known whether the age-related increase in glutaminase activity is causal or consequential to the presumably increased frequency of glutamate secretory discharges. A third possible mechanism is one based on neurotransmitter inhibition of GnRH pulsatility. This inhibitory effect, which is observable only before the onset of puberty, involves glutamate and

NMDA receptors, thus pointing to their dual role in the regulation of GnRH secretion (Figure 3C).<sup>21</sup> Such inhibition is conceivably mediated through interneurons expressing NMDA receptors and inhibitory to the pulse generator. Based on recent observations in the monkey, it can be postulated that those interneurons are GABAergic.<sup>22</sup> At the onset of puberty, disclosure of the facilitatory effect of glutamate might result from the marked reduction in activity of those inhibitory interneurons. We think that the latter mechanism is likely to play a major role at the pubertal onset, while the other mechanisms, which are still working following the onset of puberty, may contribute to the regulation of the frequency of pulsatile GnRH secretion in the adult hypothalamus.

## THE GONADAL ROLE IN THE NEUROENDOCRINOLOGY OF PUBERTAL DEVELOPMENT

The role of sex steroids in puberty has been studied extensively in pursuit of clarifying the gonadostat hypothesis.<sup>23</sup> Since sex steroids undoubtedly influence the neuroendocrine system, it is particularly difficult to delineate whether neuroendocrine manifestations of puberty are primary maturational events in the hypothalamus that account for increased pituitary-gonadal activity or secondary events resulting from increased sex steroid secretion. Currently, the neuroendocrine mechanism of puberty is believed to involve 2 components with respect to the role of the gonads. The gonadal-dependent component is illustrated by the qualitative effects of sex steroids on the activity of opioid peptides, neuropeptide Y, norepinephrine, and dopamine. These neurotransmitters exhibit opposite actions on LH or GnRH secretion in the absence or in the presence of sex steroids.<sup>20</sup> The gonadal-independent component of

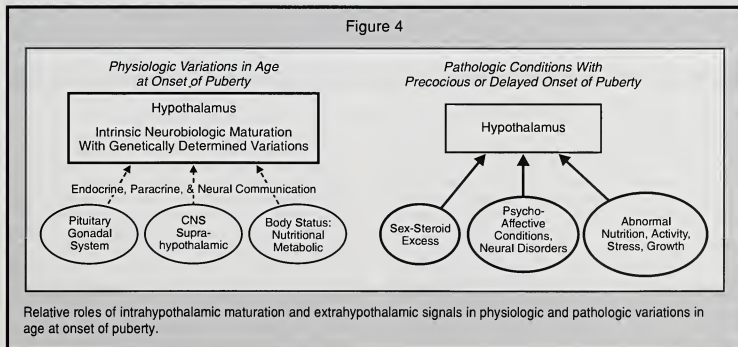
neuroendocrine maturation is illustrated by the data obtained in orchidectomized monkeys<sup>24</sup> and in agonadal patients,<sup>25</sup> who show developmental changes in gonadotropin secretion similar to normal subjects. Gonadotropin secretion is greater during infancy and at the time of adolescence than during the juvenile pause. This gonadal-independent component of maturation is quantitatively dependent on sex steroids since gonadotropin secretion during infancy and adolescence is greater in agonadal patients or animals than in normal subjects. It is critical to consider whether any change in the neuroendocrine control of GnRH-LH secretion at puberty is causal or consequential to increased sex steroid secretion. In the rat, we found that the developmental pattern of changes in NMDA receptor-mediated control of GnRH secretion was similar in both intact and orchidectomized animals, thus indicating that glutamate effects were qualitatively independent of sex steroids.<sup>26</sup> There was also a quantitative effect of sex steroids irrespective of age, since prepubertal and adult orchidectomized animals showed evidence of increased glutamatergic stimulation of GnRH secretion compared with intact animals.<sup>26</sup> Therefore, glutamate effects on GnRH secretion appear to involve an age-related, gonadal-independent component, as well as a gonadal-dependent component.

#### INTRINSIC HYPOTHALAMIC MATURATION AND EXTRAHYPOTHALAMIC SIGNALS: THEIR INFLUENCE IN STIMULATING PUBERTAL DEVELOPMENT

In addition to gonadal factors, the hypothalamus is directly under the influence of the suprahypothalamic CNS and affected by the nutritional and metabolic state of the body (Figure 4). Signals from those

systems may be communicated to the hypothalamus along paracrine, endocrine, or neural pathways. That changes in these impact on the mature hypothalamus is well established, but the signals remain to be clearly identified. However, it is not clear that the same pathophysiologic mechanisms that modulate the adult reproductive axis play any role in the neuroendocrine mechanism of the onset of puberty. This is a most difficult question to study because there are major species and sex differences.<sup>27</sup> For instance, the light/darkness cycle plays a critical role in seasonal breeders, while little or no such effect exists in humans. In humans, the impact of systems external to the hypothalamus seems to be rather low in physiologic conditions, compared with most animal models. Thus, under optimal conditions, physiologic variations in the age at onset of puberty, for example, could be related to genetic variations in the process of intrinsic neurobiologic maturation in the hypothalamus. In contrast, there is evidence that acute as well as chronic deviations from normal conditions of nutrition, activity, stress, and/or growth result in striking effects on pulsatile LH secretion and puberty. Abolition of LH pulsatility occurs during periods of fasting or strenuous physical activity.<sup>28,29</sup> Delayed puberty occurs in patients with isolated growth hormone (GH) deficiency.<sup>30</sup> Early puberty is seen following catch-up growth in adopted children recovering from nutritional and psychoaffective deprivation.<sup>31</sup> Sex steroid excess or premature increase, such as that seen in untreated congenital adrenal hyperplasia or gonadotropin-independent sexual precocity, also may result in secondary precocious hypothalamic maturation. While these extrahypothalamic signals may play a prominent role in abnormally precocious or delayed puberty, they probably play a much less important role in normal physiologic maturation.

Figure 4



## SUMMARY

In summary, the neuroendocrine mechanisms involved in triggering the onset of puberty produce an acceleration of pulsatile GnRH secretion resulting from complex anatomic interactions among GnRH neurons, other regulatory neurons, and glial cells. Many different neuromodulators may play inhibitory, facilitatory, or dual inhibitory-facilitatory roles, with glutamate and GABA currently leading this list. While the influence of extrahypothalamic factors may be minor in physiologic conditions, their effects could be prominent in disorders of puberty. Much remains to be delineated. However, the building blocks for further investigation are now in place.

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## Letter From the Editor

Dear Colleague:

Genetics indeed is a very broad area of interest. The abstracts submitted by Drs. Judith Hall and William Horton for this issue of *GROWTH, Genetics, & Hormones (GGH)* particularly emphasize to me that a disease is usually not just a disease, but a symptom complex that may have multiple etiologies. For that reason, the following abstracts have been grouped together. Such a grouping emphasizes the similarities and dissimilarities of various chondrodysplasias in

relation to their phenotypes and in relation to their genetic and/or biochemical cause. Hopefully, you will enjoy reading them as a group because, to use an analogy, you can better comprehend the trees by seeing the forest and better comprehend the forest by seeing the trees. Perhaps we can better enjoy and learn by studying the substantive relationships within and among these abstracts. We will attempt to do more grouping of this type in *GGH* in the future. We hope you approve.

Robert M. Blizzard, MD  
Chairman, *GGH* Editorial Board

## Abstracts From the Literature

### A Constitutively Active Mutant PTH-PTHrP Receptor in Jansen-Type Metaphyseal Chondrodysplasia

Jansen-type metaphyseal chondrodysplasia (JMC) is a rare but distinctive autosomal dominant skeletal dysplasia. Patients have severely short limbs, prominent skull bones, and enlarged joints that become severely deformed in adulthood. Skeletal radiographs show demineralization and rachitic-like changes in the metaphyses in infancy and severe widening and irregularities of the metaphyses in childhood. These radiographic changes and the hypercalcemia and hypophosphatemia that these patients display suggest a link to hyperparathyroidism. However, parathyroid gland histology and blood parathyroid hormone (PTH) and PTH-related protein (PTHrP) levels are unremarkable.

There are other types of metaphyseal chondrodysplasia that appear clinically similar to JMC, such as the Schmidt type; however, these 2 now have been demonstrated to be of totally different genetic origins. The Schmidt type results from heterozygous mutations of the type X collagen gene, and these are thought to diminish the amount of this protein in the growth plate. JMC, as stated in the current study, is reported to be attributable to a receptor defect that changes a strictly conserved histidine residue at position 223 in the receptor protein's first intracellular loop to arginine.

Persistent suspicion of a disturbance in the PTH-PTHrP-calcium axis prompted the investigation of the receptor for this



axis in a patient with JMC. The investigators determined that a heterozygous mutation prompted the arginine substitution at position 223. This histidine is highly conserved among members of the G protein-coupled transmembrane receptor family to which the PTH-PTHrP receptor belongs. Findings provide evidence that the recently isolated receptor is the major mediator of PTH and PTHrP action. The authors believe that a paracrine-autocrine role for PTHrP may exist, as PTHrP and the PTH-PTHrP receptor are both expressed in adjacent cells within the metaphyseal growth plate.

Intriguingly, the authors present findings that support the hypothesis that "activating" receptor mutations may cause abnormal formation of endochondral bone as well as abnormalities in mineral anion homeostasis.

One of the fascinating aspects of the report is that in COS-7 cells transfected with the receptor DNA containing the mutation, ligand-independent accumulation of cyclic adenosine monophosphate was observed. This was not found in control cells that had been transfected with the wild-type (normal) receptor cDNA.

Schipani E, et al. *Science* 1995;268:98-100.

**Editor's comment:** The etiology of the hypercalcemia in JMC, which is not seen in the Schmidt-type metaphyseal chondrodysplasia, has always been somewhat controversial. Endocrinologists suspected that it was due to hormonal causes, while chondrodysplasia experts, who were mainly geneticists, considered it to be secondary to the severe metaphyseal abnormalities. It is ironic that the endocrinologists used genetic technology to demonstrate that they were correct.

JMC now joins a growing list of genetic disorders resulting from mutations that activate receptors. These diseases include polyostotic fibrous dysplasia with or without sexual precocity (McCune-Albright syndrome); rare forms of retinitis pigmentosa; congenital nonautoimmune hyperthyroidism; gonadotropin-independent male precocious puberty (testotoxicosis); a hyperparathyroidism-like syndrome in which there is a defect of the calcium sensing receptor; congenital stationary night blindness; and others. A feature article in GGH discussing these various receptor defects will be forthcoming sometime in the next year.

William A. Horton, MD

## Genetic Heterogeneity in Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) is an autosomal dominant chondrodysplasia characterized by mild to moderate shortness of the limbs, waddling gait, genu valgum, and early onset osteoarthritis. Two autosomal dominant types have been described: the severe Fairbank type and the milder Ribbing type. Although previously it was suspected that variability occurred within the same disorder, ie, allelic disorders, linkage analysis has shown that there are at least 2 forms: Fairbank-type MED maps to chromosome 19, which may be allelic with pseudoachondroplasia; Ribbing-type MED maps to chromosome 1 near the locus for the alpha 2 chain of type IX collagen (COL9A2). These have been designated EDM1 and EDM2, respectively.

The current report describes 2 intriguing families with clinically similar findings in certain respects and dissimilar findings in others—particularly stature and mode of inheritance.

In family 1, the proband (a 5-year-old female) and 2 siblings (an 8-year-old sister and a 12-year-old brother) presented for genetic evaluation because of painful hips and waddling gait. The heights of these children were not short (35th through 70th percentiles). None were disproportionate since the arm spans approximated the heights. Radiographic findings for all 3 subjects showed typical features of Fairbank-type MED, as the epiphyses were small, irregular, and flat, especially at the knees. The femoral necks were short and broad and the capital femoral epiphyses were small and round. The bones of the hand were normal, but with delayed ossification of the distal ulnar epiphyses and carpal bones. An autosomal dominant inheritance pattern was unequivocal. The authors demonstrated that this family had Fairbank-type MED, with linkage to chromosome 19. All affected individuals had heights within  $\pm 2$  standard deviations (SD).

The 43-year-old white female proband in family 2 was evaluated because of short stature, which was disproportionate in type, and joint pain. The proband was  $< 2$  SD in height, and the arm span was short for the length (length = 151 cm; span =

143.5 cm). Two siblings had joint pain and short stature. The parents had no symptoms of MED and were not short. A recessive inheritance or possibly autosomal dominant inheritance with germline mosaicism was responsible. No abnormalities associated with chromosome 19 or with the cartilage-specific candidate collagen genes (COL) were demonstrable. COL9A2 has recently been reported to be linked in one family with autosomal dominant MED.

In summary, the authors confirm that autosomal dominant Fairbank-type MED maps to chromosome 19. However, they also studied another large MED family in which linkage to the chromosome 19 locus was excluded. They further excluded linkage of MED in this family to the chromosome 1 (EDM2) locus using markers for COL9A2. Thus, at least 1 additional genetic locus remains to be identified for conditions having the clinical and radiographic criteria of MED.

Deere M, et al. *Am J Hum Genet* 1995;56:698-704.

**Editor's comment:** With the recent identification of genes and mutations associated with multiple chondrodysplasias and similar disorders, a trend seems to be emerging. Disorders with similar clinical phenotypes involving genes that encode extracellular matrix proteins exhibit considerable genetic heterogeneity. Mutations tend to occur in different genes and at different sites within the same gene. This is not a new observation. Disorders manifesting spondyloepiphyseal dysplasia and MED phenotypes are good examples.

In contrast, disorders due to mutations of growth factor receptors, such as fibroblast growth factor receptors, exhibit much less heterogeneity. Mutations tend to cluster in relatively few sites, as in achondroplasia, where almost all patients have the same mutation. Time will tell if these impressions are correct.

William A. Horton, MD



## A Cluster of Sulfatase Genes on Xp22.3: Mutations in Chondrodysplasia Punctata (CDPX) and Implications for Warfarin Embryopathy

Chondrodysplasia punctata (CDP) refers to a group of skeletal dysplasias characterized by abnormal calcium deposition in regions of enchondral bone formation. This results in the "stippling" of epiphyses, which tends to disappear within the first few years of life. One type of chondrodysplasia punctata is X-linked recessive (CDPX). It is characterized by aberrant bone mineralization, severe underdevelopment of nasal cartilage, and distal phalangeal hypoplasia. The authors demonstrated that some of these patients have an inherited deficiency of a novel sulfatase (arylsulfatase E, or ARSE). However, not all patients with the clinical syndrome have this defect. Other patients have a recessive form of CDP. CDPX shows remarkable phenotypic similarities to 2 well-characterized disease entities involving vitamin K metabolism: warfarin embryopathy and a congenital metabolic error of vitamin K epoxide reductase deficiency. Warfarin embryopathy is caused by the administration of warfarin, an anticoagulant drug, during a critical period of pregnancy: the sixth through ninth weeks. The vitamin K epoxide reductase deficiency disease, also known as pseudowarfarin embryopathy, is a rare autosomal recessive disorder affecting the recycling of vitamin K. By extensively analyzing DNA from overlapping yeast artificial chromosome clones that spanned the critical Xp22.3 region, Franco et al identified 3 adjacent genes that encoded previously unrecognized sulfatase enzymes. Because of predicted structural similarities to arylsulfatases A, B, and C, the novel sulfatase genes were named ARSD, ARSE, and ARSF. The authors concluded that mutations of the ARSE gene account for many cases of CDPX and that the phenotype results from reduced ARSE enzyme activity. Warfarin probably produces a CDPX-like syndrome because it inhibits ARSE activity. The authors demonstrated a significant decrease of ARSE activity and postulated that ARSE activity is

inhibited by warfarin. Patients with CDPX had demonstrably deficient ARSE activity. The ARSE gene is mutated in some cases of CDPX. Intriguingly, the congenital deficiency of vitamin K epoxide reductase, the enzyme recycling vitamin K epoxide to vitamin K, produces an identical picture. The striking similarities among CDPX, warfarin embryopathy, and vitamin K epoxide reductase deficiency phenotypes and the evidence that warfarin inhibits ARSE suggest that these disorders are due to abnormalities in the same metabolic pathway but are of different etiologies.

Franco B, et al. *Cell* 1995;81:15-25.

**Editor's comment:** This paper begins to tie together a number of loose ends for biochemists interested in the arylsulfatase family of enzymes, clinicians interested in sorting out the different forms of CDPX and related conditions, and for geneticists interested in the ancestry of the pseudoautosomal region of the X chromosome, which is where not only the gene for ARSE but also the genes for ARSC and ARSD exist. The patients themselves have underdevelopment of nasal cartilage and distal phalangeal hypoplasia, as well as short stature.

William A. Horton, MD

**2nd Editor's comment:** The saying that if it looks like an elephant and walks like an elephant, then it is an elephant may apply to elephants but does not apply to patients with CDP. Drugs obviously can induce enzymatic deficiencies identical to those induced by genetic mutations or the absence of genes.

Robert M. Blizzard, MD

## Trisomy 18, Molecular Studies, Parental Origin and Cell Division in the Extra Chromosome 18 Material

Trisomy 18, or Edwards syndrome, was first described in 1960. It is the second most common autosomal trisomy. Individuals with trisomy 18 present with characteristic facial features, growth retardation, severe mental retardation, clenched hands with

overlapping fingers, and renal and cardiac anomalies. Trisomy 18 has an incidence of 0.18% in all clinically recognized pregnancies and, like other autosomal trisomies, is associated with advanced maternal age. The majority of pregnancies with trisomy 18 abort spontaneously, and only 5% survive to birth. The mean survival after birth is 1 to 3 months, and 95% of those born alive die within the first year of life.

The gene or genes responsible for the trisomy 18 phenotype are not known. While the features of trisomy 18 are most often associated with duplication of the entire chromosome, there are a number of cases in which individuals with a partial duplication of chromosome 18 present with the same or similar features. An effort to identify the regions of chromosome 18 that are critical in producing the phenotype was reported by Boghosian-Sell et al, who analyzed 6 patients with partial duplications of chromosome 18. Fluorescent in situ hybridization with DNA-specific probes to chromosome 18 was used to determine the precise duplication in these patients. The clinical features and the extent of the duplication were compared with 4 previously reported partial trisomy 18 patients. This

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permitted identification of the regions of chromosome 18 that may be responsible for the clinical features of trisomy 18. They concluded that the critical region lies between 18q12.1-18q21.2 and 18q22.3.

The parental origin of the additional chromosome as well as the cell division leading to trisomy 18 are important for understanding the etiology of trisomy 18 (Fisher et al.).

Trisomy 18 occurs because of nondisjunction during cell division (Antonarakis). Nondisjunction occurs during meiosis when a homologous pair of chromosomes has failed to separate during the first meiotic division or when the double-stranded chromosome has failed to separate into single-stranded chromatids at the second meiotic division. Nondisjunction also can occur during mitotic somatic cell division. The result of nondisjunction is an abnormal number of chromosomes (aneuploidy) for a specific chromosome. Other trisomies associated with nondisjunction include trisomy 21 and trisomy 13.

The analysis of inherited DNA markers, restriction fragment length polymorphisms (RFLPs), and microsatellite repeat polymorphisms has allowed for the tracking of the parental origin and thereby the identification of the mechanism(s) leading to the additional chromosome 18 in individuals with trisomy 18 (Antonarakis; Sherman). In the majority of cases, the additional chromosome is maternal in origin and occurs during the second meiotic division. Recently, Fisher et al. documented that in 63 cases of trisomy 18, the maternal chromosome was duplicated in 61. Both paternal cases were attributable to a postzygotic mitotic error. Of 54 maternal cases identifiable for testing, 16 were attributable to an error in the first meiotic division, 35 were due to a second meiotic error, and 3 were the result of a postzygotic mitotic error. Of the cases due to first meiotic

error, one third lacked recombination, which apparently made them prone to nondisjunction. All maternal errors were associated with advanced maternal age; however, only the examples of nondisjunction in second meiosis were calculated to be statistically significantly increased because of maternal age.

Antonarakis SE. *N Engl J Med* 1991;324:872-876.

Boghossian-Sell L, et al. *Am J Hum Genet* 1994;55:476-483.

Fisher JM, et al. *Am J Hum Genet* 1995;56:669-675.

**Editor's comment:** *New molecular techniques allow the tracking of genes and chromosomes in such a way as to give important clues to the mechanisms causing disease. In the case of trisomy 18, just as in Down syndrome, only part of the chromosome seems to produce the abnormal phenotype seen when present in triplicate. Probably lots of the chromosome 18 is either active or not important because only a few bands on the long arm seem to be required. Since the area of the chromosome producing the phenotype has been narrowed, it seems likely to expect that the specific gene(s) will soon be identified. The DNA markers also allow determination that chromosomal errors can occur at many different times. In the case of trisomy 18, maternal second meiosis (while the egg sits waiting to ovulate) seems to be the most vulnerable time for things to go wrong. However, if the chromosomes have not undergone recombination (crossover), they may malsegregate during meiosis I. At this point in time, it is hard to predict how these errors can be prevented, but it is important to know when they occur.*

Judith G. Hall, MD

## Thanatophoric Dysplasia (Types I and II) Caused by Distinct Mutations in Fibroblast Growth Factor Receptor 3

Achondroplasia (ACH) is the most common of the chondrodysplasias. Thanatophoric dysplasia (TD) is the most common of the neonatal lethal skeletal dysplasias. Homozygous ACH and TD are comparably lethal. The clinical and radiographic features of the ACH and TD entities are similar except that heterozygous ACH is less severe. Classic features of both syndromes include micromelic shortening of the limbs; relative macrocephaly with frontal bossing; reduced height of the vertebral bodies; poor cellular proliferation and column formation in the cartilaginous growth plates of the long bones; and shortened ribs, resulting in a reduced thoracic cavity and a bell-shaped abdomen.

Based primarily upon specific radiologic differences, newborns with TD have been classified as having either type I or type II. Those with TD type I have curved, short (telephone receiver-shaped) femora with or without cloverleaf skull deformity. Those with type II TD have relatively longer and straighter femora and the cloverleaf skull deformity is constant.

When mutations of the fibroblast growth factor receptor 3 (FGFR3) gene were identified in ACH, the search was on for FGFR3 mutations in TD. The highest levels of expression of FGFR3 are in the cartilage growth plates and central nervous system; lower levels of expression are seen in the lung, intestine,

and kidney. Because of the striking phenotypic similarities between homozygous ACH and TD and because of recent evidence demonstrating an important role for FGFRs in skeletal development, the investigators extensively analyzed FGFR3 in individuals with TD to determine if mutations in this gene cause one or more forms of this severe skeletal dysplasia. In the present paper, 22 out of 39 TD type I patients harbored amino acid substitutions in the extracellular domain of FGFR3 at codon 248. In contrast, a heterozygous mutation of codon 650 in all 16 cases of TD type II was found. All of these had a lysine in the intracellular tyrosine kinase domain of the receptor replaced by glutamic acid. None of the TD mutations were identified in normal individuals. Moreover, no mutations were detected in parental DNA from 3 sets of parents tested (1 set from a TD type I patient and 2 sets from TD type II patients). This demonstrates the sporadic nature of the mutations.

Tavormina PL, et al. *Nature Genet* 1995;9:321-328.

**Editor's comment:** *This paper settles several long-standing debates regarding TD and ACH. FGFR3 is involved in both instances. Different heterozygous mutations are responsible at the FGFR3 locus to produce TD types I and II and ACH. An*

intracellular domain mutation is responsible for TD type II, a mutation of the transmembrane domain is responsible for ACH, and a mutation in the extracellular domain is responsible for TD type I. While the story unravels, it becomes more complex. This report does demonstrate conclusively that the TD phenotypes result from new heterozygous mutations at the FGFR3 locus. It also confirms the long-standing hypothesis that TD

and ACH are biologically related and are, in fact, allelic disorders. Finally, the paper substantiates the existence of 2 distinct forms of TD and identifies a biologic basis for the difference. The rapidity with which the story is unfolding offers hope that the end is in sight, but don't hold your breath.

William A. Horton, MD

## Pelvic Ultrasonography: Early Differentiation Between Isolated Premature Thelarche and Central Precocious Puberty

The authors previously reported the normal increases in uterine volume/length and ovarian volume that occur during normal growth and sexual development (*Pediatr Radiol* 1994;24:11-13). In the current article, measurements in girls with premature thelarche (PT) and central precocious puberty (CPP) are reported. The sensitivity (the probability that a test result will be positive when the disease is present) and the specificity (the probability that a test result will be negative when the disease is not present) were compared with ultrasonographic measurements and indices of hormonal control. Fifty-five children with PT between the ages of 0.3 and 7.4 years who were followed for 18 months without progressive sexual maturation and 20 subjects between 2.1 and 7.7 years of age with CPP were studied for uterine length/volume and ovarian volume. All measurements were significantly greater in patients with CPP, compared with patients with PT. No significant differences were found between children with PT and the control group. No overlap was observed in uterine volume between the CPP and PT groups. The sensitivity and specificity values for CPP versus PT were as follows:

| Parameter              | Sensitivity (%) | Specificity (%) |
|------------------------|-----------------|-----------------|
| Uterine volume         | 100             | 100             |
| Uterine length         | 90              | 100             |
| Ovarian volume         | 82              | 95              |
| Peak LH/FSH after GnRH | 33              | 100             |

FSH, follicle-stimulating hormone; GnRH, gonadotropin hormone-releasing hormone; LH, luteinizing hormone

The authors conclude that measurement of uterine volume is a sensitive method for differentiating girls with PT from those with CPP, in contrast to the use of the vaginal smear and reversal of the serum LH:FSH ratio following LH-releasing hormone stimulation.

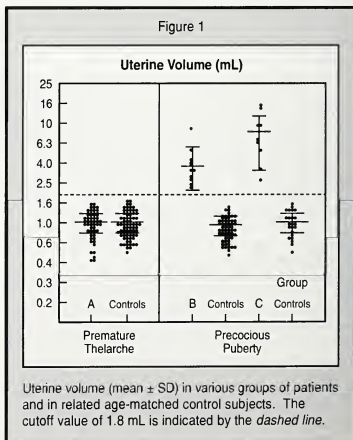
Haber HP, et al. *Eur J Pediatr* 1995;154:182-186.

**Editor's comment:** Distinguishing girls with PT and those with early CPP may be difficult during the initial evaluation. Clinical findings such as the growth pattern, the height, and the degree of sexual maturation assist in differentiating the diagnoses. Haber et al suggest that measurement of uterine volume, which is an index of estrogen activity, distinguishes between PT and CPP. However, pelvic ultrasonography is inconvenient and

expensive, although noninvasive. Rectal examinations to determine uterine size and the possibility of ovarian masses were performed for many years before ultrasonography was developed, and worked well. In 1995, physicians should routinely be using the simple tests and resorting to ultrasonography as a backup. Incidentally, uteri are not felt in normal female children from shortly after birth to the onset of secondary sexual characteristics. PT patients usually do not have palpable uteri.

It was surprising that ovarian volume was not as sensitive or specific a distinguishing characteristic as uterine volume, since gonadal enlargement is presumably the earliest response to gonadotropin stimulation. I suspect that with more experience even the measurement of uterine volume will prove to be less specific and sensitive than reported here, given the broad spectrum of pituitary-ovarian function noted in young girls. Regardless, the use of ultrasonography to supplement the rectal examination when necessary is appropriate.

Allen W. Root, MD



## Metabolic Modulation of the Growth Hormone-Releasing Activity of Hexarelin in Man

Maccario and colleagues studied the mechanism of action of hexarelin by investigating its interaction with glucose and free fatty acids. They specifically questioned whether the growth hormone (GH)-releasing effect of hexarelin could be reduced by factors known to inhibit basal and GH-releasing hormone (GHRH)-stimulated GH secretion. Six normal men participated in the study and underwent 6 treatment sessions separated by washout periods of at least 3 days. All subjects participated in each of the 6 different protocols, which included: (1) hexarelin, 2 µg/kg IV at 0 minutes; (2) GHRH, 2 µg/kg IV at 0 minutes; (3) hexarelin, 2 µg/kg IV at 0 minutes plus glucose (100 g orally at -45 minutes); (4) hexarelin, 2 µg/kg IV at 0 minutes plus lipid-heparin infusion (250 mL of a 10% lipid solution plus 2,500 U heparin from -30 to +120 minutes); (5) GHRH, 2 µg/kg IV at 0 minutes plus glucose; and (6) GHRH, 2 µg/kg IV at 0 minutes plus lipid-heparin infusion. Blood samples were taken every 15 minutes from -60 to +120 minutes. Serum GH, plasma glucose, and plasma free fatty acid levels were measured.

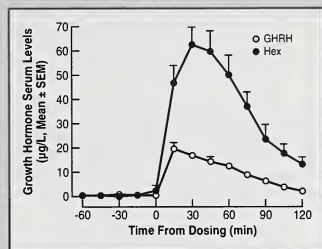
No significant decreases in basal GH were observed during the study. Hexarelin induced a much higher GH peak than did GHRH ( $62.6 \pm 8.0$  µg/L vs  $19.8 \pm 2.4$  µg/L). The increase in plasma glucose after the oral load was similar during hexarelin and GHRH testing, but the GH-releasing effect of GHRH was more inhibited by glucose (peak,  $5.6 \pm 0.9$  µg/L vs  $38.4 \pm 7.9$  µg/L) than that of hexarelin. The lipid-heparin infusion increased plasma free fatty acids similarly during both hexarelin and GHRH treatment and basal GH levels were reduced similarly during both studies. The GH released by stimulation with GHRH was reduced to  $4.9 \pm 1.0$  µg/L ( $P < 0.01$ ) while that of hexarelin was reduced to  $34.2 \pm 4.5$  µg/L ( $P < 0.05$ ). The GH response to hexarelin after glucose was similar to that during lipid-heparin infusion and much higher than the GH response after GHRH alone ( $P < 0.05$ ).

This study demonstrates a greater effect of oral glucose and lipid-heparin infusion on the GH-releasing effect of GHRH than that of hexarelin. The authors state that the results showing that the GH response to hexarelin is blunted but not abolished by glucose indicate that the stimulating effect of GHRH is partially resistant to an increase in endogenous somatostatin. The potential inhibitory effect of free fatty acids on basal and GHRH-induced GH secretion may be explained by a direct action on the pituitary. The GH-stimulating effect of hexarelin is partially resistant to the inhibitory effect of free fatty acids. Thus, unlike GHRH, the GH-releasing effect of hexarelin is partially resistant to the inhibitory effects of both glucose or free fatty acids. This resistance may be due to antagonism of somatostatinergic activity within the hypothalamus or directly at the pituitary. The authors caution that other unknown mechanisms cannot be ruled out.

Maccario M, et al. *Metabolism* 1995;44:134-138.

**Editor's comment:** More and more information is rapidly becoming available regarding the actions of GH-releasing peptides. As the authors point out, GH-releasing peptides release more GH than GHRH, and apparently have some action on specific non-GHRH, nonopioid receptors in both the pituitary and hypothalamus. The present study provides information with regard to the mechanism of action of these hormones and the level at which these hormones may act. Hexarelin is one of

Figure 1

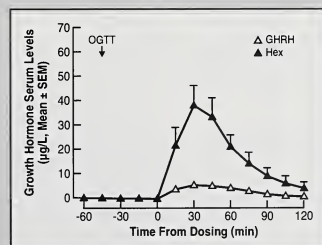


Growth hormone responses to hexarelin ([HEX] 2 µg/kg IV) or growth hormone-releasing hormone ([GHRH] 2 µg/kg IV) in 6 healthy men.

the most recently and most frequently studied GH-releasing peptides in humans. It can be given either IV, subcutaneously, intranasally, or orally. We anticipate reports of the use of this hormone to treat patients with GH deficiency due to hypothalamic abnormalities. It is realistic to assume that such data should soon be forthcoming and that GH-releasing peptides may provide a new and potentially more practical method than GHRH for treating some children with growth failure.

William L. Clarke, MD

Figure 2



Growth hormone responses to hexarelin ([HEX] 2 µg/kg IV) or growth hormone-releasing hormone ([GHRH] 2 µg/kg IV) administered in combination with oral glucose (100 g) in 6 healthy men. OGTT, oral glucose tolerance test.



## Recommendations for Standardized Human Pedigree Nomenclature






























Significant inconsistencies in the usage of common pedigree symbols lead to inaccurate reporting and poor interpretation of genetic events. Consequently, a Pedigree Standardization Task Force (PSTF) was established by the National Society of Genetic Counselors, and input was solicited from the American Board of Medical Genetics and the American Society of Human Genetics, among others. The article clarifies and standardizes

the symbols to be used to describe almost any familial relationship, and also demonstrates specifically how each symbol should be used. Consistent use of such standardized pedigree nomenclature will reduce the chances for incorrect interpretation of patient, family, medical, and genetic information. It also will improve the quality of patient care and facilitate communication among researchers.

Figure 1  
Common Pedigree Symbols, Definitions, and Abbreviations

### Instructions:

- Key should contain all information relevant to interpretation of pedigree (eg, define shading)
- For clinical (nonpublished) pedigrees, include:
  - a) family names/initials, when appropriate
  - b) name and title of person recording pedigree
  - c) historian (person relaying family history information)
  - d) date of intake/update
- Recommended order of information placed below symbol (below to lower right, if necessary):
  - a) age/date of birth or age at death
  - b) evaluation
  - c) pedigree number (eg, I-1, I-2, I-3)

|   | Male   | Female   | Sex Unknown  | Comments   |
|---|--|--|--|--|
| 1. Individual                           | <br>b. 1925   | <br>30 y  | <br>4 mo  | Assign gender by phenotype   |
| 2. Affected individual                  | <br> | <br> | <br> | Key/legend used to define shading or other fill (eg, hatches, dots, etc)<br>With ≥2 conditions, the individual's symbol should be partitioned accordingly, each segment shaded with a different fill and defined in legend |
| 3. Multiple individuals, number known   |   |   |   | Number of siblings written inside symbol (affected individuals should not be grouped)  |
| 4. Multiple individuals, number unknown |   |   |   | "n" used in place of "?" mark  |
| 5a. Deceased individual                 | <br>d. 35 y  | <br>d. 4 mo  |    | Use of cross (†) may be confused with symbol for evaluated positive (+); if known, write "d." with age at death below symbol   |
| 5b. Stillbirth (SB)                     | <br>SB<br>28 wk   | <br>SB<br>30 wk   | <br>SB<br>34 wk   | Birth of dead child with gestational age noted   |
| 6. Pregnancy (P)                        | <br>LMP: 7/1/94   | <br>20 wk   |   | Gestational age and karyotype (if known) below symbol; light shading can be used for affected and defined in key/legend  |
| 7a. Proband                             |   |   |   | First affected family member coming to medical attention   |
| 7b. Consultand                          |   |   |  | Individual(s) seeking genetic counseling/testing   |

Because the information is so pertinent, 2 of the numerous figures in the article are reproduced here to encourage interested readers to obtain a complete copy of the article and the inclusive figures for their own use.

Other important figures in the article include a systematic presentation of pedigree line definitions; assisted reproductive technology symbols and definitions; pedigree symbolization of genetic evaluations/testing information; and a hypothetical clinical pedigree, using recommended nomenclature.

**Editor's comment:** Reviewing charts or pursuing the literature about family trees, etc., reveals many inconsistencies in symbols and other designations used in constructing pedigrees. This paper provides needed guidelines for standardization. Pediatric endocrinologists, geneticists, and all pediatricians need to at least understand the symbology used in constructing and reading genetic pedigrees. Obviously, students and residents similarly need this information. It should be incorporated into appropriate teaching programs for all involved medical personnel.













Bennett RL, et al. *Am J Hum Genet* 1995;56:745-752.

William A. Horton, MD

Figure 2  
Pedigree Symbols and Abbreviations for Pregnancies Not Carried to Term

**Instructions:**

- Symbols are smaller than standard ones and individual's line is shorter. (Even if sex is known, triangles are preferred to a small square/circle; symbol may be mistaken for symbols 1, 2, and 5a/5b of Figure 1, particularly on hand-drawn pedigrees.)
- If gender and gestational age known, write below symbol in that order.

|                                   | Male  | Female  | Sex Unknown  | Comments  |
|-----------------------------------|---|---|--|---|
| 1. Spontaneous abortion (SAB)     | <br>male | <br>female | <br>ECT   | If ectopic pregnancy, write ECT below symbol                                    |
| 2. Affected SAB                   | <br>male | <br>female | <br>16 wk | If gestational age known, write below symbol; key/legend used to define shading |
| 3. Termination of pregnancy (TOP) | <br>male | <br>female |           | Other abbreviations (eg, TAB, VTOP, Ab) not used for sake of consistency        |
| 4. Affected TOP                   | <br>male | <br>female |           | Key/legend used to define shading   |

## Chronic Metabolic Acidosis Decreases Albumin Synthesis and Induces Negative Nitrogen Balance in Humans

Ballmer et al measured the effects of experimentally induced metabolic acidosis on nitrogen balance and protein synthesis in 8 male subjects on a constant metabolic diet. Two different degrees of chronic metabolic acidosis were induced using low-dose  $\text{NH}_4\text{Cl}$  (2.1 mmol/kg body weight;  $n=4$ ) and high-dose  $\text{NH}_4\text{Cl}$  (4.2 mmol/kg body weight;  $n=4$ ) orally for 7 days. Albumin synthesis rates were determined by a labeled phenylalanine technique after an overnight fast. Urinary nitrogen excretion was measured, as well as plasma concentrations of insulin-like growth factor 1 (IGF-1), free thyroxine ( $\text{fT}_4$ ), and triiodothyronine ( $\text{T}_3$ ).

In the low-dose group, a mean pH of 7.375 and a mean bicarbonate level of 19.1 mEq/L were achieved. The plasma albumin concentration did not decrease significantly. Albumin synthesis in 3 of the 4 subjects was slightly lower than during the control period and definitely decreased in the fourth subject. Nitrogen excretion averaged  $977 \pm 116$  mmol/24 h during

the control period and increased, but not significantly, with  $\text{NH}_4\text{Cl}$  administration.

In contrast, plasma albumin concentrations fell significantly in the high-dose group, in whom a mean pH of  $7.303 \pm 0.053$  occurred, in addition to a significantly lower plasma bicarbonate level of  $15.1$  vs  $19.1$  mEq/L. Albumin synthesis was significantly lower than during baseline in the high-dose group, and nitrogen excretion increased significantly from  $1,012 \pm 180$  mmol/24 h to  $1,377 \pm 236$  mmol/24 h ( $P<0.001$ ). Plasma levels of IGF-1,  $\text{fT}_4$ ,  $\text{T}_3$ , and thyrotropin all showed small but statistically significant declines during acidosis, but only when the low- and high-dose groups were combined.

The authors state that these data demonstrate for the first time that metabolic acidosis in humans decreases albumin synthesis and induces a state of sustained negative nitrogen balance. Thus, as stated by the authors, metabolic acidosis could be an important mediator of negative nitrogen balance,

increased protein breakdown, and decreased protein synthesis in acidotic patients. The effect of acidosis on albumin synthesis could be mediated in part by suppression of IGF-1,  $\text{fT}_4$ , and  $\text{T}_3$ .

Ballmer PE, et al. *J Clin Invest* 1995;95:39-45.

**Editor's comment:** This is a very interesting and provocative study. More and more pediatric endocrine clinics are treating children with chronic acidosis from chronic renal failure with recombinant human growth hormone. However, the mechanism for the reduction in growth during chronic acidosis remains

unclear. This paper contributes to a better understanding of the possible mechanisms involved in growth failure in these children, ie, negative nitrogen balance and decreased albumin synthesis. In addition, it is important to note that, especially in the low-dose group, the reductions in pH and bicarbonate levels were not great, but were comparable to those seen in conditions such as renal tubular acidosis. The number of patients studied in each group was small. Therefore, the trends observed in the low-dose group may be significant with a larger number of subjects.

William L. Clarke, MD

## Endocrinology of the Carbohydrate-Deficient Glycoprotein Syndrome Type 1 From Birth Through Adolescence

Carbohydrate-deficient glycoprotein (CDG) syndrome type 1 is a newly recognized inborn error of glycoprotein metabolism (Jaeken et al. *Acta Paediatr Scand* 1991;375[suppl]:1-71). The biochemical hallmark is a partial carbohydrate deficiency in a wide range of glycoproteins, including binding proteins, enzymes, and coagulation factors. The clinical picture is dominated by the affliction of the central and peripheral nervous system, resulting in psychomotor retardation, seizures, ataxia, and stroke-like episodes. An abnormal pattern of subcutaneous fat occurs, along with feeding difficulties, retinitis pigmentosa, hypoalbuminemia, pericardial effusion, and/or ascites. The diagnosis is confirmed by isoelectric focusing of serum sialotransferrins. CDG syndrome type 1 and type 2 are etiologically distinctly different. This report of 26 CDG type 1-affected children presents pertinent endocrine data.

Serum follicle-stimulating hormone (FSH) levels were normal in newborns and prepubertal children, but elevated in female toddlers and adolescent females and males with CDG syndrome type 1. Serum luteinizing hormone (LH) was similar and was age-dependent. In adolescent girls, serum estradiol remained low while FSH bioactivity was low normal, as was the bioactive/immunoreactive FSH ratio. Exogenous gonadotropins evoked an estradiol response and induced ovarian follicular growth. Male patients virilized at puberty, although testicular volume was subnormal. The thyroid axis was hallmarked by thyroid-binding globulin (TBG) deficiency and,

during infancy, increased serum thyrotropin concentrations were observed. A subgroup of female patients presented with hypersomatotropism and/or hyperprolactinemia. The hypothalamic pituitary area appeared intact on magnetic resonance imaging. Circulating insulin-like growth factor 1 (IGF-1) levels were low normal and transcortin levels were decreased.

The etiology is an inherited metabolic error in the posttranslational glycosylation of a variety of glycoproteins. The primary defect for CDG syndrome type 1 may be located at the level of the endoplasmic reticulum. The data are compatible with impaired *in vivo* function of FSH.

de Zegher F, Jaeken J. *Pediatr Res* 1995;37:395-401.

**Editor's comment:** Abnormalities related to defective glycosylation during posttranslational processing of synthesized proteins expand the list of molecular faults that one must consider when confronted with atypical clinical problems. Glycosylation is essential for normal function of secreted hormones, carrier proteins, and receptors. The defects *in vivo* of FSH function in children with CDG syndrome type 1 is most striking. Decreased glycosylation of FSH resulted in a prolonged half-life, perhaps leading to downregulation of gonadal FSH receptors and impaired ovarian and testicular responsiveness. Exogenous (glycosylated) FSH reversed the endocrine (ovarian) abnormalities, possibly because the endogenous FSH was suppressed, permitting the receptors to recycle to the plasma membrane and become responsive to stimulation once more.

The question must be raised whether there are children with less complete defects in glycosylation than those present in CDG syndrome type 1, such as those with an unexplained and persistent modest rise in thyrotropin found during neonatal screening for congenital hyperthyroidism. Possibly there are patients with hypergonadotropic hypogonadism without a specific identifiable primary gonadal abnormality who have a glycosylation defect. The authors of this paper suggest that defective glycosylation and impaired activity of FSH may be present in females with galactosemia and possibly in some women with the hyperandrogenism/polycystic ovary syndrome. A new form of diseases has been encountered and more of this type will probably be described.

Allen W. Root, MD

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## Telomeres and Telomerase: Cancer, Immortality, and Mental Retardation

The word telomeres comes from the Greek "telos," which means "end." When applied to chromosomes, it means the end tip of a chromosome. Repetitive DNA sequences (TTAGGG) are located at the end or tip of a chromosome and are called telomeric sequences. Telomeric repeats are highly conserved, with the same sequences found in protozoa, nematodes, lower and higher plants, and vertebrates. Telomeres were first recognized as short repeated sequences at the end of ciliate chromosomes and in lower eukaryotes such as yeast. These repetitive sequences were later recognized and documented in human chromosomes.

Recent evidence has shown that telomeres are involved in a large number of biologic functions. Two among those suggested are very important: (1) protection of the linear chromosome end from degrading, recombining, and ligating to other chromosome ends; and (2) completion of the replication of chromosome DNA sequences at the chromosome ends (Biessman and Mason).

The cloning and characterization of the repetitive sequences that make up human telomeres have greatly benefited from new DNA cloning techniques and have led to interesting observations regarding cancer and aging. For example, the length of a telomere, ie, the number of repetitive sequences, is known to be associated with the number of cell divisions that particular cell has gone through. Telomeres in human germline cells, eg, sperm and egg, are known to be longer than those seen in somatic tissue cells, such as in blood. The telomeres of the human chromosomes shorten with each cell division. Shortened telomeres (in comparison with those of adjacent nontumor mucosa) have been documented in Wilms' tumors and colorectal carcinomas. The telomeric hypothesis (originally called the marginotomy theory) stated that the gradual loss of chromosome ends leads to cell arrest. This theory was based on progressive telomeric shortening with aging and on the observation that if a telomere became too short, cell growth would arrest.

The enzyme that synthesizes the telomeric sequences is a ribonucleoprotein enzyme called telomerase. Telomerase has been shown to be abnormally increased in some cancer cells. The gene for telomerase in humans has not been mapped. Telomerase expression is directly related to telomeric conservation. Excessive expression of telomerase has the potential to stop or delay the normal shortening of the telomeres and, consequently, delay cell cycle arrest. Aberrant telomerase expression has been suggested as a mechanism for producing the "immortality" of cancer cells.

The shortening of telomeres of human chromosomes with each cell division has been thought to serve as some sort of mitotic clock that can be used as a direct marker for the number of times a cell has divided. The exact role shortened telomeres play in aging is still unclear; however, telomeric loss with a successful series of cell divisions has been referred to as a "genetic time bomb" (Harley), since it will eventually lead to cell death.

An abnormality of telomeres also has been associated with mental retardation. In a recent report, Flint et al studied the subtelomeric regions of 99 mentally retarded individuals. They hypothesized that since the telomeric end of the chromosome is an area of active recombination, it would be expected to be at risk for small deletions. To detect chromosomal abnormalities

within the subtelomeric region, they used hypervariable DNA polymorphism probes. They compared the DNA of both parents with the mentally retarded offspring. They found that 3 of 99 patients had abnormalities. One arose from an interstitial or terminal deletion and 2 from the de novo derivative translocation of 2 chromosomes. They suggest that at least 6% of all unexplained mental retardation may be the result of these small telomeric abnormalities.

**Editor's comment:** Telomeres have been studied for many years but only lately have we become aware that they are involved in much more than just making up the ends of a chromosome. Telomerase expression may provide the means for diagnosing cancer or identifying the presence of malignant cells. Downregulating telomerase as a molecular therapeutic intervention may be applicable to a wide range of cancers. The findings of Flint et al address a different aspect of telomeres. The suggestion that as many as 6% of cases of idiopathic mental retardation can be explained by a telomeric loss provides a new and important diagnostic tool in mental retardation for families with previously unexplained mental retardation who are concerned about the risk of recurrence. Looking for telomeric abnormalities may allow a definitive diagnosis with a low recurrence risk for the parent but with as much as a 50% risk to the offspring of the affected individual. It will now be necessary to counsel families that a search for telomeric loss may be appropriate in nonspecific mental retardation.

Judith G. Hall, MD

Biessmann H, Mason JM. *Adv Genet* 1994;30:185-249.  
Flint J, et al. *Nature* 1995;9:132-138.  
Harley CB. *Mutation Res* 1991;256:271-282.

**2nd Editor's comment:** Daniel Haber discussed the topic of telomeres, cancer, and immortality in a brief commentary in the New England Journal of Medicine (April 6, 1995). He states that, among other things, the progressive shortening of telomeres correlates with the absence of expression of telomerase and that continuing expression of telomerase correlates with the presence of cancer cells. In humans, germ cells express telomerase and maintain their ability to divide throughout life. In other cells, an estimated 15 to 40 nucleotides are lost each year. Kim et al (*Science* 1994;266:2011) demonstrated that 90 of 101 specimens from primary tumors representing 12 different types of cancer contained telomerase activity, in contrast to none of 50 normal tissues. The extreme sensitivity of the polymerase chain reaction-based enzymatic assay allows the detection of 1 cancer cell expressing telomerase among 4,000 normal cells. Haber points out that an effective inhibitor of telomerase might induce prompt senescence in rapidly dividing tumors. Whether clinical applications will be forthcoming in the near future is unknown at this time. You, the reader are urged to learn more about telomeres and telomerase. Haber's commentary is a good place to start. The references listed above are excellent as follow-ups. Dr. Hall has done her usual proficient job in calling these phenomena to our attention.

Robert M. Blizzard, MD



## Meetings Calendar

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**September 27-30, 1995** Molecular and Developmental Biol of Cartilage, Bethesda, MD. Info: Conf Dept, NY Acad Sci. Tel: 212-838-0230, ext. 324; Fax: 212-838-5640.

**October 18-20, 1995** Intl Symp on Growth, Santiago de Compostela, Spain. Info: Prof FF Casanueva, C Dieguez, or M Pompo. Fax: 34-81-572-121.

**October 24-28, 1995** 45th Ann Mtg of the Amer Soc of Human Genet, Minneapolis, MN. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

**November 8-11, 1995** APS Conf: New Discoveries Within the Pancreatic Polypeptide Family: Molecules to Medicine, Location TBA. Info: Amer Physiol Soc, Membership Services. Tel: 301-530-7171; Fax: 301-571-8305.

**November 25-28, 1995** 17th Mtg of the Intl Study Group for Steroid Hormones, Berlin, Germany. Info: Dr V Toscano. Tel: 39-6-494-0568; Fax: 39-6-490-530.

**December 9-13, 1995** 35th Ann Mtg of the Amer Soc for Cell Biol, Washington, DC. Info: ASCB. Tel: 301-530-7153.

**January 5-11, 1996** Integrins and Signaling Events in Cell Biol and Disease, Keystone, CO. Info: Keystone Symposia. Tel: 303-262-1230; Fax: 303-262-1525.

**January 5-11, 1996** Small GTP-Binding Proteins and Growth Factor Signaling Pathways, Tamarron, CO. Info: Keystone Symposia. Tel: 303-262-1230; Fax: 303-262-1525.

**January 7-10, 1996** 6th Wkshp on Cells and Cytokines in Bone and Cartilage, Davos, Switzerland. Info: H Triet. Tel: 41-31-632-8766 or 41-31-632-2518; Fax: 41-31-382-3038; Email: pphysecrpphy.unibe.ch.

**January 11-13, 1996** 3rd Wkshp on Bisphosphonates, Davos, Switzerland. Info: H Triet. Tel: 41-31-632-8766 or 41-31-632-2518; Fax: 41-31-382-3038; Email: pphysecrpphy.unibe.ch.

**March 11-14, 1996** Amer Coll of Med Genet and the March of Dimes, San Antonio, TX. Info: Amer Coll of Med Genet, M Ryan. Tel: 301-571-1825.

**April 13-16, 1996** Amer Academy of Ped Mtg, Chicago, IL. Info: M Francis. Tel: 800-433-9016; Fax: 708-228-5059.

**May 6-9, 1996** Amer Ped Soc/Soc for Ped Res Mtg, Washington, DC. Tel: 708-427-0206; Fax: 708-427-1305.

**May 16-19, 1996** Review Course for Genetics, Houston, TX. Info: Office of Cont Ed, 4 Beaudet. Tel: 713-798-6020; Fax: 713-798-7955.

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# GROWTH

## Genetics & Hormones

Vol. 11 No. 4

December 1995

### Letter From the Editor

#### To Our Readers:

This issue of *GROWTH, Genetics, & Hormones (GGH)* is comprised, to a significant extent, of a comprehensive index for *GGH* Volume 1, Number 1 (1985) through Volume 10, Number 4 (1994). Many readers have requested this because *GGH* has evolved as an educational tool for teaching residents and fellows, as well as a reference journal for review articles presenting new information and con-

cepts abstracted from the most recent journal publications. Hopefully, each of you will find this index to be valuable.

We look forward to serving you in 1996. We, the Editorial Board, appreciate, as we are sure you do, the unrestricted educational grant from Genentech, Inc. that makes this publication possible.

For the Editorial Board,

Robert M. Blizzard, MD  
Chairman, *GGH* Editorial Board

### Letter to the Editor

In the most recent issue of *GROWTH, Genetics, & Hormones (GGH 1995;11[3]:12-13)*, the new recommendations for standardized human pedigree nomenclature were presented as a summary of an article by Bennett RL et al (*Am J Hum Genet* 1995; 56:745-752). The summary included a figure from the article with symbols and definitions.

For the most part, the recommendations in the Bennett et al article are excellent and will go a long way toward standardizing pedigree nomenclature. However, there is one exception: the definition of "proband" as "the first affected family member coming to medical attention" (item 7a in the figure published in *GGH* on page 12). This definition is more properly that of "index case." The term "proband" has a specific meaning, which is different from the Bennett et al definition. One difference, for example, is that there can be only one index case per pedigree, whereas there can be, and often is, more than one proband per pedigree. It has been well documented that proband misidentification can introduce serious bias into any analysis of family data.

I made these points in a letter to the editor of the *American Journal of Human Genetics*. Bennett et al replied and agreed that the proband definition should be modified. If possible, it would be helpful for a clarification to be published in *GGH*. *GGH* is widely read and respected, and it would help in spreading the word about the change in proband definition from the Bennett et al original definition.

Sincerely yours,

Mary L. Marazita, PhD, FACMG  
Director, Cleft Palate-Craniofacial Center  
Associate Professor, Oral and Maxillofacial Surgery  
Associate Professor, Human Genetics  
University of Pittsburgh

**Editor's comment:** Thank you, Dr. Marazita, for your letter and the constructive criticism of the new recommendations for standardized human pedigree nomenclature. My review of the original article by Bennett et al and their reply regarding your concern concurs that confusion can exist by using the term "proband" instead of "index case." The readers are urged to modify Figure 1 (*GGH* 1995;11[3]:12) as you suggest and to modify their use of the terms "index case" and "proband" as you suggest.

Sincerely,

Robert M. Blizzard, MD  
Chairman, *GGH* Editorial Board

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## Predictive Factors in the Determination of Final Height in Boys With Constitutional Delay of Growth and Puberty

Albanese and Stanhope hypothesized that boys with constitutional delay of growth and puberty (CDGP) form a heterogeneous diagnostic category composed of children with varying degrees of impairment of final height. Consequently, they analyzed the patterns of growth in height and the changes in body proportions in 78 prepubertal or early pubertal boys with CDGP. The characteristics of those in this group were that the chronological age was  $\geq 13$  years and bone age delay was  $>1.5$  years. These boys were treated for 4 months with either 50 mg of sustained-action testosterone every 2 weeks or 1.25 mg daily of oxandrolone, or received neither drug. The mean height standard deviation score (SDS) was  $-2.7 \pm 0.7$  ( $140.6 \pm 8.6$  cm) at the initial evaluation and  $-2.0 \pm 0.9$  ( $160.5 \pm 6.7$  cm) at final height. The latter was significantly below either the mean predicted adult height or the corrected midparental height (MPH), although much overlap occurred. The final height of 45 (58%) of the 78 patients did not achieve the target height range. Of the 33 (42%) of patients whose final heights fell within the target height range, the heights of only 3 (0.7%) exceeded the corrected MPH.

At final height, several (26%) of the boys had eunuchoid habitus, with short spines relative to lower limb lengths at diagnosis and at final height. Using multiple regression analyses, the authors determined that standing height, growth velocity, and the difference between the sitting height and the subischial leg length present at the initial evaluation could be used as predictors of impaired final height. Neither the chronological age, the delay in bone age at the initial examination, nor treatment for 4 months with androgens influenced this analysis.

The authors conclude that decreased spinal growth is present in many boys with CDGP, and that the presence of a short spine relative to leg length suggests that final adult stature will be impaired.

Albanese A, Stanhope R. *J Pediatr* 1995;126:545-550.

**Editor's comment:** As the authors point out, the 78 boys studied represent only a fraction of those with CDGP, and possibly only those with the most severe impairment of growth were followed in the authors' clinic. Therefore, their conclusions may be applicable only to a subset of patients with CDGP. Nevertheless, the observation of impaired prepubertal spinal growth leading to impaired final height prompts the question whether some patients with CDGP have a subtle spinal chondrodystrophy. The report indicates the need to routinely measure sitting heights or upper to lower ratios in such patients.

Allen W. Root, MD

**2nd Editor's comment:** The authors were unable to explain the failure to achieve target height in 58% of their patients. Speculation is appropriate that this subgroup may have a variant of CDGP, one with growth hormone insufficiency that is not revealed by pharmacologic tests of growth hormone secretion, or they possibly may have an unclassified skeletal dysplasia. The authors also conclude that treatment with androgens, at the doses used, does not improve final height but only accelerates the growth spurt. They do suggest that the use of low doses of oxandrolone may prevent reduced spinal growth and, consequently, improve final height. In this editor's opinion, the problem with some of these speculations is that only 4 months of androgen therapy were used, and this short period of therapy may not affect either predicted height or ultimate height. Earlier androgen therapy over a prolonged period but at a dose absolutely not higher than that recommended by the authors may be beneficial in increasing ultimate height. Studies need to be done concerning this.

Robert M. Blizzard, MD

## Zinc Deficiency in a Breast Fed Premature Infant

Zinc is an essential element for a variety of biochemical functions of the human body, including normal function of skin, the gastrointestinal system, and the immune and central nervous system (CNS) systems. Individuals with severe zinc deficiency present with erosive skin changes, particularly of the face and anogenital area, and with alopecia of scalp hair. Failure to thrive, irritability, and immunodepression are also common.

Several causes of zinc deficiency syndrome are known. These include: (1) deficient exogenous zinc supply, either from breast milk when the mother is deficient in zinc or in her diet; (2) increased intestinal or urinary zinc loss; (3) inadequate absorption in preterm infants; and (4) poor storage. Since breast milk usually is a good source of zinc, severe zinc deficiency in full-term infants is very rare. However, a number of investigators have reported zinc deficiency in breast-fed, preterm infants (Aggett et al; Bilinski et al; Buehning and Goltz).

A recent paper by Heinen et al reports a typical case of a preterm infant who was exclusively breast-fed and suffered from severe zinc deficiency syndrome. The neonatal period was complicated by bronchopulmonary dysplasia, cerebral hemorrhage with subsequent hydrocephaly, and ventriculitis. A zinc-containing formula was given only for the first 4 days of life. After that he was breast-fed and received parenteral nutrition without zinc supplements. At 20 weeks, erosive skin changes, developmental retardation, and muscular hypotension were noted. Blood zinc levels measured in both mother and infant were significantly low. Oral zinc therapy was instituted. Marked improvement in the skin lesions occurred in 2 days. Heinen et al concluded that a diet based exclusively on breast milk may in some cases, depending on the mother's nutritional status, lack sufficient zinc and lead to severe zinc deficiency in the infant.

Aggett P, et al. *Arch Dis Child* 1980;55:547-550.  
 Bilinski DL, et al. *Arch Dermatol* 1987;123:1221-1224.  
 Buehning LJ, Goltz RW. *J Am Acad Dermatol* 1993;28:499-501.  
 Heinen F, et al. *Eur J Pediatr* 1995;154:71-75.

**Editor's comment:** Zinc deficiency is rare without a predisposing disease such as acrodermatitis enteropathica. However, it must be considered in the premature infant who may have less than normal zinc absorption. This particular case was complicated by prematurity and maternal zinc deficiency. He responded really well to therapy. Interestingly, the authors point out that if zinc oxide paste is used for diaper rash, the zinc may be absorbed transcutaneously from the anogenital area.

Judith G. Hall, MD

**2nd Editor's comment:** Zinc deficiency in premature, breast-fed infants has been previously described in the literature. It is not apparent why the authors state that this case is an example of a "distinct form of zinc deficiency syndrome." The infant described received markedly inadequate zinc intake from day 4 through 21 of life by being treated parenterally without zinc supplementation. The zinc content of the breast milk also was poor: <50% of the usual zinc content of human milk. No information was presented regarding the amount of breast milk that the baby was receiving. Since the infant was failing to thrive, energy requirements of the infant may not have been met secondary to inadequate breast milk feedings.

No information was given to explain why the mother was zinc deficient. Did she have a genetic disposition to zinc

deficiency or possibly a medical condition that interfered with zinc absorption or utilization? Moreover, there is no discussion about the mother's nutritional status during pregnancy. Speculation that the marginal zinc status of the mother may have contributed to the baby's congenital abnormalities is appropriate. In animal models, zinc deficiency during gestation has resulted in teratogenic fetal abnormalities, including neural tube defects. Epidemiologic data also link maternal zinc deficiency and CNS malformations in the fetus. Women with acrodermatitis enteropathica have a high incidence of spontaneous abortions and fetal alterations, including skeletal abnormalities and anencephaly.

Zinc needs of growing infants may be best met by breast milk even when there is intestinal malabsorption. However, low-birth-weight infants may be at risk for zinc deficiency and for these infants, the quality of the breast milk must be considered. Zinc content of human milk normally falls as lactation progresses. Thus, banked human milk may contain less zinc than the mother's own milk and should be analyzed and supplemented with breast milk fortifier before use in feeding premature infants. Providing parenteral nutrition to a premature infant without zinc supplementation is inappropriate medical care. Current recommendations call for 400 mg/kg/d of zinc in total parenteral nutrition solutions. Poor nutrition during gestation will have a great impact on the health of the baby, and knowledge of the mother's nutritional health will assist in treating the infant appropriately.

Fima Litshitz, MD

## Adrenal Insufficiency and Bronchopulmonary Dysplasia in Low Birth Weight Infants

The causes of bronchopulmonary dysplasia are not clear, but it has been associated with injuries due to mechanical ventilation. However, chronic lung disease and bronchopulmonary dysplasia can develop in small premature infants who show little initial respiratory distress and who have never needed respiratory assistance.

Glucocorticoids produced by the adrenal glands play an important role in the resolution of inflammation and the response to stress. The trigger for the release of glucocorticoids from the adrenal glands is the hypothalamic-pituitary-adrenal axis. If this axis is functioning normally, inflammatory reactions are easily resolved and the damage repaired. However, if the axis is not functioning properly, impaired inflammatory

reactions can be excessive and damage repair progresses very slowly.

Some studies done in animal models have shown that in the first few hours of life the response to adrenocorticotrophic hormone (ACTH) may be absent (Guillet et al; and Walker et al). Studies in humans show that in the first hours of life some low-birth-weight neonates may also lack the appropriate response mediated by the hypothalamic-pituitary-adrenal axis. This has been called the neonatal stress "nonresponsive" or "hyporesponsive" period. During this period the adrenal gland shows a diminished or absent response to ACTH. This nonresponsiveness is thought to resolve within the first week of life.

A recent study by Watterberg and Scott suggested that some premature infants may not recover from the neonatal stress nonresponsive period as fast as expected and are thus predisposed to greater damage from inflammatory reactions. In order to confirm this, they tested the cortisol response to ACTH by measuring blood cortisol secretion levels in a population of very-low-birth-weight infants.

At the end of the first week of life, the infants who had higher blood cortisol levels recovered without bronchopulmonary dysplasia while the infants with lower cortisol levels eventually developed bronchopulmonary dysplasia and

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remained dependent on oxygen supplementation. Watterberg and Scott concluded that the infants in the latter group may not be able to secrete an adequate amount of cortisol in response to stress and thus inflammation goes unchecked and renders them susceptible to long-term lung injuries.

Guillet C, et al. *Endocrinology* 1980;106:991-994.  
Walker CD, et al. *Endocrinology* 1986;118:1445-1451.  
Watterberg KL, Scott SM. *Pediatrics* 1995;95:120-125.

**Editor's comment:** These observations could have important ramifications for preventive therapy in premature infants. Bronchopulmonary dysplasia is a very crippling disorder and its prevention would certainly be welcome. The observations need to be confirmed. An appropriate method of screening and trial of therapy could then lead to prevention of this dreaded complication of prematurity.

Judith G. Hall, MD

## Reduced Growth Hormone Secretion With Maintained Periodicity Following Cranial Irradiation in Children With Acute Lymphoblastic Leukaemia

Lannering et al obtained growth hormone (GH) determinations every 20 minutes for 24 hours in a group of 34 children with acute lymphoblastic leukemia (ALL) who had received cranial irradiation with 18 to 24 Gy. These children (12 boys and 22 girls) had been diagnosed 4 to 10 years previously; their mean age at diagnosis was 3.9 years. Fourteen (5 boys and 9 girls) were prepubertal at the time of the study (using Tanner staging). Height was expressed as standard deviation scores (SDS) in comparison with Swedish reference values for healthy children. A control group of 208 children was utilized. The GH profiles were analyzed using the Pulsar pulse detection program and Fourier time-series analysis.

The estimated GH secretion rate in all irradiated ALL children was below the median of that of controls for pubertal stage and sex. The difference between patients and controls was more pronounced in late puberty than before puberty. GH secretion as expressed by the area under the curve was also reduced in irradiated children. However, the number of GH peaks over

24 hours was within the normal range for both boys and girls. Before puberty a broad range of cycles per 24 hours was seen; these synchronized during puberty to approximately 1 every 3 to 4 hours. Lower peak amplitudes were observed in the irradiated children. There was no correlation between time from diagnosis and GH secretion or the maximal GH level during the 24-hour period. There were no obvious influences of the time of diagnosis on GH secretion. Children who were still prepubertal at the time of the study had lost an average of 0.2 SDS. Children who had entered puberty lost an average of 1.0 SDS.

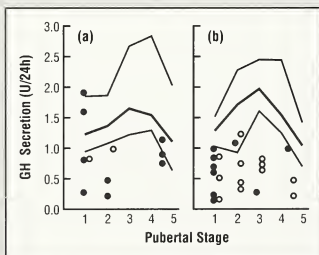
The authors state that their results indicate not only that cranial irradiation in the range of 20 to 24 Gy alters GH secretion (as determined by Moell et al, 1988), but also that irradiation with 18 Gy both before and during puberty reduces GH secretion. Specifically, there was lower pulse amplitude in the irradiated patients, suggesting a physiologic GH insufficiency. Height of the children at a mean follow-up age of 7 years fell within the normal range for the Swedish population. Final heights were not reached in a majority of patients. The authors further state that the impairment observed in growth is small before puberty. The recommendation is made that ALL patients should be studied repeatedly as adults to evaluate the effects of decreased GH secretion on organs other than the growth plate.

Lannering B, et al. *Clin Endocrinol* 1995;42:153-159.

**Editor's comment:** More and more information regarding the effects of cranial irradiation on pituitary function is becoming known. Although most pediatric endocrinologists recognize that irradiation with 24 Gy could be expected to be associated with pituitary dysfunction, it is not generally felt that lower dosages will be detrimental. However, few investigators have performed the careful type of analysis that Lannering and coworkers presented. Their data suggest that there are indeed significant reductions in GH secretion with smaller doses of radiation that may not be clinically observable (no obvious reduction in stature) until puberty, and that there is little difference between the effects of 18 and 24 Gy. It will be interesting to review final heights in the patients reported in this study. One may then be able to better counsel families whose children have received even modest doses of cranial irradiation.

William L. Clarke, MD

Figure 1



Individual values of growth hormone (GH) secretion rate are shown for (a) boys and (b) girls with acute lymphoblastic leukemia irradiated with ● (18 Gy) or ○ (24 Gy). GH secretion rate of healthy, normally-growing children at pubertal stages 1 through 5 is also given (75th, 50th, and 25th percentiles).

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## Longitudinal Data on Growth and Final Height in Diabetic Children

Growth and development were analyzed in 2 cohorts of diabetic children. In one cohort ( $n=46$ ; 22 girls and 24 boys) children with a mean age at diagnosis of diabetes of 7.5 years were followed until they attained final height; in the other cohort ( $n=27$ ; 11 girls and 16 boys) diabetic children were followed from less than 7 years of age to age 10 years for evaluation of early pubertal growth. All children were treated with conventional insulin therapy (2 injections a day of both long-acting and short-acting insulin). Metabolic control was assessed by glycosylated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>). Height (Ht) was evaluated every 6 months and transformed into standard deviation scores (SDS) using the 1966 standards for height developed by Tanner, applying a correction for the secular trend. Onset of puberty, final height, total pubertal height growth, body mass index and skeletal age were also recorded. The whole group of patients showed a mean final Ht SDS lower than Ht SDS at onset of disease ( $0.27 \pm 0.97$  vs  $0.41 \pm 0.99$  in girls and  $0.48 \pm 0.89$  vs  $0.56 \pm 0.68$  in boys). Final Ht was significantly lower than target Ht in girls ( $163.7 \pm 5.9$  cm vs  $167.1 \pm 5.0$  cm,  $P<0.05$ ) but boys did not have significant differences ( $177.1 \pm 6.1$  vs  $178.1 \pm 6.0$  cm).

Prepubertal growth was not affected by diabetes mellitus in either sex, but there was pubertal delay in boys. Ht SDS evolution showed a significant drop over the last 2 years of prepubertal growth in both, associated with the delay in the onset of puberty. Total pubertal height gain was negatively correlated with the chronologic age at onset of puberty in both boys and girls.

Girls gained weight excessively during pubertal growth. Their body mass index SDS increased from  $0.26 \pm 0.98$  at Tanner stage 2 for breast development to  $0.69 \pm 0.97$  at

final height. Bone age did not deviate from chronologic age at 10 years and at the time of pubertal stage 2.

The authors concluded the following: (1) Diabetic children have normal height at the onset of their diabetes. (2) Final height in girls was slightly reduced from target height. (3) Diabetic girls had a tendency to become obese during puberty. (4) Boys with diabetes showed a marked delay in the onset of puberty but attained an appropriate final height for their target. No correlation was found between the degree of metabolic control (HbA<sub>1c</sub>) and the total pubertal height gain.

Du Caju MVL, et al. *Pediatr Res* 1995;38:607-611.

**Editor's comment:** This is a unique paper describing growth and development in a small group of children with diabetes mellitus on a longitudinal basis, from onset of diabetes until completion of growth. Other papers assessing growth in diabetic children are cross-sectional studies, thus yielding data that cannot be as reliable as that described here.

However, it is hard to understand the precise significance of the authors' findings, since there were no measurements of growth para-factors in this study that could help ascertain possible pathophysiologic mechanisms to explain some of the differences mentioned above. Why is it that girls had a loss of final height whereas boys did not? Also, why did females become obese while males did not?

The authors did not find alterations of growth at the time of disease onset nor did they find a correlation with the degree of metabolic control. Other studies have shown improvement of growth with tight control of diabetes.

Fima Lifshitz, MD

## Zinc Supplementation and Growth of Infants Born Small for Gestational Age

Sixty-eight full-term small-for-gestational-age (SGA) infants were randomized into 2 groups: 1 of zinc (Zn) supplemented (S) and 1 of placebo (P) infants, in a double-blind manner. Group S received Zn 3 mg/d PO between feedings as a solution of Zn acetate containing 1 mg Zn/mL; group P received an equivalent volume of placebo solution. Both solutions were added to aspartame to make their taste similar. Data analysis was done with 35 infants in group S and 33 infants in group P. Before starting the Zn supplementation or placebo, and at 30, 60, 120, and 180 days of life, all infants had a blood sample drawn for measurement of plasma Zn; samples of occipital hair for measurement of hair Zn and accurate measurements of length, weight, and head circumference were obtained. All infants were initially breast-fed. Supplementation with cow's milk-based formulas was done at different ages according to perceived needs and not knowing the group of assignment. Weight (Wt)-for-age standard deviation scores (SDS) showed differences in catch-up growth between the 2 groups of patients. Patients in group S showed better catch-up growth than patients in group P.

The best improvement in Wt-for-age SDS in relation with Zn supplementation was seen among girls. An additive effect for increased Wt-for-age catch-up growth was seen in infants exclusively breast-fed for 4 months, Zn supplemented, and of the female gender. Length (Lt)-for-age SDS showed similar improvements in both groups. Plasma and hair Zn

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decreased in both groups over 6 months, but the decline was less pronounced in group S.

The authors conclude that Zn supplementation during the first 6 months of life of SGA infants in the population studied had beneficial effects for growth in both genders, but was more pronounced in girls. They attributed this effect to a deficiency in Zn nutritional status in SGA.

Castillo-Durán C, et al. *J Pediatr* 1995;127:206-211.

**Editor's comment:** Micronutrients, particularly Zn, have been receiving increasing attention in the last few decades in regards to their role in human growth and development. Zn accretion occurs mainly during the third trimester and has been calculated to be 0.85 mg/d. However, full-term infants born SGA may be at a particular risk for Zn deficiency. The American Academy of Pediatrics Committee on Nutrition has recommended that formulas for full-term infants supply at least 0.5 mg Zn per 100 kcal.<sup>1</sup> Recommendations of up to 1 mg/kg/d have been given for preterm infants in the stable/postdischarge period. The recommendation also includes to adding only 0.5 mg/kg/d when the infant is fed human milk.<sup>2</sup> The authors supplemented these infants with 3 mg/d Zn regardless of whether they were being breast-fed or formula fed; they showed an improved catch-up growth when given Zn

supplementation. This paper suggests that the recommendations for Zn supplementation in this group of infants should be reconsidered and would be higher than the current recommendations. This is applicable even to breast-fed patients.

The difference between girls and boys remains unexplained, and as the authors point out, deserves further exploration.

**2nd Editor's comment:** These infants were SGA infants for the most part, and not IUGR as defined and described by Warshaw in GGH (1992;8[1]:5-8), which the readers are encouraged to review. The concern this editor has is the wide SDS seen in both groups at each time point evaluated. A tighter statistical difference may be needed to be certain that the findings were not spurious. Regardless, the data gained may assist in evaluating the role of Zn in relation to growth parameters in SGA infants.

Robert M. Blizzard, MD

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## Effects of Differences in Dietary Fat on Growth, Energy and Nutrient Intake From Infancy to 8 Years of Age

Boulton and Magarey, as part of the ongoing Adelaide (Australia) Nutrition Study, evaluated retrospectively growth and energy intake in a cohort of 140 randomly selected children. Subjects were seen at 3, 6, and 12 months of age and at 2, 4, and 8 years of age. Before each of the visits, the parents kept a record of the child's diet. Up to 2 years of age, a 7-day weighed food record was kept. At 4 years, a 3-day record was kept; and at 6 and 8 years, a 4-day weighed food record was kept. The diet composition was analyzed using a computer program. Energy and nutrient intakes were expressed as mean intake per day. Fatness was evaluated by the sum of 4 skin-fold thickness measurements (left mid-biceps, triceps, subscapular, and suprailiac). At each age, the sample was divided into 3 groups according to the percentage of food energy derived from fat: <30%, 30% to 34.9%, and >35%. These cutoffs were chosen since 30% corresponds to the fat intake target for Australian adults, and 35% is the recommended maximum level of fat intake for young children in some countries.

The authors state that there were no significant differences in energy or nutrient intake or attained height and weight through infancy to 8 years of age according to the proportion of fat in the diet, and those in the low-fat group did not have lower essential mineral intake. They speculate that the boys in the low-fat group at 2 years of age may have had a slower growth velocity, thus accounting for their slightly lower height at age 15. They conclude that a shift to a low-

fat intake in early childhood is unlikely to have any deleterious effects on growth.

Boulton TJC, Magarey AM. *Acta Paediatr* 1995;84:146-150.

**Editor's comment:** This is a very interesting and important retrospective study. The current dietary recommendation for adults in the United States is to derive <30% of our daily caloric intake from fat. Whether this level of fat intake will have a significant effect on growth and the timing of puberty has been the subject of some controversy. The fact that in the present report boys with lower fat intakes at age 2 were somewhat shorter at age 15 than those with higher fat intakes suggests that there may be some validity to these concerns. However, what is not clear in this study is whether these 140 children remained in the same fat intake group throughout childhood. Children were not randomly assigned to a specific level of fat intake but rather their natural eating habits were evaluated using 4- to 7-day food records. Thus, there is a strong possibility that some children switched from group to group throughout childhood. There is also some concern with regard to the validity of dietary food records, although recent studies suggest that this is a relatively accurate means for measuring nutrient intake. Despite these potential shortcomings, the information in this study is of significant interest and importance to physicians who prescribe dietary regimens for children.

William L. Clarke, MD

# Role of Steroidogenic Acute Regulatory Protein in Adrenal and Gonadal Steroidogenesis

Lin et al identified the molecular defect that causes lipid adrenal hyperplasia, an autosomal recessive disorder that is associated with feminization of male external genitalia, severe salt loss in both sexes, and excessive amounts of cholesterol and impaired steroidogenesis in the adrenal and gonads. The abnormality was suspected to be in the cholesterol side chain cleavage enzyme (P450scc), but analysis of the P450scc gene (*CYP11A*) has been normal in affected subjects. Lin et al provide evidence that the primary defect is in the steroidogenic acute regulatory (StAR)

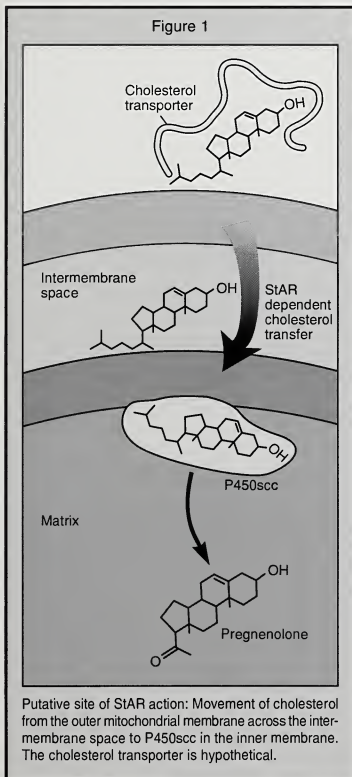
protein. This mitochondrial protein escorts cholesterol from the interior surface of the outer mitochondrial membrane to the inner mitochondrial membrane, where it serves as substrate for P450scc and initiates steroidogenesis (Waterman MR. *Science* 1995;267:1780). StAR is required for steroidogenesis in the adrenal and gonads but not in the placenta. StAR, which is responsive to corticotropin, is a 285 amino acid protein with a 25 amino acid mitochondrial targeting sequence. It is cleaved after entering the mitochondrion.

In 3 unrelated patients with lipid adrenal hyperplasia, 2 separate base pair changes were found in the StAR gene: (1) in patient 1, a C → T transition in codon 193 (Arg) resulted in a premature stop codon that is 93 amino acid residues shorter than the mature product; (2) in patients 2 and 3, a C → T transition at codon 258 (Gln) resulted in a premature stop codon and a truncated protein 28 amino acids shorter than the mature protein. Expression of these truncated products in COS-1 cells revealed absent steroidogenesis with cholesterol as substrate. Interestingly, with 20 $\alpha$ -hydroxycholesterol as substrate, steroidogenesis was normal in these cells.

Lin D, et al. *Science* 1995;267:1828-1831.

**Editor's comment:** The essential role of the StAR accessory protein in the mobilization and transmembrane transport of cholesterol for steroidogenesis is in the adrenal and gonads but not in the placenta, since progesterone production is normal in pregnancies in which fetuses have lipid adrenal hyperplasia. Since StAR is not expressed in the placenta and brain, another mitochondrial cholesterol transport system must be present in these tissues. The observation that when 20 $\alpha$ -hydroxypregnenolone is employed as substrate steroidogenesis is normal in defective cells suggests that this compound may be of therapeutic benefit to patients with lipid adrenal hyperplasia. Of the several forms of congenital adrenal hyperplasia, lipid adrenal hyperplasia is the only one that is not due to a molecular defect in a steroidogenic enzyme.

Allen W. Root, MD



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## Evidence for Partial Growth Hormone Insensitivity Among Patients With Idiopathic Short Stature

Reported in this study are 511 children with idiopathic short stature (ISS) (height standard deviation score [SDS] of  $\leq -2$ ; maximum stimulated growth hormone [GH]  $>10 \mu\text{g/L}$ ; and no other reason for short stature) who were treated with GH. Growth hormone-binding protein (GHBP) was measured before GH treatment. In 101 (20%) patients GHP SDS  $\leq -2$ , whereas in the remaining 410 (80%) patients GHP SDS  $> -2$ . Patients with low GHBP levels had lower mean extracted insulin-like growth factor 1 (IGF-1) SDS ( $-3.3 \pm 1.1$  vs  $-2.5 \pm 1.4$ ;  $P < 0.0001$ ) and higher mean 12-hour GH values ( $2.8 \pm 1.1$  vs  $2.3 \pm 1.1 \mu\text{g/L}$ ;  $P < 0.0001$ ) when compared with patients with normal GHBP levels. A direct correlation was found between GHBP SDS and extracted IGF-1 SDS, whereas an inverse correlation was present between GHBP SDS and mean 12-hour GH values. Growth velocity before and after 1 year of treatment with GH was not different between prepubertal patients with low and normal GHBP. No correlation was found between first-year growth rate with GH treatment and GHBP SDS. The authors conclude that ISS patients who have low levels of GHBP are partially insensitive to GH, as suggested by a lower IGF-1 and a higher 12-hour mean GH concentration. The authors also present a proposal for a redefinition of normal growth and growth disorders based on the evaluation of endogenous GH secretion and GH responsiveness assessed by the GHBP.

Attie, KM, et al. B.M. J *Pediatr* 1995;127:244-250.

**Editor's comment:** This is an excellent study with a large number of short-statured patients studied in a sophisticated prospective manner. Unfortunately, the authors did not separate the results by growth velocity measured before and after treatment with GH. They reported a mean pretreatment growth velocity of  $4.0 \pm 1.7 \text{ cm/y}$  in the low GHBP group and of  $4.2 \pm 1.9 \text{ cm/y}$  in the normal GHBP group. The great variability implied by the mean  $\pm 2 \text{ SD}$  (ie, growth velocities before initiation of therapy ranging from 0.6 to 7.7 cm/y and from 0.4 to 8.0 cm/y, respectively) indicates that there were some patients who were growing very well and some others who were growing poorly. The responses to GH were also reported as a mean of the whole group, thus individual variations cannot be discerned. Patients growing at a decreased rate who significantly increased their growth after GH treatment would differ from those patients originally growing at normal rates who had a minimal increase of growth rate after GH treatment. These data are important to understand the significance of the findings reported.

The more we look for magic bullets to diagnose growth abnormalities, the more compelling becomes the old adage: careful measurements of growth velocity are necessary to ascertain the need for therapy and the response to it. The reader is referred to a recent article in the *New England Journal of Medicine* (1995;333:1093-1098); for a report of the mutations of the GH receptor in children with ISS.

Fima Lifshitz, MD

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# GROWTH

## Genetics & Hormones

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### Allelic Expansion Underlies Many Genetic Diseases

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For decades, most genetic diseases were little more than medical curiosities. The genes harboring the responsible mutations, the nature of the mutations, and the mechanisms by which the mutations produced clinical disease were either unknown or poorly understood. However, this is now rapidly changing. Nowhere is our accelerated understanding more evident than in disorders that result from instability of trinucleotide repeats of DNA.

Indeed, **observations over the past 4 years have revealed that mutations involving instability of trinucleotide repeats, or DNA tracts of tandemly repeated 3-bp units, are responsible for numerous human genetic disorders** (Table 1, page 2).<sup>1</sup> Since the instability usually involves an increase in the number of repeats in only 1 of a person's 2 alleles for a given gene, **the phenomenon is often called "allelic expansion."** Delineation of this phenomenon, combined with the recent discovery that mutations in DNA repair genes leading to familial cancer syndromes may contribute to such genetic instability, has increased both the awareness of and interest in these repeat DNA sequences, and in the consequences of their instability. In this article, some aspects of the data regarding instability of simple repeats during DNA

replication and genetic transmission are reviewed and current hypotheses regarding mechanisms leading to this remarkable phenomenon are presented.

Among the trinucleotide repeat disorders, **2 general classes of instability of the repeat sequences exist.** The **first class** has mutations leading to only small-scale alterations of up to 2-fold the original length in repeat size; in the **second class**, both small-scale and quite large-scale changes of as much as 20-fold occur (Table 1, page 2). **No mechanism has been demonstrated to account for either type of mutation.** While it is tempting to view these 2 general types of mutations as different mechanistically, there is no direct evidence as yet to support this view.

#### DISEASES RELATED TO SMALL-SCALE CHANGES

Disorders characterized by exclusively **small-scale instability** include **spinobulbar muscular atrophy (SBMA), Huntington disease (HD), spinocerebellar ataxia type 1 (SCA1), dentatorubral pallidoluysian atrophy (DRPLA), and Machado-Joseph disease (MJD).**<sup>2-7</sup> Each disorder exhibits mutations that change the number of CAG repeats. Since the trinucleotide-CAG codes for glutamine, these mutations are presumed to alter the length of polyglutamine tracts in the respective gene products, which leads to degeneration that is specific for subsets of central or spinal neurons. These disorders recur when the expanded repeat sequence is transmitted from parent to child. Little or no variation in repeat size is observed in somatic tissues of an individual, ie, there is no somatic mosaicism. It is tempting to attribute the instability observed during parent-to-child transmission to mutations occurring during meiosis, and to attribute the instability observed during somatic growth of an individual to mutations occurring during mitosis. However, it is not clear whether the alterations observed before conception occur during meiosis or in the mitotic divisions that precede or even follow sperm or egg production.

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Small-scale expansions can reach a doubling of the repeat number, and occasionally reductions in repeat number are found. Expansions vastly exceed reductions, and the likelihood and magnitude of change are related to whether they are inherited from the mother or the father. In general, paternal transmissions result in greater changes in size and more frequently involve increases. Measurement of expanded repeat lengths in sperm demonstrate directly that these increases occur in the germ line of the father rather than in the embryo. It also is known that **patients who carry large repeat lengths often have early onset of their disorder**.<sup>4-9</sup> These phenomena help explain the greater likelihood of juvenile onset of HD, SCA1, and DRPLA when the expansion is inherited from affected fathers rather than affected mothers. The propensity for expansion provides a clue in considering the mechanism of instability.

The alleles containing the maximum number of repeats found in patients with SBMA is 62. In HD, the maximum number of repeats found is 121. These sizes are below the low end of repeat sizes in disorders

subject to large-scale changes (Table 1). This size restriction may reflect the presence of the CAG repeat in the protein-coding regions of these genes. Alteration might simply abolish the functions of such proteins. However, this does not seem to be the case, since mutations known to abolish function of these proteins produce different clinical manifestations compared with those involving allelic expansion. As noted earlier, individuals carrying expanded repeats in this general class rarely show mosaicism, ie, multiple sizes of alleles within a tissue. This is in marked contrast to what is found in individuals carrying the large amplifications, where multiple sizes of alleles within a tissue occur.

## DISORDERS RELATED TO LARGE-SCALE CHANGES

In fragile site-related mutations, such as in the fragile X syndrome<sup>10-12</sup> and in myotonic dystrophy,<sup>13-15</sup> increases in repeat number as much as 20-fold have been observed in a single parent-to-child transmission.

Table 1  
Characteristics of Unstable Trinucleotide Repeats Identified to Date

| Disease                             | Chromosome | Locus        | Location in Associated Gene | Repeat    | Size in Normal | Size in Carrier | Size in Affected | Change in Gene Function |
|-------------------------------------|------------|--------------|-----------------------------|-----------|----------------|-----------------|------------------|-------------------------|
| Kennedy disease (SBMA*)             | Xq11-12    | AR           | Exon 1                      | CAG (gln) | 12-34          | —               | 40-62            | Gain                    |
| Huntington disease                  | 4p16.3     | HD           | Exon 1                      | CAG (gln) | 6-37           | —               | 35-121           | Gain                    |
| Spinocerebellar ataxia type 1       | 6p22-23    | SCA1         | Exon 8                      | CAG (gln) | 6-39           | —               | 41-81            | Gain                    |
| Dentatorubral pallidolusian atrophy | 12p12-13   | DRPLA        | Exon 5                      | CAG (gln) | 7-34           | —               | 54-70            | Gain                    |
| Machado-Joseph disease              | 14q32.1    | MJD          | Internal exon?              | CAG (gln) | 13-36          | —               | 68-79            | Gain                    |
| Fragile X syndrome                  | Xq27.3     | FRAXA (FMR1) | 5' untranslated             | CGG       | 5-52           | 43-200          | 230->2,000       | Loss                    |
| Dystrophin myotonia                 | 19q13.3    | DM           | 3' untranslated             | CTG (CAG) | 5-37           | 44, 46          | 50->2,000        | RNA stability?          |
| Mental retardation?                 | Xq27.3     | FRAXE        | ??                          | GGC (CGG) | 6-25           | 116-133         | 200->850         | ??                      |
| (None)                              | Xq28       | FRAXF        | ??                          | GGC (CGG) | 6-29           | —               | 300-500          | ??                      |
| (None)                              | 16p13.11   | FRA16A       | ??                          | GGC (CGG) | 16-49          | —               | 1,000-2,000      | ??                      |

Small-scale polyglutamine (gln) disorders are in the top half of the table, and large-scale mutations are in the bottom half. Repeat sequences are listed in the coding strand and frame, where known, or as reported by the authors of the study; however, only 2 triplets (CAG and CGG) are represented.

\* Spinal and bulbar muscular atrophy



It is possible that **large-scale expansions** result from the same mechanism as the small-scale changes. However, in these instances they **are the consequence of many rounds of expansion during the DNA replication preceding meiosis or mitosis occurring in the early embryo**. In support of this notion is the finding of significant levels of mosaicism in the absence of ongoing instability in the fragile X syndrome, suggesting an early embryonic expansion limited to somatic tissues.<sup>16</sup> Evidence in this syndrome also suggests that the male germ line is spared the expansion event. However, it should be noted that sufficient differences exist in the characteristics of the large-scale expansions to raise questions as to whether one mechanism is common to all. For example, such expansions occur only with maternal transmission in fragile X syndrome, while both maternal and paternal transmissions can result in large increases in myotonic dystrophy. Reductions are observed rarely in myotonic dystrophy and in fragile X syndrome.

## LESSONS FROM OTHER REPEAT SEQUENCES

Because of their utility in genetic mapping, a vast number of repeats of the dinucleotide CA have been isolated and characterized. The fact that their sizes are polymorphic, ie, their sizes vary in the population, indicates that they are unstable. Moreover, the likelihood and degree of this polymorphism correlate with the length and purity of the repeats. However, the instability of CA repeats is largely historical, since in today's population they are genetically transmitted with high fidelity. Indeed, this fidelity allows their use as genetic markers. Mutations can be found in CA repeats, and the frequency has been estimated from family studies at  $\sim 5 \times 10^{-4}$  per gamete per generation.<sup>17</sup> Direct analysis of cell lines provides a measurement of  $\sim 1 \times 10^{-5}$  per cell per generation for a CA<sub>17</sub> repeat.<sup>18</sup> While these values are significantly higher rates of mutation than are found in DNA sequences outside of a simple repeat sequence, they are well below the rates found in the trinucleotide repeat disorders mentioned earlier ( $\sim 10^0$ ).

Mutations in CA repeats involve additions or deletions of one repeat unit or a few repeat units, analogous to the small-scale changes found in trinucleotide repeats described previously. The frequency of mutation may relate to length, as is suggested by the higher levels of polymorphism found in the longer repeats. **Most CA repeats are less than 30 repeats in length (60 bp), while the instability found in trinucleotide repeats becomes significant above 35 repeats (100 bp).** In accord with this is the observation of greater mutation rates in tetranucleotide repeats assayed in family studies ( $2.1 \times 10^{-3}$ ).<sup>17</sup> Tetranucleotide repeats tend to be longer overall than CA repeats (40 to 100 bp).

## MISMATCH REPAIR PROCESSES

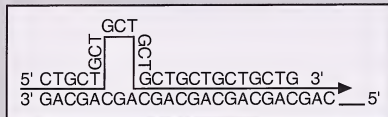
Recent efforts to characterize familial cancer syndromes have identified **defects in repair of DNA in which bases are mismatched during replication**. These are termed **mismatch repair processes**. For example, Lynch syndrome, or hereditary nonpolyposis carcinoma of the colon (HNPCC), was mapped to sites on chromosomes 2 and 3 by analyzing how tumors were inherited in families and detecting loss of heterozygosity (LOH) in tumors. The latter refers to the fact that certain genes, known as tumor suppressor genes, need to be present in at least one copy (heterozygosity) to prevent tumors. LOH for such protective genes is often found in tumors. The LOH analyses utilized CA repeat DNA markers, and the number of repeats was often found to have changed in the tumors compared with normal tissues from the same individual. These alterations are similar to the small-scale changes found in the trinucleotide repeat disorders, involving increases and decreases by one or a few repeat units. The instability found in HNPCC tumors suggested a possible role for defective mismatch repair in these families, since mutations of mismatch repair pathways in yeast (*Saccharomyces cerevisiae*) lead to similar instability of dinucleotide repeats. Indeed, mutations in 4 human genes equivalent to bacterial mismatch repair genes located on chromosomes 2, 3, and 7 have been found in Lynch syndrome families,<sup>19-23</sup> and cell lines derived from such individuals exhibit defects in mismatch repair. **It is postulated that defective mismatch repair allows accumulation of mutations that promote tumorigenesis.** Observed changes in the number of CA repeats are in essence a side effect of the repair defects, but this offers insight into the mechanism responsible for these conditions.

## SLIPPERY DNA

**One mechanism for generating mismatched DNA involves slipped mispairing or slippage of repeated DNA sequences during replication.** Figure 1 (page 4) shows generation of slipped structures. When these errors occur during DNA replication in yeast and *Escherichia coli*, they are recognized and repaired by the mismatch repair system. When unrepaired, such structures generally result in increases or decreases of one or a few slipped repeat units, resembling the small-scale changes seen in trinucleotide repeat disorders. Slipped mispairing is enhanced significantly by greater length and purity of repeat sequences, correlating with the increases seen in trinucleotide repeat instability. **It is likely that the small-scale changes result from slipped structures that are not adequately repaired.** There is no evidence to suggest that mismatch repair is defective in families with trinucleotide repeat disorders. In fact, the preponderance of the



Figure 1  
Example of Slipped Mispairing During  
DNA Replication



DNA sequences with repetitive elements are found to have a higher likelihood of exhibiting slipped structures, which can result in both increases and decreases in repeat number. This mechanism has a role in trinucleotide repeat instability, which may be enhanced by the ability of the CTG and CGG sequences to adopt alternative DNA structures, helping to stabilize slipped structures.

evidence suggests otherwise. However, the peculiar developmental timing and tissue location of these changes may implicate certain times and locations where mismatch repair is ineffective, such as early in embryogenesis or in the germ line.

## WHY INCREASES?

The excess of DNA repeat expansion over contraction in disorders characterized by trinucleotide repeats is not consistent with the model of slipped strand mispairing. Theoretically, slippage should be possible on both the newly synthesized and the template strand, resulting in both expansion and contraction. However, recent data demonstrating polar variation in the fragile X repeat suggest a higher frequency of mutation in the newly replicated strands. If newly synthesized fragments on the lagging strand (Okazaki fragments) are more prone to these changes, it might be expected that increases would be found more frequently, and that the direction of replication would affect the mutability and location of mutations.

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## WHY ARE CGG AND CAG PREDOMINANTLY AFFECTED?

If length alone is the determinant of propensity of instability, then other simple sequence repeats of similar lengths might be expected to show similar levels of instability. There are no reports of mutation rates of similar levels in other simple sequence repeats, although the number of loci that would meet the criteria and that have been thoroughly investigated is not large. The limitation to these sequences suggests specific structural features of these repeats that contribute to the mechanism of instability.

## MODELING THE LARGE-SCALE CHANGES

Slipped mispairing is not a compelling explanation for the large increases found in the fragile site syndromes and myotonic dystrophy. While multiple rounds of expansion could account for these events, it seems likely that a different mechanism is operative here. A number of models have been proposed,<sup>10</sup> and in the absence of an experiment system to reproduce this behavior, it is difficult to test these models.

## CONCLUSION

Mismatch repair defects play a significant role in both human tumor genesis and some of the dramatic variations of the genomes associated with human genetic disease. As demonstrated by the number of diseases listed in Table 1 (page 2), this association can be anticipated to account for additional diseases that are not currently classified as resulting from the mismatch repair process. As the process is further studied we can anticipate understanding better the genetic variations in the normal and diseased states.

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# Genetics of $\beta$ -Hydroxysteroid Dehydrogenase Deficiency Disorder

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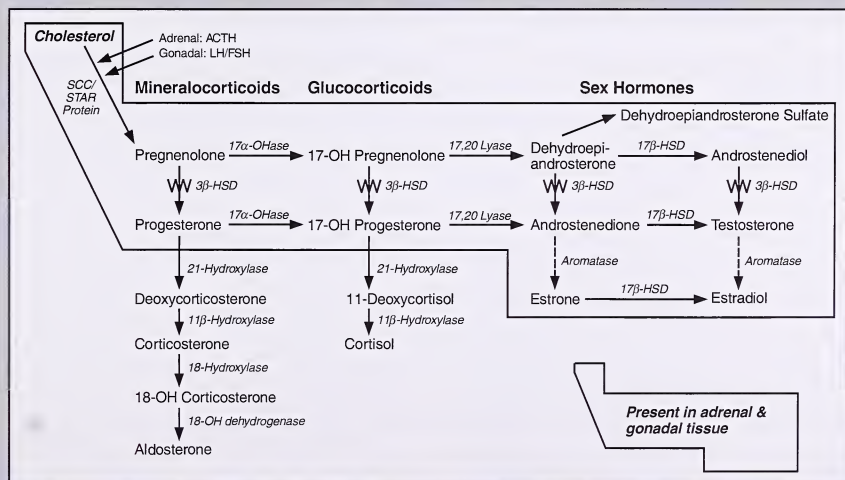
$\beta$ -Hydroxysteroid dehydrogenase/ $\Delta 5 \rightarrow \Delta 4$  isomerase ( $\beta$ -HSD) catalyzes the conversion of  $\Delta 5$ - $\beta$ -hydroxysteroid to  $\Delta 4$ - $\beta$ -ketosteroids in the adrenals, gonads, and in many extra-adrenal and extra-gonadal tissues (Figure 1).<sup>1,2</sup> The genetic control of  $\beta$ -HSD expression differs between the intra- and extra-adrenal and gonadal tissues.<sup>3,4</sup> Disorders involving decreased  $\beta$ -HSD activity in the adrenals and gonads have long been recognized. Recently, the discovery of  $\beta$ -HSD genes has led to the understanding of the molecular basis of  $\beta$ -HSD deficiency disorder. This review relates the clinical, biochemical, and molecular basis of severe, classic  $\beta$ -HSD deficiency disorder, and relates the molecular findings in patients exhibiting hormonal evidence of mildly decreased adrenal  $\beta$ -HSD activity. The latter led to the diagnosis in the last decade of mild, late-onset  $\beta$ -HSD deficiency disorder.

## PATHOPHYSIOLOGY OF $\beta$ -HSD DEFICIENCY

Classic  $\beta$ -HSD deficiency in humans occurs concomitantly in the adrenals and gonads, and is transmitted by an autosomal recessive trait.<sup>3-5</sup> Thus, regulation of adrenal/gonadal  $\beta$ -HSD activity in humans is under single-gene control. The enzyme deficiency in the adrenals results in cortisol deficiency, with or without aldosterone deficiency, and leads to increased corticotropin secretion and increased production of pregnenolone ( $\Delta 5$ -P), 17-hydroxypregnenolone ( $\Delta 5$ -17P), dehydroepiandrosterone (DHEA), and androstenediol (Figure 1).<sup>3-8</sup> Severe  $\beta$ -HSD deficiency produces congenital adrenal hyperplasia (CAH) with salt-wasting and ambiguous genitalia in male infants due to fetal testicular testosterone deficiency<sup>5</sup> and in female infants probably due to the effect of excess DHEA metabolites produced from the fetal adrenals.<sup>3-7</sup> Not all affected female infants have virilization.

The clinical spectrum of  $\beta$ -HSD deficiency at birth, however, includes both salt-wasting and non-salt-wasting forms independent of the extent

Figure 1  
Schematic of Adrenal and Gonadal Steroidogenesis



Dotted arrow: major pathway in ovaries and minor pathway in testes and adrenal cortex.  
FSH, follicle-stimulating hormone; LH, luteinizing hormone; SCC, cholesterol side chain cleavage; STAR, steroidogenic acute regulatory protein; 17 $\beta$ -HSD, 17 $\beta$ -hydroxysteroid dehydrogenase; 17 $\alpha$ -OHase, 17 $\alpha$ -hydroxylase.

of genital ambiguity.<sup>3,5,8</sup> Non-salt-wasting severe 3 $\beta$ -HSD deficiency during childhood is associated with premature acne and/or premature sexual hair growth, and with growth acceleration in both sexes.<sup>3,8</sup> Clitoromegaly occurred in 1 affected girl.<sup>3</sup> During adolescence and adulthood, varying degrees of hypogonadism occur in males,<sup>4,7,9</sup> and hirsutism, irregular menses, and polycystic ovaries occur in females.<sup>8,9</sup> During the last decade, however, a concept of a so-called mild, late-onset 3 $\beta$ -HSD deficiency disorder was introduced and reported to occur in 3% to 60% of hirsute females,<sup>10-15</sup> and in 1.5% to 13% of premature pubarche children.<sup>12,16</sup> Diagnosis was based on  $\Delta 5$ -17P and DHEA levels >2 standard deviations (SD) above normal mean corticotropin-stimulated levels and on ratios of  $\Delta 5$ -17P:17-hydroxyprogesterone (17-OHP),  $\Delta 5$ -17P:cortisol, and/or DHEA:androstenedione ( $\Delta 4$ -A). Questions regarding the validity of the hormonal diagnostic criteria for mild 3 $\beta$ -HSD deficiency disorder remain, however, since no definite genetic evidence is available to document that such mild  $\Delta 5$  steroid abnormalities to corticotropin stimulation result from mild variants of 3 $\beta$ -HSD deficiency CAH.<sup>10-16</sup>

Biochemically, 3 $\beta$ -HSD deficiency in both salt-wasting and non-salt-wasting patients is present only in the adrenals and gonads and not in the extra-adrenal/gonadal tissues. This indicates independent genetic regulation of the 3 $\beta$ -HSD enzyme between the intra- and extra-adrenal/gonadal tissues.<sup>3,4</sup> The presence<sup>4-6</sup> or absence<sup>3,6-8</sup> of 3 $\beta$ -HSD deficiency in the aldosterone biosynthetic pathway in patients with severe 3 $\beta$ -HSD deficiency suggests varying genetic make-up among the forms of the disorder.

## THE GENETICS OF 3 $\beta$ -HSD GENES AND PROTEINS

In humans, 2 genes (type I and II) encoding 3 $\beta$ -HSD protein have recently been characterized.<sup>17-20</sup> Both genes are located in the chromosome 1p11-13 region, are 7.84 to 7.88 kb in length, and consist of 4 exons and 3 introns.<sup>19,21</sup> The type I and II 3 $\beta$ -HSD proteins are 93.5% homologous in amino acid sequence.<sup>17-20</sup> **Type I gene expression occurs primarily in the placenta, mammary gland, and skin. Type II gene expression occurs in the adrenals and gonads.** The 3 $\beta$ -HSD is membrane-bound in the endoplasmic reticulum and mitochondria.<sup>22,23</sup> The putative functional domains in both genes include 2 predicted membrane-spanning segments in exons III (codons 73 to 90) and IV (codons 286 to 305) and 2 suggested membrane-spanning segments in the 5' and middle regions of exon IV. In the type I gene, an additional membrane-spanning segment is suggested in exon II. These putative membrane-spanning domains are predicted to be critical gene sites.

In vitro kinetics of human type I and II 3 $\beta$ -HSD exhibited greater type I than type II 3 $\beta$ -HSD activity.<sup>18,19,24</sup>

The Km values for  $\Delta 5$ -P, DHEA, and dihydrotestosterone of the type I 3 $\beta$ -HSD were approximately 5-, 6-, and 10-fold lower, respectively, than the Km values of the type II 3 $\beta$ -HSD. The maximum velocity ( $V_{max}$ )/Km values for  $\Delta 5$ -P, DHEA, and dihydrotestosterone of the type I 3 $\beta$ -HSD were approximately 6-, 4-, and 3-fold greater, respectively, than the  $V_{max}$ /Km values of the type II 3 $\beta$ -HSD.<sup>18,19,24</sup> Thus, the type I enzyme should efficiently convert the predictably low  $\Delta 5$  steroid concentrations in the peripheral tissues. 3 $\beta$ -HSD in the liver, kidney, lung, brain, and adipose in humans is not yet characterized. Additionally, three 3 $\beta$ -HSD pseudogenes or related genes have been identified by screening a human leukocyte genomic DNA library.<sup>25</sup> These pseudogenes, which contain stop codons and/or deletions in the coding regions, were suggested to have diverged from the type I gene millions of years ago.<sup>25</sup>

In other species, several types of 3 $\beta$ -HSD genes have been identified.<sup>22,23</sup> In rats and mice, the type I gene encodes for adrenal/gonadal 3 $\beta$ -HSD, the type II gene for liver 3 $\beta$ -HSD, the type III gene for liver and liver/kidney 3 $\beta$ -HSD, and the type IV gene for placental/skin and kidney 3 $\beta$ -HSD. The macaque type I gene encodes for gonadal 3 $\beta$ -HSD, and the bovine type I gene encodes for ovarian 3 $\beta$ -HSD. Thus, it is apparent that the **regulation of 3 $\beta$ -HSD expression in various tissue sites is under tissue-specific and independent genetic control in humans, rodents, and other mammalian species.**

## MOLECULAR BASIS OF 3 $\beta$ -HSD DEFICIENCY CAH

**The human type II 3 $\beta$ -HSD gene encodes specifically for adrenal/gonadal 3 $\beta$ -HSD enzymes.** Thus, CAH resulting from 3 $\beta$ -HSD deficiency was predicted to result from deleterious mutations in the type II 3 $\beta$ -HSD gene. Analysis of the type II gene in all but 2 alleles from unrelated patients of various ethnic background with salt-wasting 3 $\beta$ -HSD deficiency revealed a premature stop codon or frameshift and subsequent premature stop codon in the gene due to a homozygous or compound heterozygous point mutation involving codons 171, 273, and 318, an insertion mutation between codons 186 and 187, or combined mutations at codons 248 and 249.<sup>26-29</sup> The resulting truncated 3 $\beta$ -HSD protein lacks 3 $\beta$ -HSD activity, causing the salt-wasting disorder. Two patients, however, had a missense mutation at codon 142 (Glu→Lys) or 253 (Thy→Asn) in 1 allele, and a 186/Ins C/187 mutation or W171X premature stop codon in the second allele.<sup>30</sup> These mutant 3 $\beta$ -HSD proteins translated by the cells transfected with the mutant genes via mutagenesis exhibited no enzyme activity in vitro (Table 1). The regions of codons 142 and 253 are well conserved throughout species, suggesting that these regions of amino acid residues are critical for 3 $\beta$ -HSD activity.<sup>30</sup>



**Analysis of the type II  $\beta$ -HSD gene from patients with non-salt-wasting but severe  $\beta$ -HSD deficiency revealed primarily missense mutations in coding regions of the gene in all<sup>30-35</sup> but 2 alleles (Figure 2, page 8),<sup>32, 34</sup> including a point mutation involving codons 82 (Ala→Thr), 100 (Asn→Ser), 129 (Gly→Arg), 173 (Leu→Arg), 245 (Ala→Pro), and 254 (Thy→Asp).<sup>31-35</sup> In vitro, the apparent relatively specific efficiency to convert  $\Delta 5$ -P to progesterone (P) and DHEA to  $\Delta 4$ -A in monkey kidney transformed (COS) cells or homogenates transfected with the mutant genes by mutagenesis was compared with the 100% activity of wild-type  $\beta$ -HSD, revealing activities of 11.9% and 13.1%, respectively, by the codon 245 mutant protein (Ala→Pro); of 2.7% and 11% respectively by the codon 100 mutant protein (Asn→Ser); and of 2% and 4.7%, respectively, by the codon 129 mutant protein (Gly→Arg) (Table 1). These degrees of  $\beta$ -HSD activity were sufficient to prevent aldosterone deficiency, resulting in the non-salt-wasting disorder. The codon 254 (Thy→Asp) mutation exhibited no**

enzyme activity in vitro.<sup>34</sup> A second allele mutation in the type II gene, however, was not identified in this case to compare the phenotype with the genotype. The mutant 173 (Leu→Arg) and 82 (Ala→Thr) genes were not sufficiently studied to compare with the phenotype of the patients.<sup>31,33</sup> In non-salt-wasting sibs with the codon 129 missense mutation in 1 allele, the second allele had a G→A mutation in intron 3 at nucleotide 6651, 6 bases upstream from exon IV.<sup>32</sup> This may create a new splicing junction and affect the normal splicing of the mRNA. Type I gene sequences were normal in all alleles of patients with severe  $\beta$ -HSD deficiency who were examined.<sup>27,28,32</sup>

In general, review of the type II  $\beta$ -HSD gene mutations in  $\beta$ -HSD deficiency CAH indicates that the salt-wasting form results from a homozygous or compound heterozygous mutation involving grossly altered  $\beta$ -HSD gene structures or alteration in amino acid residues in the conserved region of the gene. The non-salt-wasting form appears to result from amino acid substitution mutations in the

Table 1  
In Vitro  $\beta$ -HSD Activity of Missense Mutant Type II  $\beta$ -HSD Genes and Its Comparison to the Phenotype<sup>30-35</sup>

| Mutant Gene Tested         |                   |                      |              | Clinical Phenotype             |                          |                                       |  |                            |
|----------------------------|-------------------|----------------------|--------------|--------------------------------|--------------------------|---------------------------------------|--|----------------------------|
| Genotype                   | Protein Synthesis | V <sub>max</sub> /Km |              | Second Allele Genotype         | SW or NSW                | Genetic Male With Ambiguous Genitalia | Genetic Female With Hirsutism and Menstrual Disorder | Premature Pubarche         |
|                            |                   | Δ5-P→P               | DHEA→Δ4-A    |                                |                          |                                       |  |                            |
| Wild-type                  | +                 | 100%                 | 100%         |                                | —                        | —                                     | —  | —                          |
| Mutant gene: 253 (Tyr→Asn) | +                 | 0                    | 0            | 186/Ins C/187 frameshift/ stop | SW                       | Yes                                   | —  | N/A                        |
| 142 (Glu→Lys)              | +                 | 0                    | 0            | 171 Trp→stop                   | SW                       | Yes                                   | —  | N/A                        |
| 245 (Ala→Pro)              | +                 | 11.9%                | 13.1%        | Homozygous                     | NSW                      | Yes                                   | —  | N/A                        |
| 100 (Asn→Ser)              | +                 | 2.7%                 | 11.0%        | Homozygous                     | NSW                      | Yes                                   | —  | N/A                        |
| 129 (Gly→Arg)              | +(↓)              | 2.0%                 | 4.7%         | n6651 Intron 3 mutation        | NSW<br>NSW               | Yes<br>—                              | —<br>Yes   | Yes<br>Yes                 |
| 254 (Thy→Asp)              | +                 | 0                    | 0            | Not found(?)                   | NSW                      | —                                     | Yes  | No                         |
| 173 (Leu→Arg)              | Not done          | Not done             | Not done     | Homozygous                     | NSW<br>NSW               | Yes<br>—                              | —<br>N/A   | N/A<br>N/A                 |
| 82 (Ala→Thr)               | Not reported      | Not reported         | Not reported | Homozygous                     | NSW<br>NSW<br>NSW<br>NSW | Yes<br>Yes<br>—<br>—                  | —<br>—<br>N/A<br>No                                  | Unknown<br>No<br>Yes<br>No |

$V_{max}/K_m$ , the first order rate constant is the index for apparent relative specific efficiency; SW, salt-wasting; NSW, non-salt-wasting; N/A, not applicable due to young age



less conserved region of the gene in at least 1 allele. These **type II  $\beta$ -HSD gene findings in patients with classic  $\beta$ -HSD deficiency suggest that CAH with this enzyme deficiency results from type II  $\beta$ -HSD gene mutation**, and the phenotype correlates well with the genotype in classic  $\beta$ -HSD deficiency disorder.

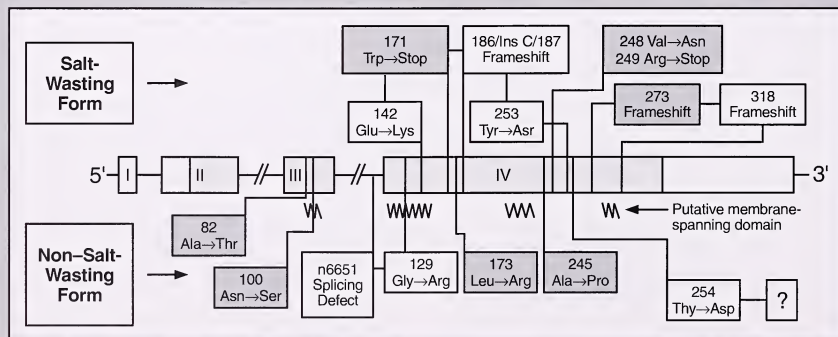
# **TYPE II $\beta$ -HSD GENE FINDINGS IN PATIENTS WITH HORMONAL EVIDENCE OF MILDLY TO MODERATELY DECREASED ADRENAL $\beta$ -HSD ACTIVITY**

During the past decade, the so-called mild, late-onset variant of  $\beta$ -HSD deficiency has been diagnosed in premature pubarche children<sup>12,16</sup> and in hirsute females, with or without menstrual disorders,<sup>10-15</sup> when corticotropin-stimulated  $\Delta$ 5-17P and DHEA levels and ratios of  $\Delta$ 5-17P:17-OHP,  $\Delta$ 5-17P: F, or DHEA: $\Delta$ 4-A were  $\geq 2$  SD above mean values of pubertal stage-matched normal subjects. The hormonal criteria, however, have not been universally accepted for diagnosing the mild, late-onset disorder because the hormonal abnormalities were not outstanding compared with mild 21-hydroxylase deficiency.<sup>36,37</sup> In mild 21-hydroxylase deficiency, corticotropin-stimulated 17-OHP levels were  $>21$  SD above homozygous normal mean values, and  $>6$  SD above mean levels of 21-hydroxylase deficiency carriers.<sup>36,37</sup> In addition, unusually large numbers of hirsute females and premature pubarche children had mild variants of  $\beta$ -HSD deficiency by the previous hormonal criteria.<sup>10-16</sup> Further, such hormonal diagnosis was not based on any genotypic proof. **Thus, the validity of the hormonal criteria published previously for diagnosing mild**

**$\beta$ -HSD deficiency disorder, including this author's work, is questionable.**

The mild variants of  $\beta$ -HSD deficiency producing CAH would likely result from a less deleterious mutation in the type II  $\beta$ -HSD gene. The type II  $\beta$ -HSD gene sequences—including regions of a putative promoter, all exons, and exon and intron boundaries—were normal in 5 premature pubarche children and 5 hirsute females whose corticotropin-stimulated levels were at 2.5 to 6.5 SD above the normal mean for 5-17P; 2.5 to 7 SD for DHEA; 2.5 to 4.3 SD for  $\Delta$ 5-17P:F ratio; and 3 to 8.6 SD for DHEA: $\Delta$ 4-A ratio, indicating mild, nonclassic  $\beta$ -HSD deficiency by the previous hormonal criteria.<sup>38</sup> Further, corticotropin-stimulated hormonal profiles of 3 carrier mothers for severe  $\beta$ -HSD deficiency with a single allele mutation in the type II  $\beta$ -HSD gene were appropriately normal.<sup>38</sup> These findings suggest that, **hormonally, mildly or moderately decreased adrenal  $\beta$ -HSD activity is not caused by mild variants of  $\beta$ -HSD deficiency resulting from type II  $\beta$ -HSD gene mutations in 1 or both alleles.** Furthermore, **proven carriers of severe  $\beta$ -HSD deficiency do not appear to express decreased adrenal  $\beta$ -HSD activity.** Recently, Zerah et al<sup>39</sup> reported normal type I and II gene sequences in hirsute females with variably mildly decreased adrenal  $\beta$ -HSD activity as having nonclassic  $\beta$ -HSD deficiency.<sup>39</sup> **It is now apparent that children with premature pubarche and hirsute females with mildly decreased adrenal  $\beta$ -HSD activity and normal type II  $\beta$ -HSD gene sequences do not have the mild variant of  $\beta$ -HSD deficiency CAH.** The etiology of this mildly decreased adrenal  $\beta$ -HSD activity remains unknown.

Figure 2  
Reported Mutations in the Type II  $\beta$ -HSD Gene in Patients With Severe (Classic)  $\beta$ -HSD Deficiency Congenital Adrenal Hyperplasia



## CONCLUSIONS

Except for 1 allele of 1 patient, the patients with  $\beta$ -HSD deficiency disorder exhibiting unequivocal clinical and hormonal abnormality had type II  $\beta$ -HSD gene mutation in all alleles.<sup>30-35</sup> The patients exhibiting mildly to moderately decreased adrenal  $\beta$ -HSD activity, which led to the diagnosis of so-called mild, late-onset  $\beta$ -HSD deficiency disorder by the previously published hormonal criteria, had no mutation in the type II  $\beta$ -HSD gene in any allele.<sup>38,39</sup> Therefore, the previously published hormonal criteria recommended for diagnosing mild, late-onset  $\beta$ -HSD deficiency are not appropriate for diagnosing late-onset or nonclassic  $\beta$ -HSD deficiency in premature pubarche children or in females with hirsutism and/or menstrual disorders.

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## Abstracts From the Literature

### The Role of the *obese* Gene and Its Product in Obesity

Much attention is being given to the genetic predisposition to obesity. A review of several pertinent papers and the interrelationship of the data presented therein follows. Zhang et al<sup>1</sup> cloned the *obese* (*ob*) gene in mice and humans. A mutation found in the *ob/ob* mouse gene prompted studies of the biologic effects of its protein product (termed the OB protein, or leptin, a name derived from the Greek word leptos, or thin) in normal, heterozygous, and homozygous abnormal mice. The *ob* gene, sited on mouse chromosome 6, has 2,852 bases and codes for a 167 amino acid propeptide and a mature protein of 146 amino acids. Messenger RNA of the *ob* gene is expressed only in white adipose tissue of the mouse. Morbid obesity occurs in the C57BL/6J *ob/ob* mouse due to increased feeding and decreased activity. Type II diabetes mellitus and hyperinsulinemia also occur. A C→T transversion in codon 143 of the *ob* gene alters arginine to a stop codon (Arg143Stop), and prevents translation of this gene product.<sup>1</sup>

Utilizing recombinant mouse OB (rmOB) protein, Halaas et al<sup>2</sup> raised a rabbit polyclonal antibody, and by immunoprecipitation and SDS-PAGE gel electrophoresis demonstrated its presence in the plasma of wild-type C57BL/Ks *db/+* and C57BL/Ks *db/db* mice and in normal slim human subjects, but not in C57BL/6J *ob/ob* mice. (C57BL/Ks *db/db* mice resemble the *ob/ob* mice phenotypically, but as will be noted later are resistant to the effects of OB protein.) Halaas et al then administered rmOB protein (5 mg/kg intraperitoneally daily for 15 to 33 days) or phosphate-buffered saline (PBS) to female C57BL/6J *ob/ob* and C57BL/Ks *db/db* mice. The following

findings were observed in the rmOB protein-treated C57BL/6J *ob/ob* mice: (1) significant weight loss within 4 days and a sustained weight loss reaching 40% of initial body weight by 33 days of treatment; (2) a 60% decrease in food intake; (3) a 95% decrease in body fat content in the C57BL/6J *ob/ob* mice; (4) a 50% decrease in plasma glucose concentration; (5) significantly greater weight loss than in pair-fed C57BL/6J *ob/ob* mice; and (6) equivalent weight and body fat reducing potencies of recombinant human OB and rmOB proteins in this model. No effect of rmOB protein was noted in C57BL/Ks *db/db* mice, indicating that this strain is resistant to the biologic effects of rmOB protein, perhaps due to an abnormality in the as yet unidentified receptor for rmOB protein. In wild-type female CBA/J *+/+* mice receiving twice daily injections of rmOB protein (12.5 mg/kg/d), body weight decreased approximately 10% but fat mass declined 95%.

Pelleymounter et al<sup>3</sup> demonstrated in C57BL/6J *ob/ob* mice: (1) a dose-response relationship between weight loss and rmOB protein; (2) a decrease in food and water intake, serum glucose and insulin concentrations, and oxygen consumption in mice receiving rmOB protein; and (3) an increase in body temperature and locomotor activity. Campfield et al<sup>4</sup> reported that: (1) as weight increased, the effects of rmOB protein administered intraperitoneally in C57BL/6J *ob/ob* mice were reversible when the protein was withdrawn; (2) rmOB protein decreased food intake and weight of diet-induced obese mice; (3) acute intravenous injection of rmOB protein lowered food intake for more than 7 hours in C57BL/6J *ob/ob* mice; and

(4) injection of rmOB protein into the lateral ventricle of C57BL/6J *ob/ob* mice decreased food consumption for at least 7 hours.

1. Zhang Y, et al. *Nature* 1994;372:425-432.
2. Halaas JL, et al. *Science* 1995;269:543-546.
3. Pellemounter MA, et al. *Science* 1995;269:540-543.
4. Campfield LA, et al. *Science* 1995;269:546-549.

**Editor's comment:** Data reported in these 3 articles<sup>2-4</sup> indicate that the OB protein (leptin): (1) circulates in mice and humans and is thus a hormone (and that fat is a gland of internal secretion and hence an endocrine gland); (2) induces weight loss and decline in body fat mass by both a decrease in caloric intake and an increase in activity; and (3) acts through a receptor,

probably located within the central nervous system, and is active in obese syndromes associated not only with absence of this protein but also in diet-induced obesity. Establishing immunologic methods for the measurement of plasma levels of leptin; defining its normal physiologic role in the regulation of body composition, carbohydrate metabolism, the secretion of insulin, and other glucoregulatory hormones; elucidating its mechanisms of action; and exploring the legion of possible malfunctions of leptin production, action, or therapeutic applicability in a variety of diseases such as type II diabetes mellitus, polycystic ovary syndrome, and anorexia nervosa auger an exciting future for this peptide.

Allen W. Root, MD

## Gonadoblastoma: Molecular Definition of the Susceptibility Region on the Y Chromosome

It has been known for some time that gonadoblastomas are more common in Turner syndrome patients who have some of the Y chromosome still present. Gonadoblastomas are rare neoplasms composed of aggregates of germ cells mixed with smaller epithelial cells resembling mature Sertoli and granulosa cells. Gonadoblastomas arise within dysgenetic gonadal tissue of individuals who possess a Y chromosome or part of a Y chromosome. The authors of this paper used molecular markers from the Y chromosome to demonstrate the area of the Y chromosome that was missing in the individuals who developed gonadoblastomas, thus mapping the gonadoblastoma locus (GBY) on the Y chromosome. All individuals with gonadoblastomas had region 3 and region 4 of the Y chromosome present in their Y chromosomal material. All other regions of the Y chromosome were missing. Some of the study population were lacking the *SRY* gene, while others had it. Copies of 2 Y-linked gene families: *TSPY* (testis-specific protein, Y-encoded) and *YRRM* (Y-chromosome RNA recognition motif) were present in all patients. These 2 gene families have sequences dispersed over many regions of the Y chromosome. It seems likely that all the copies are not active; however, this is currently unproven. It is likely that most, if not all, patients with gonadoblastoma will have at least some copies of the genes present, despite large deletions of their Y chromosome.

The analysis of the DNA in this part of the Y chromosome is complicated because the interval lies in a region of XY homology and the PCR product from both the Y and X chromosomes are almost the same size. The authors estimate that the GBY critical region is about 1 to 2 megabases. Copies of the *TSPY* gene but not the *YRRM* gene fall within the GBY critical region according to the deletion mapping.

Two tumors were sampled that showed expression of both *TSPY* and *YRRM* genes. Interestingly, in one patient with unilateral gonadoblastoma, the contralateral unaffected streaked gonad did not show expression of either gene.

These studies have not directly implicated either gene in the etiology of gonadoblastoma. However, they certainly raise the issue of whether there is only one gene or a critical region or possibly multiple loci on the Y chromosome involved in the production of gonadoblastoma.

**Editor's comment:** Pediatric endocrinologists have wondered for some time why individuals with Turner syndrome are at risk for gonadoblastoma. It was suggested in the 1980s that only those individuals with Turner syndrome who had Y chromosome material still present in the gonadal streak were at risk. The present study isolates the specific area of Y chromosome (regions 3 and 4) that puts these individuals at risk. Thus, the gonadoblastoma region of the Y chromosome has been identified and gonadoblastoma tissue shows expression of at least 2 genes not expressed in nongonadoblastoma tissue.

*TSPY* falls within the critical region and has 30% identity and 56% similarity with the SET protein. The SET gene has been implicated in acute undifferentiated leukemia and thus may play some kind of role in tumorigenesis.

A great deal more will have to be learned regarding the presence of multiple copies of genes on the Y chromosome. It is not clear whether there are individual or polymorphic variations in copy number or variations in these Y sequences. More needs to be known about the expression of alternate transcripts and the presence of the untranscribed control elements of these genes. Nevertheless, the significance of this study for Turner syndrome patients is that it shows that deletions of the Y chromosome that leave regions 3 and 4 intact put these individuals at risk for gonadoblastoma.

Judith G. Hall, MD

### Editorial Board

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Tsuchiya K, et al. *Am J Hum Genet* 1995;57:1400-1407.



## Microphallus: Eventual Phallic Size Is Dependent on the Timing of Androgen Administration

Husmann and Cain produced 2 animal (rat) models of hypogonadotropic hypogonadism in utero. Persistent microphallus and sexual infantilism occurred. Treatment with dihydrotestosterone (DHT) in large doses was begun at 7, 28, 56, and 84 days of age. To evaluate the effect of treatment, the length (stretched) and weight (autopsied) of the penis was measured. The androgen receptor protein found in the penile corpora, which is necessary for penile growth and which disappears as the penis reaches end-stage growth, was measured immunohistochemically.

Early exposure to androgens (before 56 days) resulted in diminutive penile growth, apparently due to accelerated downregulation of the androgen receptor. Although late administration of androgen enhanced penile length significantly (Figure 1), penile weights (reflecting penile widths) remained subnormal (<2.5 standard deviations [SD] below the normal mean).

The authors believe, based on current clinical and experimental data, that brief androgen therapy of the neonate with micropenis is necessary to determine if the phallus will respond; this is necessary in considering the sex of rearing. Interval therapy during childhood is not recommended. Treatment with androgens to stimulate maximal phallic growth should be initiated when the child is >12 years of age. The statement is made that evaluation of the adult population with a history of micropenis reveals that interval androgen therapy during childhood does not result in any significant size advantage of the penis compared with that of the untreated child. Unfortunately, delaying pharmacologic therapy does not result in complete development of phallic growth (weight, therefore width). Further studies reportedly are underway using the 2 rat models.

Husmann DA, Cain MP. *J Urol* 1994;152:734-739.

**Editor's comment:** Although published in 1994, this article only recently was called to my attention (by Dr. Dan Metzger

of the University of British Columbia, and British Columbia Children's Hospital, Vancouver). A subsequent abstract was presented at the American Academy of Pediatrics meeting in April 1995 dealing with analogous studies in humans (Cain et al. "Micropenis Secondary to Hypogonadotropic Hypogonadism: Clinical Evaluation of Early Versus Late Hormonal Therapy").

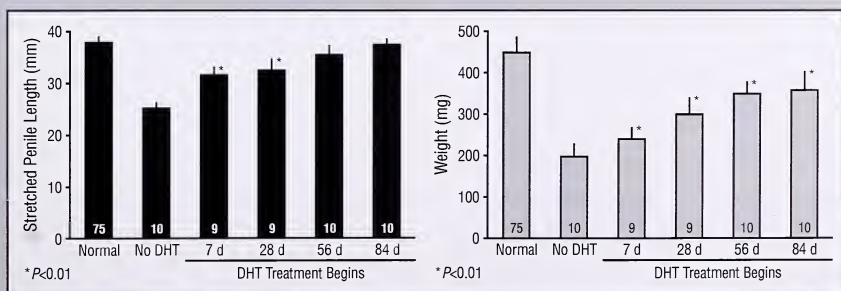
Twenty-five patients met the criteria of micropenis, ie, stretched penile length at diagnosis <2.5 SD below the mean and laboratory criteria consistent with gonadotropin hormone-releasing hormone deficiency. Early hormonal therapy was defined as >20,000 IU of human chorionic gonadotropin (HCG), <6 months treatment with testosterone cream, or combined HCG and testosterone treatment >3 months, by 7 years of age. Delayed treatment was defined as initiation of treatment after 11 years of age. Ten and 15 patients were categorized into each group, respectively.

The median age at diagnosis in the early treatment group was 4 years (range, birth to > 7 years). Final evaluation was at 20.5 years (16 to 28 years). All continued to have micropenis (<2.5 SD). Fifteen received late treatment (median age, 15 years). Final evaluation was at 21.3 years (15 to 37 years). Stretched penile length was within normal range in 13 of the 15 patients treated late. The authors concluded that the clinical data support the following hypothesis: Improved phallic growth occurs with delayed hormonal therapy for micropenis secondary to hypogonadotropic hypogonadism.

These data from humans and the conclusions drawn are intriguing because of the similarity to the conclusions reached from studying the animal model.

Is the parallelism justified? My answer is: Possibly, but only possibly. The studies are important because of the attempt to study biochemical and anatomic science with treatment in the rat, and make clinical observations to support or reject the hypotheses derived from the animal studies. Congratulations are extended to the authors for their approach and efforts. However, questions to be raised include: (1) Were the phalluses

Figure 1



Penile length and weight are given for hypogonadotropic rats in relation to when dihydrotestosterone (DHT) treatment was begun. DHT treatment was effective in increasing length in all, but the increase was greater in rats treated late.



in the older age group relatively larger before treatment? The median age at diagnosis was 12 years, in contrast to 4.0 years for the early treatment group. (2) Were the widths of the phalluses greater in the late treatment group—before and/or after? Widths of the flaccid phallus could have been measured in the human, although this was not possible in the rat. (3) Were any of the children in the early treatment group deficient in growth hormone (GH)? GH deficiency is associated with micropenis, and particularly so when luteinizing hormone and GH are both deficient.

Regardless of the answers to these questions, extended delay of treatment (usually with androgens) for individuals

with micropenis is advisable. However, in my experience there are some young children who have significant psychological consequences as a result of not being able to bare themselves in the dressing room and/or stand to urinate with their peers. These children should be treated early, in my opinion, if they are being raised as males. Emphasis should be made in respect to interpreting these data that the subjects all allegedly had micropenis secondary to hypogonadotropic hypogonadism, and the observation and deductions should not be construed outside micropenis of this origin.

Robert M. Blizzard, MD

## The Role of the Sulfonylurea Receptor in Insulin Secretion

Review of several articles permits the following deductions. In response to glucose, there is depolarization of pancreatic  $\beta$  cells, transient increase in cytoplasmic levels of  $Ca^{++}$ , and release of insulin. These processes are regulated by adenosine triphosphate (ATP)-sensitive  $K^+$  channels that are blocked by glucose-induced increase in the cytosolic ratio of ATP:adenosine diphosphate (ADP), thus resulting in membrane depolarization and increased release of stored intracellular  $Ca^{++}$ .<sup>1</sup> Sulfonylureas stimulate insulin secretion by blocking ATP-sensitive  $K^+$  channels, thus depolarizing pancreatic  $\beta$  cells. Aguilar-Bryan et al<sup>2</sup> have identified the genes for the endogenous sulfonylurea receptors (SURs) of the rat and hamster; they code for 1,582 amino acid proteins with a molecular weight of 177 kd and 13 transmembrane domains that share homology with the cystic fibrosis transconductance regulator (CFTR) and P-glycoprotein multidrug resistance (MDR) genes, both membrane transport proteins. The SUR is associated with but is not the  $K^+$  receptor; it may regulate activity of this monovalent cation channel by affecting the phosphorylation of the  $K^+$  channel or by sensing the ATP:ADP ratio.

In humans, the gene for the SUR is localized to chromosome 11p15.1, the same chromosomal location to which persistent hyperinsulinemic hypoglycemia of infancy (PHHI) has been linked. In patients with PHHI, an autosomal recessive disease of severe hypoglycemia and unregulated insulin secretion, Thomas et al<sup>3</sup> demonstrated that the gene for the human SUR was abnormal. In 13 families, a homozygous guanine to adenine (G→A) mutation was present in the region of the gene coding for the second nucleotide binding fold (a portion of the

protein that interacts with cytosolic nucleotides), leading to an abnormal frameshift and inclusion of a stop codon, thus resulting in a truncated protein. In one family a G→A mutation in a codon preceding the exon coding for the second nucleotide binding fold region resulted in abnormal splice sites within this important region. These studies demonstrate the importance of the endogenous SUR in the regulation of insulin secretion. Inactivation of this receptor results in unregulated insulin secretion, implying that normally this receptor inhibits insulin release by maintaining the activity of the ATP-dependent  $K^+$  channels within the pancreatic  $\beta$  cell.

1. Philipson LH, Steiner DF. *Science* 1995;268:372-373.
2. Aguilar-Bryan L, et al. *Science* 1995;268:423-426.
3. Thomas PM, et al. *Science* 1995;268:426-429.

**Editor's comment:** These articles illustrate the principle that many therapeutic agents reflect the action of endogenous substances as yet undiscovered. Thus, identification of the SUR implies the presence of an endogenous ligand for this receptor that must be involved in the regulation of insulin secretion and carbohydrate metabolism. One wonders about the chemical composition and source of this endogenous ligand and whether the endogenous ligand and/or its receptor might be aberrant not only in subjects with PHHI but also in patients with other disorders of energy homeostasis, perhaps with islet cell tumors or some forms of obesity.

Allan W. Root, MD

## Lymphocytic Hypophysitis: Clinicopathological Findings

A clinicopathologic description of 16 (2 male and 14 female) patients with lymphocytic hypophysitis was presented. In 10 of the 14 female cases, the presentation was associated with pregnancy (2 in the second trimester, 2 in the third trimester, and 6 postpartum). Clinical presentations were diverse: 9 patients (56%) exhibited signs of expanding pituitary mass; 10 (63%) showed anterior pituitary hypofunction; 3 (19%) had diabetes insipidus; and 6 (38%) displayed hyperprolactinemia (4 associated with pregnancy and 2 attributable to a stalk effect). Three

(19%) died due to progressive unrecognized hypopituitarism. In 1 patient (6%), elevated growth hormone (GH) levels with a resultant increase in insulin-like growth factor 1 were demonstrated. In 4 patients (25%), autoimmune thyroiditis was found. In 10 patients (63%) a pituitary mass mimicking an adenoma on computed tomography scans or magnetic resonance images was demonstrated, with 8 showing evidence of suprasellar extension. Antipituitary antibody testing was performed in 2 patients and yielded negative results. The

diagnosis of hypophysitis was made by pathologic studies in all patients. Light microscopy revealed lymphoplasmacytic infiltrate accompanied by varied numbers of neutrophils, eosinophils, and macrophages. Preserved cells were grouped in small islands surrounded by inflammatory infiltrate or fibrous tissue. Immunocytochemistry performed in 14 cases revealed the presence of GH and prolactin in all but 1 patient. There was absence of corticotropin immunoreactivity in 5 patients; of these, 3 had adrenal insufficiency and 1 was receiving treatment with steroids. In addition to confirming the findings of light microscopy, electron microscopy (8 patients) identified lactotroph cell hyperplasia or hyperactivity in 3 patients (1 male and 2 females, pregnancy related). In the 3 postmortem examinations, gross pituitary atrophy along with adrenal atrophy (presumably secondary to pituitary-target organ dysfunction) was found. The authors concluded that lymphocytic hypophysitis should be considered in the differential diagnosis of females presenting with pituitary enlargement in the peripartum period, in patients presenting with GH deficiency or excess associated with autoimmune disorders, and in patients presenting with rapidly enlarging pituitary masses with or without pituitary hormone dysfunction.

Thodou E, et al. *J Clin Endocrinol Metab* 1995;80:2302-2311.

**Editor's comment:** This is an important compilation of patients with lymphocytic hypophysitis that showed the great diversity and heterogeneity of the clinical picture of this disorder. The diagnosis of lymphocytic hypophysitis was confirmed by biopsy in all instances. The authors advocate conservative treatment on the basis of clinical suspicion to avoid aggressive surgical intervention. However, it was the experience of the authors, as well as of others, that there is no way to elucidate the final diagnosis before surgery. Antipituitary antibody testing in 2 patients produced negative results. Thus, the validity of these measurements to diagnose lymphocytic hypophysitis cannot be relied upon for diagnostic purposes. This group of patients with hypophysitis did not include children with hypopituitarism; therefore, it is hard to ascertain whether autoimmune hypophysitis occurs in children diagnosed with GH deficiency who do not exhibit pituitary masses on imaging studies and in whom the diagnosis of idiopathic GH deficiency is made. Most of the literature of lymphocytic hypophysitis relates to middle-aged patients, with a higher prevalence of females than males.

Fima Lifshitz, MD

## Final Height and Predicted Height in Boys With Untreated Constitutional Growth Delay

The authors reexamined 49 males at a mean chronologic age of 22.9 years (range, 20.4 to 31.2 years) who presented to their clinic at a mean age of 13.3 years (range, 7.3 to 16.4 years) and were diagnosed with constitutional delay of growth (CDG). The reexamination included measurements of standing height, using a Harpenden stadiometer, and testicular volume. At initial presentation, the diagnosis of CDG was made by documenting a standing height <5th percentile for chronologic age and a bone age retarded by 1 year or more in a boy who was born at term and had a birth weight of 2,500 g. Seventy-five percent of the boys had a history of late maturing parents. Heights of both parents were recorded. No patient with dysmorphic features, systemic disease, nutritional disorders, or suspected hormone deficiency was included in the sample. None of these men had received any chronic medical treatment, including anabolic steroids, during the intervening years. At the initial visit, the bone ages were determined by the methods of Greulich and Pyle and of Tanner-Whitehouse Mark II (TW2). Height predictions were calculated by the Bayley-Pinneau, TW2, and Roche-Wainer-Thissen (RWT) methods. Target height (TH) was defined as midparental height with 6.5 cm added and with 1 standard deviation (SD) defined as 4.25 cm. Paired *t*-tests and linear analyses were used for comparisons.

At the reexamination, the measured final height of these men was within the lower range of normal for the population, but significantly below their THs (by an average of 1.7 cm). There was a good correlation between the final height SD score (SDS), the initial bone age deficit, and the initial height SDS for bone age. No endocrine disorders became evident in these men and the mean testicular volume on reexamination was 19.0 mL (range, 10.3 to 25 mL). Predicted height by the Bayley-Pinneau method did not differ from the mean final

height, and predictions by all 3 methods and by TH were significantly positively correlated with final height. Height predictions were not more accurate in boys with advanced versus younger chronologic age at initial presentation.

Sperlich M, et al. *Eur J Pediatr* 1995;154:627-632.

**Editor's comment:** This study provides important information for the pediatric endocrinologist counseling boys with CDG and their families. The authors have demonstrated that there is a good correlation between predicted heights, THs, and final heights. However, in untreated men with CDG the final heights are significantly lower than the THs. Interestingly, both the final heights and the THs were within the lower range of the population norm, suggesting a component of familial short stature present in these men. Their data suggest that final height in untreated CDG patients may be compromised. A table in the article summarizes similar findings from 10 other studies of final height in untreated men with CDG. All but one demonstrate a final height significantly lower than the TH.

William L. Clarke, MD

**2nd Editor's comment:** In GGH, Vol. 11, No. 4, page 2, an article entitled "Predictive Factors in the Determination of Final Height in Boys With CDGP" was abstracted. The ultimate heights recorded in this article were less than expected for midparental height. You may wish to review the data abstracted from the article and the editorial comments by 2 of our editors in GGH 11:4.

Robert M. Blizzard, MD

## One Gene, Three Chondrodysplasias: Déjà vu

It was recently shown that mutations of the *FGFR3* gene cause achondroplasia, hypochondroplasia, and thanatophoric dysplasia. This came as a surprise for some because the severity of the clinical phenotypes varies so much. Now it seems that the same phenomenon occurs with mutations of another gene, the so-called diastrophic dysplasia sulfate transporter (*DTDST*) gene. *DTDST* mutations have been detected in 3 autosomal recessive chondrodysplasias: diastrophic dysplasia (DTD), atelosteogenesis type II (AOII), and achondrogenesis type 1B (ACG-1B). The latter 2 conditions are lethal in the perinatal period. Both exhibit poor skeletal development; however, the defect is more severe in ACG-1B.

The *DTDST* gene was discovered in 1994 when mutations were found in patients with DTD.<sup>1</sup> As its name implies, the gene product acts to transport sulfate ions into cells. Although *DTDST* expression is widespread, the consequences of *DTDST* mutations are restricted mainly to cartilage, presumably because the proteins (proteoglycans) that occupy cartilage matrix are so highly sulfated. Regarding bone growth, it was suggested that defective sulfate uptake by chondrocytes leads to deficient sulfation of cartilage proteoglycans, which causes cartilage to function poorly as a template for endochondral bone growth.

Because qualitative similarities in skeletal radiographs and growth plate histology to DTD were observed, studies were carried out in both AOII and ACG-1B, which eventually led to finding mutations of *DTDST* in both recessive conditions. Five different mutations were detected in the 6 *DTDST* alleles from 3 patients with AOII, and 7 mutations were found in the 12 *DTDST* alleles from 6 patients with ACG-1B. Most interesting was that some of the same mutations were identified in the different conditions.

Thus, the 3 disorders are not only allelic, but they share common mutant alleles in different combinations. In other words, certain combinations of mutations appear to produce the clinical manifestations of DTD, other combinations result in AOII, while other combinations cause ACG-1B.

As addressed in both recent papers,<sup>2,3</sup> the simplest explanation for the findings is that all 3 disorders result from a common

pathogenesis, which involves defective sulfate uptake by chondrocytes. The degree to which uptake is disturbed, which reflects how well the combined products of the 2 alleles function to transport sulfate, determines the clinical phenotype. The 3 disorders thus constitute what is often called a phenotypic series of disorders.

1. Hästbacka J, et al. *Cell* 1994;78:1073-1087.
2. Superti-Furga A, et al. *Nature Genet* 1996;12:100-102.
3. Hästbacka J, et al. *Am J Hum Genet* 1996. In press.

**Editor's comment:** The number of chondrodysplasia gene loci seem to be shrinking. Indeed, if one considers the disorders that map to the COL2A1 (type II collagen) locus, which include the various spondyloepiphyseal dysplasias, Kniest dysplasia, Stickler dysplasia, hypochondrogenesis, and achondrogenesis type II, and to the *FGFR3* and *DTDST* loci as discussed here, one can account for a very large percentage of all patients with chondrodysplasias. It will be interesting to see if this trend continues or if the number of chondrodysplasias associated with mutations at these loci have reached their limit.

The revelations regarding the *FGFR3* and *DTDST* mutations bring up the issue of where, ie, what tissues, genes are expressed versus where disease manifestations arise when the genes are mutated. It is often true that they are the same. For example, mutations of type I and II collagen genes in osteogenesis imperfecta and spondyloepiphyseal dysplasias respectively produce manifestations in most tissues where the genes are expressed. In contrast, both *FGFR3* and *DTDST* genes are expressed in many tissues, yet the pathologic consequences of mutations are restricted mainly to cartilage, especially the growth plate. For *DTDST*, this observation apparently reflects the much greater need for sulfate in cartilage compared with other tissues. For *FGFR3*, the explanation is not yet evident.

William A. Horton, MD

## Mutations of the Growth Hormone Receptor in Children With Idiopathic Short Stature

The authors studied 14 children with idiopathic short stature who had normal growth hormone (GH) secretion but low serum concentrations of GH-binding protein. They thought it likely that these patients had abnormalities in the gene for the GH receptor. They hypothesized that the mild form of insensitivity to GH could be caused by a mildly disruptive mutation of the gene for the GH receptor as compared with children with complete GH insensitivity such as Laron dwarfism. Four of the 14 children had PCR fragments that had altered migration mobility. Sequencing of the genes showed that 3 patients had a single mutation, while the fourth patient was a compound heterozygote. All had changes in the DNA that were confined to the extracellular domain of the receptor. It seems possible that the other 10 patients also had changes in the GH receptor gene that could not be picked up by mobility changes.

The implications are that heterozygote mutations of the GH

receptor gene can have mild or severe growth consequences, depending on what the other gene is like. The patient who was a compound heterozygote was more severely affected than either of his heterozygote parents. Another child was more severely affected than his heterozygote mother, suggesting that his father might also carry an as yet undefined mutation.

These patients only had a marginal response to GH therapy, so appropriate therapy is unclear at this time.

Goddard AD, et al. *N Engl J Med* 1995;333:1093-1098.

**Editor's comment:** Good clinical criteria exist to suspect that an individual may have a problem with the GH-binding protein. These include mild to moderate short stature; the presence of normal GH levels but low serum concentration of GH-binding protein; and poor response to GH therapy. The mutations of the GH-binding protein gene that have been described are all



in the extracellular domain of the protein. It can be anticipated that there will also be intracellular mutations.

This type of problem would be expected to run in families, with interaction between the 2 genes in the individual since the functional protein is a dimer. Further family studies are needed. The biology of GH and its receptor is being revealed through this type of molecular study of the experiments of nature.

For GH to stimulate release of insulin-like growth factor (IGF), the GH-binding sites must form a proper complex and produce an intracellular signal to activate the secretion of IGF-1. The classic form of GH receptor deficiency is Laron dwarfism, wherein the receptor is absent. Several hundred cases of classic Laron dwarfism have been identified; those patients with partial deficiency are just beginning to be described. Previously described heterozygotes for Laron dwarfism have been normal. Perhaps having an abnormal gene

product is more disruptive than lacking a gene product from 1 of the 2 genes.

It is not at all clear what the optimal therapy for these patients will be. Perhaps if IGF-1 therapy is successful, it can bypass the GH receptor gene abnormality.

Judith G. Hall, MD

**2nd Editor's comment:** A related article was reviewed in the immediately previous issue of GGH (Vol 11:4) entitled "Evidence for Partial GH Insensitivity Among Patients With Idiopathic Short Stature" (J Pediatr 1995;127:244-250). You as a reader may wish to reread the abstract and Dr. Lifshitz's editorial comment.

Robert M. Blizzard, MD

## Letters to the Editor

I would like to comment about an abstract published in the June 1995 (Vol. 11, No. 2) issue of *GROWTH, Genetics, & Hormones (GGH)*, "Identical Mutations in the *FGFR2* Gene Cause Both Pfeiffer and Crouzon Syndromes Phenotypes."

In 1981, DeNegrotti and I described a girl with Pfeiffer syndrome; her mother presented with a very mild expression of the disease but only at the level of cranium and face. Her hands and feet were normal.

One of the references in that report was a paper from Jackson et al that described a large Amish kindred with several individuals affected by different types of acrocephalosyndactyly, showing a great intrafamilial phenotypic variability. Our hypothesis was then that most of the autosomal dominant syndromes of acrocephalosyndactyly were the result of

defects in just one gene, rather than the result of mutations in different genes. At that time, molecular studies were not yet available.

The abstract published in *GGH* seems to confirm our 14-year-old hypothesis, which was based only on clinical observations.

Sincerely,

Dr. José María Sánchez  
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Sánchez JM, et al. *J Med Genet* 1981;18:73-75.  
Jackson CE, et al. *J Pediatr* 1976;88:963-968.

William L. Clarke, MD, reviewed the paper of Baron et al (*GGH* 1995;11[1]:6-7) on catch-up growth in rabbits after infusion of glucocorticoid into the tibial growth plate. Clarke states, "These data suggest that catch-up growth is intrinsic to the growth plate and not the result of a systemic hormonal mechanism." This seems to be the classic error in logic, ie, generalizing from the particular. It is analogous to the blind man deciding on the elephant's

shape on the basis of feeling a single part. Baron and colleagues tend to do the same thing—but not quite. In fact, the experiment of Baron et al only shows what happens when glucocorticoids hit chondrocytes. Their experiment does confirm work extending over the past 50 years showing that excessive glucocorticoids are poison to the growth zone.

Best regards and congratulations on guiding *GGH* for a decade.

Sincerely yours,

H. David Mosier, MD  
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## Response to *Letter to the Editor*

Dear Dr. Blizzard:

Thank you for giving me the opportunity to respond to Dr. Mosier's letter. My reply follows:

The prevailing explanation for catch-up growth involves a central nervous system mechanism that compares actual body size to an age-appropriate set point and adjusts growth rate accordingly.<sup>1</sup> In contrast, we hypothesized that the mechanism governing catch-up growth resides not in the central nervous system but rather in the growth plate. To test this hypothesis, we asked whether transient suppression of growth by excess glucocorticoid within a single growth plate would lead to local catch-up growth. We administered dexamethasone directly into the proximal tibial growth plate of 6-week-old rabbits into the contralateral growth plate.<sup>2</sup> Dexamethasone slowed the proximal tibial growth rate during the 4-week infusion compared with the contralateral vehicle-treated control. After the infusion ended, the growth rate of the dexamethasone-treated side not only normalized but actually surpassed that of the control side, thus correcting approximately half of the growth deficit. This catch-up growth was observed solely in the growth plate in which the growth inhibition had occurred; growth in the distal tibia and in the femur was unaffected.

Since the catch-up growth was unilateral, it could not be explained by the prevailing neuroendocrine hypothesis. A neuroendocrine mechanism, or any systemic mechanism that involved circulating factors, would have affected all growth plates and thus could not by itself account for the observed

anatomic specificity. Thus, the data suggest that the underlying mechanism is intrinsic to the growth plate.

In his letter, Dr. Mosier seems to be suggesting metaphorically that different mechanisms might contribute to catch-up growth under different conditions and the observed local mechanism might be just one component of catch-up growth. That hypothesis is quite plausible. In fact, in our published report we noted, "Our data do not exclude the possibility that both local and systemic mechanisms may contribute to catch-up growth under other circumstances."<sup>2</sup> As Dr. William Clarke commented in his review of our study, it would be interesting to see similar studies performed in models of other disorders associated with decreased growth velocity and subsequent catch-up growth.<sup>3</sup>

Sincerely,

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2. Baron J, et al. *Endocrinology* 1994;135:1367-1371.

3. Clarke WL. *GGH* 1995;11(1):6-7.

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# GROWTH

## Genetics & Hormones

Vol. 12 No. 2

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### Osteoporotic Syndromes in Childhood

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The structural framework of the body is the skeleton. The elements of the skeleton, which are fiber, matrix substance, and mineral, coexist in a highly regulated manner in this fascinating and complex system. Many skeletal components have extraskeletal counterparts, and the pathology within the skeleton may parallel extraskeletal disease. Interactions between the skeleton and other organs can cause extraskeletal dysfunction and vice versa.

**Osteoporosis** exists when the matrix and mineral within the bone are pathologically diminished. **Osteomalacia** exists when there is failure of mineralization despite the presence of adequate matrix. **Osteopenia** describes an abnormally low density of skeleton; however, it is insufficiently severe to cause

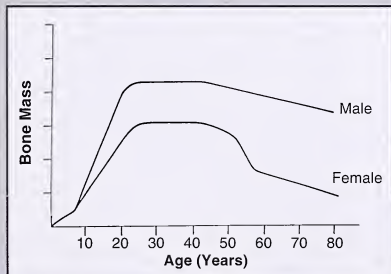
symptoms or loss of function.<sup>1</sup> It differs from osteoporosis only in degree, and the terms are used interchangeably here.

Osteoporosis usually is regarded as a disease of later life; it occurs particularly in women and is associated with the hormone changes of menopause. The main emphasis in this review is on osteoporosis occurring in children. The causes, presentation, and treatment of metabolic bone disease in the newborn will be covered in a subsequent issue.

#### SKELETAL TURNOVER AND THE DEVELOPMENT OF OSTEOPOROSIS

**Bone arises largely from cartilaginous derivatives of the mesodermal anlage.** Both cortical and trabecular bone exist in a state of constant dynamic activity. **Osteoblasts** form new matrix and promote calcification of that matrix. **Osteoclasts** remove existing bone. Bone cells regulate each other's activity in a paracrine fashion, indicating **that the processes of bone formation and resorption are coupled.** During childhood a net gain of skeletal material occurs. A plateau is reached in the early 20s, followed by a decline (Figure 1). Bone **mineral** accretion in childhood results in somatic and skeletal growth, and the bones increase in density simultaneously, ie, mass of calcified tissue per unit volume of bone. Adult osteoporosis results primarily from bone resorption. In childhood, a similar imbalance may give rise to osteoporosis, but **failure of new bone to develop as the skeleton grows may reduce bone density by a mechanism unique to childhood.**

Figure 1



Diagrammatic representation of changes in bone mass (indicated in arbitrary units on the Y-axis) with age. Note the rapid gain of bone mass during childhood and adolescence, the subsequent slow decline until middle age and the rapid bone loss in women between 50 and 60 years of age (modified from Cooper<sup>19</sup>).

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Richard Fine, MD

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## MEASUREMENT OF BONE DENSITY

Osteoporosis may be diagnosed from symptoms resulting from the collapse of a vertebral body or peripheral fracture. However, **the presence or absence of fracture is only a crude measure of the skeleton's integrity.** Thus, extreme degrees of osteopenia now can be diagnosed utilizing densitometric methods. In recent years these methods have become quite sophisticated. **Normative data for children are now available because of the low doses of radiation required.**<sup>2</sup> Both dual-energy densitometry (DXA) and quantitative computed tomography (qCT) provide usable data. The former is the most widely used method. The drawback is that the method measures the attenuation of an X-ray beam across a projected cross-section of bone (Figure 2). Therefore, the dimensions of the bone as well as the actual density of the skeletal mineral are recorded. The changes in these dimensions in childhood require that corrections based on the child's height and weight be applied.<sup>3</sup>

**True bone density, ie, mass/unit volume, is measured by qCT. Both cost and radiation dose limit its use in children and available normative data are limited.** The future development of low-dose radiation methods for qCT will prove beneficial.

## SYMPTOMATOLOGY OF CHILDHOOD OSTEOPOROSIS

Osteoporosis in the elderly has been called the silent epidemic, an allusion to the unobtrusive way in which osteoporosis, usually painless until fractures occur, develops. The prime exception is osteopenia

due to malignant infiltration of bone. **Osteoporosis in childhood is usually discovered by radiologic examination of at-risk children, after a fracture has occurred, or as a chance finding on X-ray films.** In fractures of the lower limbs, it may be difficult to determine the existence of osteopenia. In contrast, vertebral fractures in children are almost invariably due to local or generalized osteoporosis, unless there has been severe trauma. Vertebral fractures cause pain, deformity (kyphosis), and loss of height in the upper body segment.

Classification of childhood osteoporotic conditions is presented in Table 1. Detailed discussions of some of the individual causes then follow.

## CLASSIFICATION OF CHILDHOOD OSTEOPOROTIC CONDITIONS

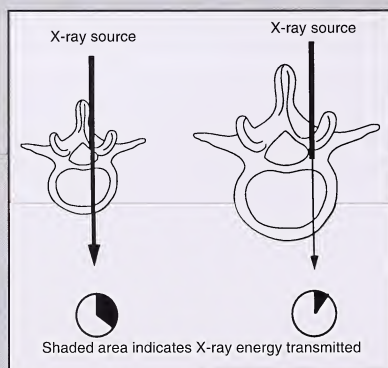
### Genetic Defects

The bone matrix consists of collagen and a large number of noncollagenous proteins. **Genetic or acquired defects in the structure and/or assembly of collagen can lead to osteopenia, as in osteogenesis imperfecta (OI).**

### *Osteogenesis Imperfecta*

This deforming bone disease is caused by heritable quantitative or qualitative disorders of type 1 collagen, the major collagen in bone.<sup>4</sup> Many cases of OI previously classified as recessive are now attributed to dominant germline mutations in a parent.

Figure 2



The diminished attenuation of a transmitted X-ray beam by a smaller bone means that body size must be taken into account in the interpretation of DXA "bone mineral density" readings.

Table 1  
Classification of Osteoporotic Conditions of Childhood

| Class                        | Disorder                            | Etiology  |
|------------------------------|-------------------------------------|---|
| Genetic Defects of Matrix    | Osteogenesis imperfecta             | Mutations in one of type 1 collagen genes                     |
|                              | Homocystinuria                      | Cystathionine synthetase deficiency                           |
|                              | Menkes's syndrome                   | Mutation in copper transporting ATPase $\alpha$ polypeptide   |
| Hormonal                     | Hypogonadism                        |   |
|                              | Glucocorticoid excess               |   |
|                              | Thyrotoxicosis                      |   |
|                              | Hyperparathyroidism                 |   |
| Nutritional and Metabolic    | Liver disease                       |   |
|                              | Vitamin D deficiency (with rickets) | Nutritional or sunshine deprivation; gastrointestinal disease |
|                              | Calcium deficiency                  | Maize-based diets   |
|                              | Copper deficiency                   | Artificial diets  |
|                              | Vitamin C deficiency (scurvy)       | Nutritional deprivation                                       |
| Immunologic and Inflammatory | Systemic mastocytosis               |   |
|                              | Rheumatoid arthritis                |   |
|                              | Hyper IgE syndrome                  |   |
| Neoplastic                   | Leukemia                            | Direct invasion of bone                                       |
|                              | Neuroblastoma                       | Direct invasion of bone                                       |
| Immobilization               | Chronic neurogenic                  | Cerebral palsy and neural tube defects                        |
|                              | Acute neurogenic                    | Traumatic paraplegia  |
|                              | Burns                               | ? Cytokine effect   |
| Miscellaneous and Unknown    | Thalassemia major                   | Transfusional hemosiderosis                                   |
|                              | Idiopathic juvenile osteoporosis    |   |

ATPase, adenosine triphosphatase

Sillence's classification of OI (Table 2, page 20) is widely used but is being superseded as advances are made in molecular genetics.<sup>5</sup>

The severity of OI depends on the type, and is variable even between individuals in the same family who have the identical genetic defect. Regardless of type, all patients are osteopenic,<sup>6</sup> and their bones are liable to fracture with minimal trauma. In severe OI, fractures may occur in utero. In milder cases, the first fracture may occur at any age up to old age (**10% of infants with mild OI are born with fractures**). Fracture or separation of the epiphysis is rare in OI, and OI is important in the differential diagnosis from trauma due to child abuse. Spinal osteoporosis may begin in the first decade, as can vertebral collapse. Dentinogenesis imperfecta causes the teeth to be grayish blue or brown, with reduced resistance to wear. Abnormalities also occur in other collagenous tissues such as ligaments

and the sclerae, which are often blue. **Despite many attempts, no effective nonsurgical therapy exists for OI. Prenatal diagnosis from the genotype of the fetus is possible if the collagen gene mutations in affected family members are known.** Prenatal diagnosis under such circumstances depends on material taken by chorionic villus biopsy performed as early as 8 weeks gestation.<sup>7</sup>

### Homocystinuria

Homocystinuria is a recessively inherited disorder causing mental retardation, a distinctive appearance, and the urinary excretion of excess amounts of the sulfur amino acid homocystine. This rare condition has provoked considerable research interest because of the high incidence of 2 very common disorders associated with it: arterial thromboses, a frequent source of morbidity, and osteoporosis. In homocystinuria, the limbs are thin and



Table 2  
Sillence Classification of Osteogenesis Imperfecta<sup>5</sup>

| OI Type | Fragility | Sclerae | Dental Involvement | Inheritance       | Comments           |
|---------|-----------|---------|--------------------|-------------------|--------------------|
| IA      | Present   | Blue    | Yes                | Aut dom           | Relatively common  |
| IB      | Present   | Blue    | No                 | Aut dom           | Variable severity  |
| II      | Extreme   | Blue    |                    | ? dom (germ cell) | Perinatal          |
| III     | Severe    | Normal  | No                 | ? dom (germ cell) | Skeletal deformity |
| IVA     | Present   | Normal  | Yes                | Aut dom           | Uncommon           |
| IVB     | Present   | Normal  | No                 | Aut dom           | Variable severity  |

Aut dom, autosomal dominant

spindly, with a decreased upper:lower segment ratio. Tall stature is present and persists into adult life. Kyphoscoliosis is common, and severe osteoporosis begins in adolescence. There is downward dislocation of the lens, leading to secondary glaucoma, myopia, and retinal detachment.

## HORMONAL OSTEOPOROSIS

### *Hypogonadism*

Both androgens and estrogens promote bone anabolism. Osteoblasts bear receptors for both classes of hormones. In both sexes there is a sharp increase in bone mineral content during puberty. **Osteopenia is seen in adolescents and young adults of either sex in a variety of settings of gonadal hormone deficiency.**

Symptomatic osteoporosis used to be common in young women with *Turner syndrome* before estrogen replacement became routine. **Recent data clearly show that with adequate estrogen replacement, osteoporosis can be avoided in Turner syndrome.** It is less clear whether growth hormone, used investigatively to promote growth in these short girls, leads to an increase in bone density before estrogens are administered.

Reduced gonadal hormone output is a hallmark of *exercise-anorexia nervosa amenorrhea* and is also present in many highly trained athletes. There are areas of physiologic overlap between anorexia and athleticism in the anorectic's urge to exercise and the athlete's concern for a trim, muscular, and efficient body. **Both in anorexia and in athletic training, males and females may suffer from diminished gonadal function.** However, the diagnosis is made far more commonly in young women, and more is known about the skeletal consequences in females than males with this syndrome. **Significant**

**osteopenia is common in anorexia,<sup>8,9</sup>** leading to concern that clinically significant osteoporosis might develop in middle age. **However, osteoporotic fractures of the vertebrae or limbs are uncommon in young anorectic girls.** It is hard to tell how much of the osteopenia of anorexia is due to hypoestrogenemia and how much to nutritional deficiency, but analogy with more clearly defined varieties of hypogonadism indicates that hormonal deficiency plays a major role.

By imposing varying loads on the skeleton, exercise promotes bone anabolism. Nevertheless, female athletes and other trained young women, such as ballerinas, lose bone and may become osteoporotic even if they are reasonably well nourished. **This bone loss stems from the negative effects of acquired hypogonadism, which override the benefit from exercise.** In contrast to the situation in anorexia, fractures—particularly cortical microfractures of the lower limbs—are not uncommon. This is related to the great strain imposed upon the limbs by the activities of these girls. Bone loss may not be fully reversed with estrogen treatment.<sup>10</sup>

Young men with *Kallmann's syndrome* and other causes of hypogonadism are often osteopenic. Their reduced bone mass is reversible upon administration of androgens. Despite the presence of androgen receptors on bone cells, doubt has been cast on the role of androgens by the fascinating reports of osteopenia in males who cannot produce estrogens (aromatase deficiency) or who are resistant to estrogens.<sup>11</sup> In both of these unusual disorders, men developed significant osteopenia despite normal testosterone levels.

### *Glucocorticoid Excess*

**Cushing's syndrome causes pathologic bone loss in children and inhibits linear growth.** The

bone loss can be severe, with frequent limb and vertebral fractures. The cellular causes of corticosteroid-induced osteoporosis relate more to the catabolic effects of glucocorticoids on matrix protein than to any effect on intestinal or renal calcium handling. Although there is no reliable treatment for corticosteroid-induced osteoporosis, attempts at prevention have been made by altering the schedule of corticosteroid administration, eg, alternate-day doses, and by using analogues such as deflazacort, designed to have an improved therapeutic ratio. **Established disease has been treated with vitamin D, calcium supplements, antiresorptive agents, and anabolic agents such as androgens and growth hormone, all to little avail.**

#### ***Thyrotoxicosis and Hyperparathyroidism***

Endogenous and factitious thyrotoxicosis in childhood may cause osteoporosis if untreated for a long period. Hyperparathyroidism may be associated with bone loss, but the sites of skeletal damage are quite specific and different from those seen in true osteoporosis.

### **NUTRITIONAL AND METABOLIC DISORDERS**

#### ***Liver Disease***

The liver is involved in calcium metabolism as the site of the first (25-hydroxylation) step in the activation of vitamin D. Additionally, bile production is essential for the normal absorption of both vitamin D and calcium. **As a result, severe bone disease resulting in a combination of rickets (a form of osteomalacia) and osteoporosis can occur in young children with biliary obstruction.**<sup>12</sup>

#### ***Vitamin D Deficiency (With Rickets) and Calcium Deficiency***

These conditions, the first being common and the second being very rare in industrialized countries, produce specific radiologic changes, mainly affecting the epiphyses. The bending of bone due to bony softening (osteomalacia) is quite different from the pathophysiology of pure osteopenia.

#### ***Immunologic and Inflammatory Disease***

Cytokines, such as those that signal between cells of the immune system, can influence the relative activities of bone-forming and bone-resorbing cells. Conditions in which cytokine production is deranged, such as systemic mastocytosis, rheumatoid arthritis, and the hyper IgE syndrome, are associated with osteoporosis. **In juvenile rheumatoid arthritis, disuse osteoporosis is combined with corticosteroid effects and a catabolic effect of immune cytokines results, leading to a particularly refractory osteoporosis.**

### **Neoplastic Diseases**

Crush fractures of the vertebrae and pathologic fractures through malignant deposits in appendicular bone occur in leukemia, neuroblastoma, and other malignancies. The radiologic appearances may be patchy, with some areas of the skeleton appearing normal.<sup>13</sup> In other instances, the osteopenia is diffuse. **Malignant osteoporosis of childhood is often painful, providing a strong clue as to the underlying diagnosis.** Excessive bone resorption may lead to hypercalciuria or even hypercalcemia, but the absence of biochemical abnormalities does not exclude malignancy as a cause for childhood osteoporosis.

### **Immobilization Osteoporosis and Osteoporosis of Miscellaneous and Unknown Causes**

*Disuse osteoporosis* occurs in congenital paraplegia (spina bifida), cerebral palsy, and acquired paraplegia. Mechanical stresses stimulate the formation of new bone and the gain of skeletal mass. Conversely, weightlessness, bed rest, and paralysis lead to bone loss. Lower limb fractures are common in nonambulatory children with spina bifida and cerebral palsy. **Often nutritional factors contribute to the development of bone disease in these multiply handicapped individuals.** A variety of orthopedic approaches may be necessary to deal with the fractures, but correction of the underlying cause is generally not an option.

Acute paraplegia causes rapid bone loss in otherwise healthy growing children, as children have higher bone turnover rates than adults. Osteoporosis of the paralyzed limbs is the rule in such cases, and a major clinical problem is the rapid rise in serum and urinary calcium after injury.

Bone loss after burn injury has been attributed to immobilization. However, **more recent work<sup>14</sup> has suggested that biochemical markers of bone formation can remain depressed for years after burn injury.** The cause of such long and lasting

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depression of skeletal function following burn injury remains to be discovered.

**Idiopathic juvenile osteoporosis (IJO)** is a term used to describe a severe and rapidly progressive form of osteoporosis seen in the years before puberty.<sup>15</sup> The disease affects both sexes and its cause is unknown. At the onset of puberty, the disease remits and, while residual deformity persists, new growth takes place in the absence of further fractures. IJO is not known to be a familial condition, but the possibility that it is due to an underlying disorder of collagen formation, much like that in OI, cannot be ruled out. **The remission of osteoporosis with puberty is a feature shared by many other types of osteoporosis in adolescents, even those whose causes have nothing to do with gonadal hormone status, such as in corticosteroid-induced osteoporosis and OI.** The natural history of these conditions bears witness to the powerful effect of gonadal hormones on bone.

## PEDIATRIC ASPECTS OF ADULT OSTEOPOROSIS

If bone mass increases steadily throughout childhood and the early 20s and is then subject to an inevitable decline, it follows that measures to promote skeletal accretion in youth may limit the effects of bone loss later in life. **The factors contributing to**

**the gain in bone mass in childhood include calcium nutrition,<sup>16</sup> the timing of puberty, other hormonal influences, and innate genetic traits.** A start in unraveling these genetic factors may have been made by the observation that polymorphisms in the noncoding region of the vitamin D receptor gene are predictors of adult bone mass.<sup>17</sup> Doubtless other genetic influences will be discovered since there is certainly a familial component to postmenopausal osteoporosis.<sup>18</sup>

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## Abstracts From the Literature

### Identification of a Stimulator of Steroid Hormone Synthesis Isolated From Testis

A follicle-stimulating hormone (FSH)-dependent product of the rat Sertoli cell that stimulates Leydig cell function through paracrine mechanisms was identified. A 70-kD protein complex was resolved into 2 proteins of 28 kD and 38 kD. The 28-kD fraction expressed Leydig cell-stimulating activity. The 38-kD protein permitted maximal expression of this activity. The 28-kD fraction also stimulated steroidogenesis in isolated rat granulosa cells and mouse adrenocortical cells. Further studies revealed that the 28-kD fraction was identical to the tissue inhibitor of metalloproteinase-1 (TIMP-1) and the 38-kD fraction to the proenzyme form of cathepsin L/Sertoli cell cyclic protein-2 (CP-2).

TIMP-1 is present in many tissues. Among other functions, it binds to matrix metalloproteinases or interstitial collagenases and influences cell migration, angiogenesis, embryo implantation, and cell growth. The mechanisms by which TIMP-1 stimulates steroidogenesis are as yet unknown. Procathepsin L enters lysosomes through the mannose-6-phosphate receptor (the type II insulin-like growth factor receptor) and is metabolized to cathepsin L, a cysteine proteinase

that is involved in prohormone activation, bone resorption, and sperm maturation. Since TIMP-1 contains 6 disulfide bonds, cathepsin L may be involved in full expression of the steroidogenic activity of TIMP-1 by modifying its 3-dimensional structure.

Boujrad N, et al. *Science* 1995;268:1609-1612.

**Editor's comment:** The importance of TIMP-1/cathepsin L in steroidogenesis in humans is uncertain, although human Sertoli cells have been reported to secrete an FSH-responsive factor that stimulates Leydig cell function.<sup>1</sup> Whether this factor may be involved in the physiology of normal adrenarche or in the pathogenesis of such disorders as polycystic ovary syndrome or male limited gonadotropin-independent sexual precocity in some patients remains an issue for future study.

Allen W. Root, MD

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## Growth in Full-Term Small-for-Gestational-Age Infants: From Birth to Final Height

This study took advantage of the features of the population in the health-care and school systems of Sweden, where there is low migratory activity, 98% of the children are in school at 17 to 19 years of age, and accurate data on auxologic measures for almost all children from birth through 18 years of age are available. Followed in the study were 3,650 children without dysmorphism; none had any known reason for short stature of pathologic origin.

The aims of the study were: (1) to describe the postnatal growth pattern for small-for-gestational age (SGA) children defined as SGA by either birth weight or birth length  $<-2$  SDS, instead of by birth weight only, as is the usual criterion for SGA; (2) to determine the relative risk of ultimate short stature in children classified as SGA by either definition; and (3) to identify predictors for ultimate short stature in SGA infants by correlating the growth patterns with independent variables such as size at birth, midparental height, length of gestation, and sex.

The entire group was subdivided into 4 groups (Table 1). Group 1, the normal group for birth weight and length (N), consisted of 94.6% of the newborn population. Group 2 (SGA for weight only, SGA<sub>w</sub>) consisted of 1.6%. Group 3, which was SGA for both weight and length (SGA<sub>wL</sub>), consisted of 1.5%. Group 4, which was SGA only for length (SGA<sub>L</sub>), was a significant percentage at 2.4%. Rapid growth occurred in the first 6 to 12 months in all groups, but most rapidly in groups 3 and 4. At 2 years of age, 9.9% and 13.4% of groups 2 and 4,

respectively, remained  $<-2$  SDS for length. By 18 years of age, there still were 6.4% of group 2 and 7.9% of group 4 who were  $<-2$  SDS for length. Calculations were not given for group 3. The final height SDS for group 2 ( $-0.4$ ) was the most severely stunted of the groups. The authors reported that at final height, 22% of the short individuals in a clinic for short stature were found to be SGA as defined by birth length; 14% were found to be SGA as defined by birth weight. Birth length SDS and length of gestation were positive predictors of catch-up growth by 6 months of age; only midparental height and birth length were predictors of final height gain by 18 years of age.

The authors concluded that the majority ( $>86\%$ ) of healthy full-term singleton SGA infants will exhibit catch up in height during the first 6 to 12 months of life and that this is almost independent of whether birth weight or length is used to define SGA. They add that of the SGA infants remaining  $<-2$  SDS at 12 months, about half will be short in final height, thus constituting a high-risk population for persistent short stature.

An intriguing aspect of this study was a determination of the relative risk (RR) for short stature in SGA infants defined by birth length (groups 3 and 4), which was slightly higher (RR = 7.1) than that for SGA infants (RR=5.2) defined by birth weight (groups 2 and 3). However, there is no statistical difference between 7.1 and 5.2. The authors emphasize that SGA infants are usually defined in terms of birth weight alone, and if that definition had been used in this study, group 4 would have been eliminated. Poignantly, omission of group 4 would have had a major influence on the results, especially when the outcome is the postnatal gain in height, as the authors found that the majority (61.7%) of the short newborn population belonged to group 4. They also add that it is difficult to compare the results of this study with those of other studies, as there are differences in definitions of the study populations.

Karlberg J, Albertsson-Wikland K. *Pediatr Res* 1995;38:733-739.

Table 1  
Characteristics of Birth Size Groups

| Classification                         | N<br>Group 1 | SGA <sub>w</sub><br>Group 2 | SGA <sub>wL</sub><br>Group 3 | SGA <sub>L</sub><br>Group 4 |
|--|--------------|-----------------------------|------------------------------|-----------------------------|
| Birth length                           | $>-2$<br>SDS | $>-2$<br>SDS                | $<-2$<br>SDS                 | $<-2$<br>SDS                |
| Birth weight                           | $>-2$<br>SDS | $<-2$<br>SDS                | $<-2$<br>SDS                 | $>-2$<br>SDS                |
| Total percentage                       | 94.6%        | 1.6%                        | 1.5%                         | 2.4%                        |
| Percentage<br>$<-2$ SDS at<br>2 years  |              | 9.9%                        |                              | 13.4%                       |
| Percentage<br>$<-2$ SDS<br>at 18 years |              | 6.4%                        |                              | 7.9%                        |
| $\Delta$ SDS in first<br>12 months     |              | 0.6<br>SDS <sub>L</sub>     | 1.2<br>SDS <sub>L</sub>      | 1.3<br>SDS <sub>L</sub>     |
| Mean final<br>height                   |              | -0.4<br>SDS                 | -0.4<br>SDS                  | -0.8<br>SDS                 |

SDS, standard deviation scores

SGA, small-for-gestational age

N, normal

SGA<sub>w</sub>, SGA defined by birth weight

SGA<sub>wL</sub>, SGA defined by birth weight and length

SGA<sub>L</sub>, SGA defined by birth length

**Editor's comment:** Much has been written about growth and growth prognosis in SGA infants, but never based on data as solid as that presented in this excellent study. The authors have enjoyed the luxury of performing a longitudinal retrospective analysis of data due to the homogeneity of the study population and the supportive structure of very well-organized health-care and school systems. They showed that birth length was a predictor of final height.

The traditional definition of adequacy of size for gestational age based only upon birth weight is very elegantly challenged here, where birth length is also included. As a result of this variable, a new group of infants is considered within the SGA group, and this was the most prevalent. These are the infants who are short despite having a normal birth weight, labeled here as group 4. The final height SDS of these infants is closest to that of group 3 individuals, traditionally known as symmetric SGA. The latter are usually considered



as having a poorer growth prognosis. The catch-up growth is impressive in short-for-gestational-age babies (groups 3 and 4) during the first 6 months of life, but their final height remains affected. In contrast, babies in group 2 (normal length but low weight) did not show a dramatic catch-up growth but did end up with a better height.

These facts should stimulate us to improve the accuracy of birth length measurements in delivery rooms and nurseries. In many instances, the current practice for length measurements involves very inaccurate techniques.

Fima Lifshitz, MD

## Male Pseudohermaphroditism Due to a Homozygous Mutation of the LH Receptor Gene

The investigators report that in 2 siblings with male pseudohermaphroditism associated with Leydig cell hypoplasia (testicular histology characterized by a paucity of interstitial cells and seminiferous tubules primarily composed of Sertoli cells) there was a homozygous G→C transversion at nucleotide 1787 in exon 11 of the luteinizing hormone receptor (LHR) gene, resulting in an Ala593Pro substitution in the sixth transmembrane domain of the LHR. This mutated receptor had normal binding affinity for LH but was unable to transduce an intracellular signal (adenylyl cyclase - cyclic adenosine monophosphate), thus rendering it nonfunctional. This resulted in decreased testosterone production and failure of development of normal male external genitalia. The data demonstrate the embryologic importance of the LHR for normal Leydig cell differentiation, proliferation, and function.

Kremer H, et al. *Nature Genet* 1995;9:160-164.

**Editor's comment:** The mutation Ala593Pro in the LHR renders it nonfunctional. This is of interest because of its proximity to many other mutations of the LHR that render it constitutively active. These are presented in Table 1.

The mutation at amino acid 593 is distal to the mutations leading to constitutive activation of the LHR. This suggests that between amino acids 583 and 592 a transition point is located that alters the relationship between the LHR and its associated  $G_s$  protein. Of interest is whether this mutated LHR is able to activate other signal pathways, such as phospholipase C through  $G_q$  protein.

Allen W. Root, MD

**2nd Editor's comment:** The clinical aspects of this type of male pseudohermaphroditism, as reviewed in this article, also are worthy of comment.

The probands were the 46,XY products of first cousins. They had sexual infantilism; female external genitalia; short blind vaginas; no uterus or fallopian tubes; low testosterone levels, which failed to rise with human chorionic gonadotropin (hCG) stimulation; markedly elevated LH but normal follicle-stimulating hormone levels; and testes with Sertoli cells but no mature Leydig cells. Wolffian duct tissue (epididymis and vas deferens) was found, which was surprising since it is found only in the presence of Leydig cell androgens at some point in fetal development. This supports the concept that androgen synthesis in Leydig cells is initiated early in fetal life, independent of LH or hCG synthesis. The authors also conclude that the data and findings further demonstrate that at a later fetal stage the absence of a functional LHR interferes with Leydig cell proliferation and maturation. No abnormal female sex characteristics have been noted in sisters of patients with this form of pseudohermaphroditism. This is consistent with experimental results that indicate absence of a functional ovarian LHR until after birth. Several sisters of reported patients have had amenorrhea, which reflects the need for LHR to be present for normal menstruation.

Nine references are given concerning patients with absence of Leydig cells or insufficient Leydig cell differentiation occurring as an autosomal recessive condition. The phenotypes range from an extreme form of male hermaphroditism to milder forms in which males present with hypergonadotropic hypogonadism and a micropenis. Testicular LH binding was decreased or absent in some studies; this could be either the cause or consequence of Leydig cell hypoplasia. This article is well worth reviewing in its entirety for those interested in the multiplicity of genetic and/or auxologic and related biochemical alterations in male pseudohermaphrodites.

Robert M. Blizzard, MD

Table 1  
Mutations in Familial Male-Limited  
Isosexual Precocity

| cDNA | Nucleotide Change | Amino Acid Change | Location                 |
|------|-------------------|-------------------|--------------------------|
| 1624 | A→C               | Ile542Leu         | TM domain V              |
| 1691 | A→G               | Asp564Gly         | Third intracellular loop |
| 1713 | G→A               | Met571Ile         | TM domain VI             |
| 1715 | C→T               | Ala572Val         |                          |
| 1723 | A→C               | Ile575Leu         |                          |
| 1725 | G→A               | Met575Ile         |                          |
| 1730 | C→T               | Thr577Ile         |                          |
| 1732 | G→T               | Asp578Tyr         |                          |
| 1733 | A→G               | Asp578Gly         | TM domain VI             |
| 1741 | T→C               | Cys581Arg         |                          |
| 1745 | A→G               | Asp582Gly         |                          |

TM = transmembrane

## Short-Term Effect of Testosterone Treatment on Reduced Bone Density in Boys With Constitutional Delay of Puberty

This study demonstrates: (1) that boys with constitutional delay in growth and sexual maturation (CDGP) have decreased bone mineralization beyond that which can be explained by their short stature or delayed bone age, and (2) that in subjects who have received testosterone for 6 months there is increased bone mineralization 6 months later compared with untreated subjects. Bone mineral density (BMD; grams per square centimeter) and bone mineral content (BMC; grams per centimeter) were determined in the nondominant radius by single photon absorptiometry (SPA) in 17 white males with CDGP. BMD and BMC were decreased relative to control data for chronologic age ( $14.6 \pm 1.0$  years), height age ( $11.6 \pm 1.6$  years), and bone age ( $11.9 \pm 1.6$  years). Eight boys received testosterone depot, 100 mg/mo intramuscularly, for 6 months. Six months later (12 months after initiation of testosterone therapy), height, weight, and sexual maturation of the testosterone-treated youths were greater than that of the 9 control subjects, and BMD and BMC had increased substantially as well ( $P < 0.001$ ). BMD increased  $+26.2\% \pm 13.6\%$  in testosterone-treated boys versus  $+0.54\% \pm 8.7\%$  in nontreated subjects. BMC increased  $+41.1\% \pm 28.8\%$  in testosterone-treated boys and  $+5.1\%$  in the untreated subjects. The investigators concluded that boys with CDGP have decreased bone mineralization that is disproportionately greater than that related

to their short stature and delayed skeletal maturation, and that short-term administration of testosterone increases bone mineralization.

Bertelloni S, et al. *J Bone Miner Res* 1995;10:1488-1495.

**Editor's comment:** Approximately 30% of adult lumbar spine BMC is accumulated within a 3-year peripubertal interval, and half of the increase in BMC that occurs during adolescence reflects growth in bone size rather than increase in bone density. Young adult males with CDGP have lower spinal BMD than do control subjects with earlier adolescence, emphasizing the importance of the timing of adolescence as well as the secretion of sex hormones themselves for this process. Whether there are adverse clinical consequences of the lower BMD of CDGP subjects is not known. In the present report, compact bone mineralization has been measured only at the radius by SPA. It will be important to confirm these findings by more extensive evaluation of skeletal mineralization, including sites of trabecular bone such as the lumbar spine and hip, employing dual-energy X-ray absorptiometry. Whether decreased bone mineralization in the male with CDGP should lead to more aggressive therapy is uncertain as yet.

Allen W. Root, MD

## Computer-Aided Skeletal Age Scores in Healthy Children, Girls With Turner Syndrome, and in Children With Constitutionally Tall Stature

The aims of the present study were: (1) to evaluate the reliability of a computer-assisted bone age scoring system in healthy children; (2) to compare this method against a manual rating system in healthy children, as well as in subjects with 2 specific entities, manifested by short and tall stature, respectively; and (3) to determine whether a shortened version of the bone age scoring system might substitute for the original long version. Reference curves for bone maturation in Turner syndrome (TS) and constitutional tall stature (CTS) determined by the computerized system are presented.

Three groups of individuals—healthy children, girls with TS, and children with CTS—underwent bone maturation evaluation. Evaluations were conducted manually using the 13b model of the Tanner-Whitehouse method (TW-RUS), and by the long (13b) and the shortened (6b) models of the computer-aided skeletal age scoring system (CASAS), a transformation of the TW-RUS method into a computerized image analysis system.

As evaluated by the percentage of equal ratings on duplicate (within-observer as well as between-observer) assessments, reliability was high ( $\pm 90\%$ ) in healthy children and similar to those obtained by the manual ratings. The comparability of

CASAS (both 13b and 6b models) with the manual rating system was assessed by calculating the correlation coefficients and evaluating the average and the limits of the range of agreement by a method described by Bland and Altman. Although some of the mean differences of methods were statistically significant, they were not clinically significant, as they were  $< 0.4$  bone age year. Up to 8% of manual insertions occurred in all groups. The percentage was lower in the 6b model than the 13b model, particularly in the CTS children. This suggests that the 6b model of CASAS may have a comparable level of reliability, while introducing a smaller degree of inconsistency.

The authors conclude that CASAS is applicable in TS and CTS. Their data support the use of the 6b model of CASAS, as it is less time-consuming and labor-intensive and provides data almost as reliable as the manual ratings.

Van Teunenbroek A, et al. *Pediatr Res* 1996;39:360-367.

**Editor's comment:** This challenging article compares CASAS scores, particularly those obtained with the 6b model, with manually obtained TW-RUS scores, in the evaluation of the bone maturation in 3 populations of children. Although some

inconsistencies were noted, the methods were comparable. The main advantage of the computerized method over the manual rating method is that it uses a continuous scale instead of an interval scale. This diminishes the error of the interpretation of maturity stages, as the difference of 1 interval stage in the rating of a particular bone may result in an increase of 0.3 bone age years. The 6b model was less time-consuming than the 13b model, and yielded acceptable readings of bone maturation. The main disadvantage of CASAS is the need for special equipment (hardware and software),

which increases the cost. The curves for bone maturation in TS girls and CTS children presented in this article will enhance our understanding of the dynamics of growth, whether spontaneous or in response to specific therapeutic modalities. The procedure has been technically described by Tanner and Gibbons (J Pediatr Endocrinol 1994;7:141-145), in an article that is highly recommended to those of you who wish to review the details of the system and its principles.

Fima Lifshitz, MD

## Intranasal Administration of GHRP Hexarelin Accelerates Growth in Short Children

The investigators administered the growth hormone-releasing peptide (GHRP) hexarelin (His-D-2-methyl-Trp-ALA-Trp-D-Phe-Lys-NH<sub>2</sub>) to 8 short prepubertal children (7 males, 1 female; 5.3 to 11.6 years of age). All subjects had normal growth hormone (GH) secretion (>10 ng/mL) in response to provocative stimulation as well as a substantial increase in GH concentrations (>20 ng/mL) following 1 intranasal inhalation of hexarelin (20 µg/kg). Hexarelin (60 µg/kg/dose) was administered intranasally 3 times daily while the children were recumbent. Mean growth rate increased from  $5.3 \pm 0.8$  cm/y to  $8.3 \pm 1.7$  cm/y during the first 6 to 8 months of therapy ( $P < 0.001$ ). Skin-fold thickness declined and head circumference increased during therapy. Serum levels of insulin-like growth factor 1 (IGF-1), inorganic phosphate, and alkaline phosphatase increased during administration of hexarelin. No adverse local or systemic clinical or biochemical events were recorded during this treatment period. The authors concluded that, over the short term, intranasal hexarelin accelerates growth in short children with intact GH secretion.

**Editor's comment:** The natural compound whose biologic activity is mimicked by the various synthetic GHRPs is unknown. GHRP acts through a somatotrope receptor that is separate from that for GH-releasing hormone (GHRH) and through a different intracellular signaling pathway (GHRH-adenylyl cyclase/cyclic adenosine monophosphate; GHRP-phosphoinositol). The primary site of action of the GHRPs may be within the hypothalamus rather than directly at the pituitary, as they are inactive in the absence of GHRH. GHRPs are active when administered intravenously, subcutaneously, intranasally, and orally. The present report demonstrates the short-term effects of GHRP administered intranasally. We may anticipate that these agents will also be active during short- and long-term oral administration. If experience demonstrates the safety and effectiveness of oral GHRP, yet another therapeutic agent may be available for the management of the carefully selected short, GH-sufficient child.

Allen W. Root, MD

Laron Z, et al. Clin Endocrinol 1995;43:631-635.

## Nondisjunction in Human Sperm: Evidence for an Effect of Increasing Paternal Age

It is well established that increased maternal age is associated with an increased risk of chromosomal trisomy in offspring, ie, the maternal age effect. In contrast, the existence of a paternal age effect has been controversial, with most epidemiologic evidence favoring the absence of such an effect. One of the difficulties has been separating paternal from maternal age effect.

Griffin et al have taken a different approach to the question of paternal age effect. They directly analyzed sperm. Sperm are normally monosomic; they contain only 1 copy of each autosome plus an X or a Y chromosome, ie, 23 X or Y. The authors used fluorescent in situ hybridization (FISH) to count the number of X, Y, and number 18 chromosomes in about 400,000

individual sperm from 24 men, ranging in age from 18 to 60 years. By using probes for both the X and Y chromosomes and chromosome 18, they could distinguish between disomy involving 1 chromosome and diploidy involving a whole complement of chromosomes. Moreover, in cases of disomy for the sex chromosomes, they could distinguish between nondisjunction that occurred during the first meiotic division, which would produce disomic sperm carrying both an X and a Y chromosome, and nondisjunction that occurred during the second meiotic division, which would produce disomic sperm carrying 2 X or 2 Y chromosomes. When such sperm fertilize normal ova, trisomic embryos would be produced containing 47,XXX, 47,XXY or 47,YYY chromosome complements.



The results showed that there was an approximate doubling of nondisjunction for the 3 chromosomes considered together when sperm from men 18 to 29 years were compared with sperm from men 50 to 60 years of age. The numbers were small: 0.11% for the former and 0.27% for the latter. Most of the disomy involved the X and Y chromosomes, with disomic sperm containing XY outnumbering disomic sperm containing XX or YY by about 2:1.

The authors concluded that nondisjunction does occur during male meiosis. It mainly involves sex chromosomes and increases with age, approximately doubling between the ages of 20 to 60 years. However, they point out that the risk for producing trisomic offspring is low: In men over the age of 50 years, only 0.27% of sperm were disomic for the X and/or Y chromosome. The authors also caution that it is not known if disomic sperm compete equally with normal (monosomic)

sperm for fertilization. If not, then the clinical relevance of disomic sperm may be moot.

Griffin DK, et al. *Hum Mol Genet* 1995;4:2227-2232.

**Editor's comment:** This is the first direct evidence that meiotic nondisjunction increases with age in males. As the authors mention, the effect is small, which probably explains why epidemiologic studies have failed to detect a paternal age effect. They rightfully point out that their results provide little basis for suggesting that older men, like older women, be offered prenatal testing for age-related trisomy. In reality, however, since older women are usually married to older men, such testing may be undertaken anyway.

William A. Horton, MD

## A Double-Blind, Placebo Controlled Study of the Effects of Low-Dose Testosterone Undecanoate on the Growth of Small for Age, Prepubertal Boys

Twenty-three short prepubertal boys (11 to 14 years of age) with heights at or below the 3rd percentile were randomized into a double-blind study comparing the effects of oral testosterone undecanoate (TU; 20 mg qd for 6 months) versus placebo on various growth parameters. Treatment was preceded and postluded for 6-month periods. The aim was to assess whether very low doses of TU could accelerate growth velocity (GV) without unduly advancing bone age (BA) in boys with constitutional delay of growth and puberty (CDGP).

The investigators reported that 11 boys taking TU showed a significantly greater GV compared with 12 boys receiving placebo (GV = 5.84 vs 3.38 cm/y), a difference of 2.46 cm/y attributed to 6 months of TU treatment. The effect on BA, axillary and pubic hair, lean body mass, and testicular volume was negligible. Nocturnal growth hormone (GH) concentrations measured over an 8-hour period (every 20 minutes) did not change with treatment. Measurement of serum testosterone, before and following testosterone administration in the morning, revealed an average 10-fold increase at 1-hour postinjection. Within 8 hours, the increase fell to less than 4-fold. There was a projected fall to base level by 16 hours. The authors appropriately emphasized that the efficacy of anabolic or sex steroids in promoting short-term growth and increasing final height is as yet unproven, and that carefully designed, controlled, prospective trials to determine the optimal regimen for growth acceleration would be of great potential therapeutic benefit to many children.

Brown DC, et al. *Arch Dis Child* 1995;73:131-135.

**Editor's comment:** The authors are to be commended for designing a well-planned study of the type needed for the stated purposes. Unfortunately, this study was too brief to provide

adequate information regarding how best to treat CDGP patients with TU. Specifically, 6 months of treatment that provided small alterations in GV is prone to quantitative misinterpretation. A GV increase of approximately 1.25 cm in 6 months (projected to be at a rate of 2.5 cm in 12 months) is at great risk of being in error. A 0.5 cm error at one measurement and a 0.5 cm error in the opposite direction 6 months later will produce an error of 1.0 cm/y or 2.0 cm/y projected. Some smoothing of the error may occur when groups of children are studied, but the error remains significant. This is not to say that low doses of TU do not increase GV. Nevertheless, errors in measurements taken over only a 6-month period of treatment can lead to erroneous conclusions. Even if one accepts that there may be no significant error, 6 months of treatment yielding a gain of 1.25 cm, is relatively insignificant in producing the alterations that are therapeutically effective in boys with CDGP. Therefore, a 12 month study would have been much more useful.

Another possible error in the protocol was the morning administration of TU and the nocturnal measurement of GH concentrations 12 to 16 hours later, when serum testosterone levels have fallen to essentially the projected level of untreated boys. TU given in the morning may have been associated with increased GH levels during the day that went undetected because GH was not measured at that time.

Another advantage of a 12 month study is that BA acceleration often requires more than 6 months of observation to be recognized. Twelve months of treatment might have demonstrated inappropriate advancement of BA. The authors are invited to write a letter of rebuttal if they wish.

Robert M. Blizzard, MD



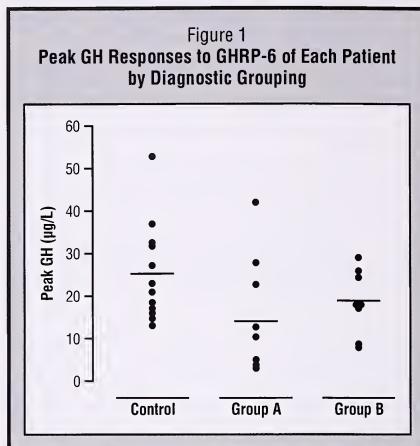
## Plasma Growth Hormone Response to Growth Hormone-Releasing Hexapeptide (GHRP-6) in Children With Short Stature

Pombo et al measured growth hormone (GH) levels every 15 minutes (for 90 minutes) following an intravenous bolus of the synthetic hexapeptide GHRP-6 (1 µg/kg). This agent is one of a group of synthetic compounds that have been shown to release GH by a non-GH-releasing hormone (GHRH)-dependent mechanism. The authors tested whether GHRP-6 could be used to diagnose GH deficiency in children with short stature. Three groups of children were studied. The first group (A) included 10 children with idiopathic GH deficiency, as determined by failure of GH levels to rise to 10 µg/L following provocative stimulation. The second group (B) included 8 children with normal GH response to provocative stimuli but with markedly reduced 24-hour integrated GH concentrations. The third group (C) included 12 normal prepubertal children (Figure 1).

All 10 patients in group A showed variable responses to GHRP-6; 50% showed a response > 10 µg/L. In group B, 6 of 8 subjects showed a GHRP-6 response > 10 µg/L. Although there were differences between mean GH secretion in response to GHRP-6 in group A patients compared with normal children, the results suggest that GHRP-6 stimulation is not an adequate method for diagnosing idiopathic GH deficiency.

Pombo M, et al. *Acta Paediatr* 1995;84:904-908.

**Editor's comment:** Interpretation of these data is that approximately 50% of children diagnosed as having idiopathic GH deficiency by provocative stimuli are able to release GH in response to GHRP-6. Furthermore, 75% of the children with neurosecretory GH deficiency release GH in response to GHRP-6. The authors concluded that GHRP-6 is of no value in diagnosing idiopathic GH deficiency. Perhaps a more



provocative conclusion is that the data demonstrate that many children diagnosed with idiopathic GH deficiency have defects in GH secretion rather than GH synthesis. The mechanism of action of GHRP-6 remains controversial. Both a direct effect at the pituitary and/or the hypothalamus by way of somatostatin or GHRH have been suggested. Studies with GHRP-6 have the potential to reveal important information concerning the normal mechanism of GH synthesis and secretion.

William L. Clarke, MD

## Does Linear Growth Occur Continuously or as "Saltatory" Growth?

Lampl et al<sup>1</sup> reported that in daily, semiweekly, or weekly measurements of crown-heel length in 31 normal infants followed for 4 to 15 months, linear growth proceeded in a start-stop manner; that is, the infant grew at rapid rates for a brief period of several days, followed by prolonged intervals (average 12 days, but as long as 63 days) without any increase in length. Thus, growth in infancy was not continuous but composed of intervals of stasis and rapid growth, a pattern termed saltatory growth. Heinrichs et al<sup>2</sup> challenged this observation. They measured crown-heel (Harpending-Holtain infantometer), knee-heel (from photographs), head circumference, and weight of 5 infants (1.6 to 4.2 months) at the same hour of every day for 1 month. These investigators concluded that their data indicated the infants grew continuously in all aspects. By their analyses of direct inspection of individual growth curves, frequency distribution of growth velocities,

cumulative probability plots, and correlation of crown-heel and knee-heel growth rates, they could find no evidence for saltatory growth and concluded that growth in infancy was continuous. Lampl et al<sup>3</sup> rebut the observations of Heinrichs et al. They point out the inter- and intra-individual variation in growth pattern in infants in their own data and that 1 month of measurements may have been insufficient to observe saltatory growth in the infants reported by Heinrichs et al. Furthermore, Lampl and colleagues reanalyzed the Heinrichs data and concluded that these infants did indeed display a saltatory growth pattern. For these and other reasons, Lampl et al reject the criticisms of Heinrichs et al.

1. Lampl M, et al. *Science* 1992;258:801-803.
2. Heinrichs C, et al. *Science* 1995;268:442-445.
3. Lampl M, et al. *Science* 1995;268:445-447.

**Editor's comment:** The implications of the 2 different models of growth—continuous versus saltatory—for the regulation of mitogenesis and growth are significant. This writer finds it difficult to conceptualize a regulatory system in which cellular growth completely ceases and then resumes. On the other hand, a system that modifies the rates of cellular growth (but not to zero) is less difficult to conceptualize because this is observed clinically in the growth of normal infants, children, and adolescents, as well as during and after intervals

of illness or suboptimal nutrition. I would prefer to consider the periods of absent growth observed by Lampl *et al* as intervals of such slow cellular replication that the measurement instruments utilized to record growth were too insensitive to recognize them—thus merging the concepts of continuous and saltatory growth.

Allen W. Root, MD

## A Novel Transcriptional Activator Originating From an Upstream Promoter in the Human Growth Hormone Gene

**Editor's comment:** The finding of a "gene within a gene" is intriguing. By analogy to large proteins that may be precursors for several peptides, eg, proopiomelanocortin, it is likely that other genes will be identified with similar construction. It is of interest to speculate that growth hormone-derived transcriptional activator (GHDTA) may be a transcription-activating factor for proopiomelanocortin; alternatively, it might serve as a repressor of human growth hormone (hGH) gene transcription in corticotropes. Now that you have read this comment, please read the abstract that follows.

Allen W. Root, MD

Labarrière and coworkers identified a second gene product that begins in the upstream promoter region of the hGH gene and includes all of the first and part of the second exon of the hGH gene. The major transcription-activating factor for the hGH gene is Pit-1; this factor binds to upstream bases -130 to -105 and -92 to -65 to initiate gene transcription for hGH mRNA. Between bases -294 and -177 is a sequence that also has the structure of a transcription-promoting region. These investigators cloned the mRNA transcribed from this

secondary promoter region, which begins at base -151 in the hGH gene and ends in the middle of exon 2 of the hGH gene at a stop codon, the result of a frameshift. This mRNA encodes a protein of 107 amino acids (molecular weight = 11.4 kd). Expression of mRNA for hGH is confined to the pituitary somatotrope, whereas the protein product of the secondary mRNA is detectable in pituitary corticotropes and placenta. The second protein has been termed GHDTA because it acts as a transcription-activating factor in cells transfected with reporter genes. GHDTA has homology with a liver-specific transcription factor and contains potential protein kinase C-dependent phosphorylation sites. The investigators suggest that GHDTA might be a DNA-binding transcription factor or an activator of a transcription factor.

Labarrière N, *et al*. *J Biol Chem* 1995;270:19205-19208.

**2nd Editor's comment:** A change in GGH's usual format was made for this abstract and the editor's comment precedes the abstract. This was done so that readers may better interpret the abstract.

Robert M. Blizzard, MD

## Insensitivity to Anti-Müllerian Hormone Due to a Mutation in the Human Anti-Müllerian Hormone Receptor

Müllerian-inhibiting substance (MIS), a product of the Sertoli cell, causes regression of müllerian duct development and prevents formation of the fallopian tubes, uterus, and upper third of the vagina in the normal male. Abnormalities in the production of MIS lead to the persistent müllerian duct syndrome (PMDS) of müllerian duct structures in phenotypic and genetic males. The present investigators have isolated and characterized the human MIS receptor and have identified a patient with PMDS due to an abnormality in the gene for this receptor. This patient thus has end-organ insensitivity to MIS. This gene is situated on chromosome 12q13 and is composed of 11 exons that encode a mature protein of 573 amino acids. Exons 1 through 3 encode the signal sequence (17 amino acids) and extracellular domain (127 amino acids); exon 4

encodes the single transmembrane domain (26 amino acids); and exons 5 through 11 encode the intracellular domain (403 amino acids), which has serine/threonine kinase activity. The human MIS receptor is homologous to that of the rat (78.5%) and rabbit (82%). In addition to the testicular Sertoli cell, RNA for MIS and its receptor is expressed in the normal ovary and some granulosa cell tumors.

In a 3-month-old boy with PMDS, the AMH gene was normal, and serum AMH was easily measurable. Analysis of the MIS receptor gene revealed a guanine to adenine (G→A) transition at a guanine-thymine (GT) dinucleotide at the splicing donor site of the 5' end of the second intron. This led to 2 abnormalities of gene transcription: (1) loss of exon 2 (exon skipping); and (2) substitution of aspartate for glycine at amino

acid 78 and the insertion of 4 extra amino acids from the first 12 bases of intron 2 into the 3' end of exon 2 (intron inclusion). Either aberration within the extracellular domain is expected to lead to abnormalities of ligand binding and hence hormone action.

Imbeaud S, et al. *Nature Genet* 1995;11:382-388.

**Editor's comment:** *MIS is a member of the transforming growth factor-6 (TGF-6) family. The MIS gene (chromosome 19p13.3) contains 5 exons and encodes a protein of 560 amino acids (including a leader sequence of 24 amino acids).<sup>1</sup> It is active as a disulfide-linked homodimer. PMDS has been associated with both normal and subnormal or absent production of AMH. In the latter group, abnormalities in the MIS gene have been detected, including deletions, nonsense mutations associated with stop codons, frameshift mutations leading*

*to downstream stop codons, point mutations leading to instability of the protein molecule, and intronic splice donor point mutations.<sup>1,2</sup> The majority of abnormalities have been found in exons 1 through 3. Identification of the receptor for MIS and documentation of its abnormality in a patient with PMDS provide further evidence of the importance of these proteins in male sexual differentiation. MIS is also produced by granulosa cells and is involved in the regulation of ovarian function. In the article by Imbeaud et al, PMDS results not from a mutated gene for MIS but a gene for its receptor. Again, the same apparent syndrome may be the same syndrome but of different gene origin.*

Allen W. Root, MD

1. Josso N, et al. *Rec Prog Horm Res* 1993;48:1-59.
2. Imbeaud S, et al. *Hum Mol Genet* 1994;3:125-131.

## No Reduction in Birth Weight in Phenylketonuria

A registry of all known children with phenylketonuria (PKU) born in the United Kingdom from 1964 onward allows the definition of growth parameters and natural history. The birth weight, sex, social class, gestational age, disease severity, and birth date were all taken into consideration when determining norms and averages. Data were available for 1,886 infants. The mean birth weight for PKU infants born in the United Kingdom was 3,306.7 g and the median 3,337 g. There are no significant differences from other births in the United Kingdom and PKU individuals show a similar pattern to the normal population.

Tillotson SL, et al. *Eur J Pediatr* 1995;154:847-849.

**Editor's comment:** *It is nice to have a proper natural history study that facilitates assessment of the natural history of a disorder. This PKU data gave completely normal growth findings in the affected newborn. This work is important since there has been a recent report suggesting impairment was already present at birth.*

Judith G. Hall, MD

## A Gene (PEX) With Homologies to Endopeptidases Is Mutated in Patients With X-Linked Hypophosphatemic Rickets

The gene for familial X-linked hypophosphatemic rickets (FHR) has been localized to chromosome Xp22.1. By positional cloning, the present investigators have detected 4 partial deletions and 3 mutations (from a total of 150 families studied) in a gene in this region that is homologous to several endopeptidases such as neutral endopeptidase, endothelin-converting enzyme-1, and the Kell antigen. The deletions ranged in size from <1 to 55 kb; the mutations included loss of a dinucleotide, resulting in a frameshift, and 2 point mutations leading to exon skipping. This gene has been termed *PEX* (phosphate-regulating gene with homologies to endopeptidases on the X chromosome). As do other members of the neutral endopeptidase family, *PEX* has many small exons, a short cytoplasmic amino-terminal domain, a transmembrane segment, and a large extracellular carboxyl-terminal region with a zinc-binding motif and 7 conserved cysteine residues. The investigators hypothesize that the *PEX* endopeptidase is important for processing a circulating factor that regulates function of the sodium-phosphate cotransporter (whose gene is situated on

chromosome 5q13). Loss of this endopeptidase would result in an inactive phosphate regulatory factor and decreased renal phosphate resorption.

The HYP Consortium. *Nature Genet* 1995;11:130-136.

**Editor's comment:** *Identification of a defective gene coding for an endopeptidase as the candidate gene for FHR leads to additional questions. For example, what is the target protein for PEX endopeptidase action and where is its gene located? How does this protein affect activity of the sodium-phosphate cotransporter? In addition, it introduces the probability that there are abnormalities in this and other factors that also lead to hyperphosphaturia, hypophosphatemia, and metabolic bone disease. Indeed, autosomal recessive and autosomal dominant forms of hypophosphatemic rickets and hypophosphatemic bone disease have been described that may involve these other proteins.*

Allen W. Root, MD



## Ob/Ob and Db/Db Gene(s), Obesity, and Sterility, and Relationships to Leptin

**Editorial introductory comment:** Grouping of related abstracts often enhances understanding of an entity or related entities. The story of genetic obesity has complexities that prompt grouping of the following abstracts by Dr. Root. For purposes of orientation, the following introductory remarks to the abstracts are given:

The ob/ob obese mouse is genetically deficient in the production of the ob gene product, leptin. Leptin administration produces loss of weight and decreases appetite in ob/ob mice. The db/db obese mouse, which has a very similar phenotype to the ob/ob mouse, is not responsive to leptin.

Therefore, a receptor defect is apparently present. Different genes have been thought to be involved in the 2 strains. New data referred to below suggest that the same gene may be responsible. We previously learned that identical auxologic phenotypic syndromes such as GH deficiency and Laron syndrome can be of different molecular origins. We are learning now that different components of the same gene can be responsible for the almost identical phenotypic ones, but syndromes that differ in response to a therapeutic agent.

Robert M. Blizzard, MD

## Correction of the Sterility Defect in Homozygous Obese Female Mice by Treatment With the Human Recombinant Leptin

The obese female mouse that is homozygous for the ob/ob mutation leading to a decrease in the synthesis of leptin also is sterile. The infertility of these animals is due to dysregulation of hypothalamic-pituitary function that is unrelated to body weight per se, because reduction of body weight to normal values does not restore fertility. There are low levels of reproductive hormones in the ob/ob animal, while the ovaries of these animals are normally responsive to gonadotropins. The present investigators demonstrated that administration of leptin (10 µg/g of initial body weight per day intraperitoneally for 30 to 42 days) resulted in a decline in body weight by 40% to 48%. When mated with normal male mice, all (n=6) female mice copulated and became pregnant (leptin administration was continued at 5 µg/g/d). All (n=28) of the pups that were delivered died by 2 days of age because the mothers did not suckle their young. In another experiment, 6 ob/ob female mice were treated with leptin and lost weight, mated, conceived, and delivered pups immediately, after which the number of pups was purposely decreased to 3 pups/litter in the 2 dams who were able to suckle. These animals survived, as did the offspring that were transferred to normal foster dams. Interpretation of the data are that (1) leptin administration to ob/ob homozygous female mice leads to weight loss, copulation, ovulation, pregnancy, and parturition that is independent of weight loss alone; and (2) lactation is only partially restored by leptin administration, either because of defective breast development in this strain of mice or because leptin adversely affects mammary development/function in the pregnant female ob/ob mouse.

Chehab FF, et al. *Nature Genet* 1996;12:318-320.

**Editor's comment:** This paper reports data that support the suggestion that leptin alters hypothalamic-pituitary-ovarian function, permitting ovulation, conception, and parturition in animals with defective synthesis of this fat-derived peptide. (Further studies of the effect of leptin on the secretion of LH,

FSH, prolactin, the sex hormones, and breast function must be carried out in order to determine the physiologic mechanisms that underlie the clinical effects of leptin in these animals.) Thus, in addition to its effects on hypothalamic regulation of appetite, leptin may also influence the synthesis/secretion of gonadotropin releasing hormone (GnRH) and/or the pituitary gonadotropins directly. The relationship between body fat content and the reproductive endocrine system, particularly in females, has long been known (eg, the "critical weight" hypothesis of Frisch, which correlates weight loss and amenorrhea accompanying the weight loss of dieting, illness, or excessive exercise), but the mechanism of this regulatory effect has not been identified. Perhaps leptin is the messenger traveling from body fat to the hypothalamus that influences GnRH synthesis/release. Very possibly leptin may play a fundamental role in normal adolescent sexual maturation, as well as in a number of states of energy deprivation.

Allen W. Root, MD

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## Evidence That the Diabetes Gene Encodes the Leptin Receptor: Identification of a Mutation in the Leptin Receptor Gene in db/db Mice

The investigators identified an abnormality in the hypothalamic receptor for the fat-derived, anorectic OB peptide (leptin) in the db/db mouse, which is responsible for the phenotype of this animal. The phenotype is similar to that of the ob/ob mouse and characterized by early-onset obesity, insulin resistance, and susceptibility to diabetes. In the normal mouse there are short and long forms of leptin receptor (designated OB-R) that arise by alternative splicing of transcripts. The short OB-R has extracellular leptin-binding transmembrane and intracellular domains of 816, 23, and 34 amino acids, respectively. The long OB-R has an intracellular domain of 302 amino acids. The latter results from the inclusion of an exon that is not transcribed in the OB-R short form. It is the long form of the OB-R that probably is involved in intracellular signaling. It is related to the class I group of cytokine

receptors and contains motifs for interaction with janus kinase (JAK) and the signal transducer and activator of transcription (STAT). The db/db mouse pathologically expresses only the short form of the OB-R. It does so because of a mutation (G→T) in the OB-R gene that creates a new splice site that leads to the incorporation of 106 extra nucleotides in the transcript of the OB-R gene, including a stop codon that results in premature termination of the long intracellular portion of the OB-R.

Chen H, et al. *Cell* 1996;84: 491-495.

**Editor's comment:** Recent papers by Lee et al<sup>1</sup> and Chua et al<sup>2</sup> add further information to the genetic defect in the db/db mouse. Lee et al reported identical findings to those of Chen et al in the db/db mouse; they also observed 9 normal splicing variants of OB-R expressed in mouse brain, hypothalamus, adipose tissue, testes, and heart. Chua et al reported that the OB-R gene and those encoding the defects in the db/db mouse and fa/fa (Zucker fatty) rat are the same. Interestingly, the OB-R also is expressed in the ovaries (Chen et al). The human OB-R has been localized to chromosome 1p31.

Allen W. Root, MD

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1. Lee G-H, et al. *Nature* 1996;379:632-635.
2. Chua SC Jr, et al. *Science* 1996;271:994-996.

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# GROWTH

## Genetics & Hormones

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### Activating Mutations in G Protein-Coupled Signaling Pathways as a Cause of Endocrine Disease

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Unraveling the mechanisms by which extracellular stimuli activate intracellular signaling pathways is an extremely active area of research. Many stimuli, including photons, hormones, neurotransmitters, odorants, proteases, and ions, act through a group of membrane-spanning cell surface receptors that are coupled to guanine nucleotide-binding proteins (G proteins) to modulate the activity of cellular effectors.<sup>1</sup> In the last few years, mutations in the genes encoding components of these signaling pathways have been shown to cause human disease.<sup>2-4</sup> Identification of these naturally occurring mutations not only has value in defining the molecular basis of disease, but also has accelerated progress in understanding the fundamental mechanisms by which G protein-coupled signal transduction occurs. Loss-of-function mutations have been described in several endocrine diseases characterized by hormone resistance, such as pseudohypoparathyroidism type Ia ( $G_{\alpha}$ ), hereditary glucocorticoid deficiency (corticotropin receptor), and nephrogenic diabetes insipidus (V2 vasopressin receptor). This review will focus on those endocrine diseases that have been shown to be due to gain of function mutations, ie, where inappropriate signaling occurs in the absence of an agonist (Table 1).

#### G PROTEIN AND RECEPTOR SIGNALING

All members of the G protein-coupled receptor (GPCR) family are predicted to share a common "serpentine" structure: a bundle of 7  $\alpha$ -helical hydrophobic regions (TM1 through TM7) connected by alternating extracellular (e1 through e3) and intracellular (i1 through i3) loops (Figure 1). Receptor

Table 1  
Endocrine Diseases Caused by Activating Mutations in G Protein-Coupled Pathways

| Gene Mutation                                | Disease  |
|--|--|
| <u>G protein <math>\alpha</math> subunit</u> |  |
| $G_s(gsp)$                                   | Somatotrope and thyroid adenomas; McCune-Albright syndrome   |
| $G_s(\text{Ala366} \rightarrow \text{Ser})$  | Combined testotoxicosis and pseudohypoparathyroidism type Ia |
| $G_{12}(gip)$                                | Ovarian and adrenocortical tumors                            |
| <u>Receptor</u>                              |  |
| Luteinizing hormone                          | Testotoxicosis   |
| Thyroid-stimulating hormone                  | Thyroid adenoma and hyperplasia                              |
| $\text{Ca}^{2+}$                             | Autosomal dominant hypocalcemia                              |
| Parathyroid hormone                          | Jansen's disease (metaphyseal chondrodysplasia)              |

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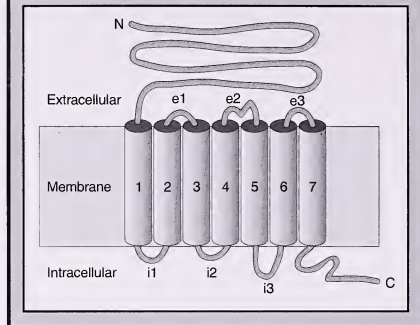
activation that accompanies agonist binding involves conformational changes in the transmembrane barrel that are relayed to the cytoplasmic surface. The concerted action of several intracellular loop regions, especially the N- and C-terminal ends of i3, has been implicated in G protein binding and activation.

Heterotrimeric G proteins are inactive in the guanosine diphosphate (GDP)-bound state, and interaction with an activated receptor is necessary to promote release of the nucleotide (Figure 2). Binding of ambient guanosine triphosphate (GTP) to the vacated site on the G protein  $\alpha$  subunit leads to a change in the conformation of the G protein, dissociation of the complex, and effector activation by  $\alpha$  and  $\beta\gamma$  subunits. Once freed, the activated receptor can promote GDP dissociation from multiple other G protein molecules, thus providing signal amplification. Effector activation is terminated when the  $\gamma$ -phosphate of GTP is hydrolyzed by a guanosine triphosphatase (GTPase) that is intrinsic to the  $\alpha$  subunit. Thus, GTP/GDP exchange is the rate-limiting step in the cycle, and GTPase serves as the critical timing mechanism. Different classes of G proteins are coupled to various effector mechanisms. The G protein that will be highlighted below is  $G_s$ , the G protein that stimulates adenylyl cyclase activity. The first disease shown to be due to a G protein defect was cholera, in which a bacterial toxin catalyzes adenosine diphosphate (ADP)-ribosylation of Arg201 in  $G_{s\alpha}$  in intestinal cells. This covalent modification inhibits GTPase activity, leads to persistent production of cyclic adenosine monophosphate (cAMP) in the presence of little or no hormone, and causes severe secretory diarrhea.

### ACTIVATING MUTATION OF G PROTEIN $\alpha$ SUBUNIT GENES IN TUMORS

The first example of an activating mutation in a G protein  $\alpha$  subunit gene was the discovery of somatic heterozygous mutations of  $G_{s\alpha}$  (*gsp* mutations) in a subset of growth hormone (GH)-secreting tumors of human pituitary.<sup>5,6</sup> One set of mutations involved Arg201 (the cholera toxin target) and another affected Gln227. Substitution of either of these amino acid residues *in vitro* has been shown to inhibit GTPase activity and lead to inappropriate stimulation of adenylyl cyclase. Recent crystallographic data show that these residues are located adjacent to the  $\gamma$ -phosphate of GTP, and it is now easy to see why changes at either of these critical positions would interfere with GTP hydrolysis.<sup>7</sup> Mutations of *gsp* were subsequently identified in some thyroid tumors.<sup>8-10</sup> The pathology associated with *gsp* mutations is consistent with the known effects of cAMP in mediating increased cell proliferation and secretion of hormone in somatotropes and thyroid follicular cells.<sup>11</sup>

Figure 1  
Predicted Structure of a  
G Protein-Coupled Receptor



### $G_{s\alpha}$ MUTATION IN McCUNE-ALBRIGHT SYNDROME

McCune-Albright syndrome (MAS) is a sporadic disease classically defined by polyostotic fibrous dysplasia, café au lait spots, sexual precocity, and other hyperfunctional endocrinopathies.<sup>12,13</sup> Endocrine tissues that function autonomously in MAS include the gonads, thyroid, adrenal cortex, and pituitary somatotropes. The sporadic occurrence of MAS, its variable presentation, and the distinctive pattern of skin pigmentation led Happle<sup>14</sup> to hypothesize that the disorder was due to a dominant somatic mutation occurring early in embryogenesis. According to this model, patients with MAS are mosaic for the mutant gene (Figure 3). Because cAMP was known to stimulate the growth or function of tissues classically involved in MAS, it was proposed that the mutant gene was one that led to excess cAMP production.<sup>12,15</sup>

#### In Future Issues

##### Genes of Growth Factors and Hormones: An Update

Victor McKusick, MD

##### The Neuroendocrinology of Stress: Its Relationship to Growth

George Chrousos, MD

##### Indications for Leg-Lengthening Procedures and Current Status of the Technique

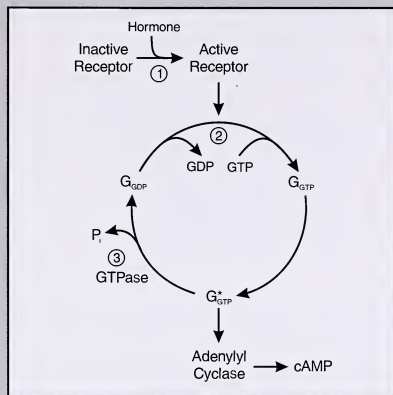
Deborah Stanitski, MD

##### Growth Hormone Treatment in Chronic Renal Insufficiency: An Update

Richard Fine, MD



Figure 2  
The G Protein Cycle Coupled to  
Adenylyl Cyclase



Inappropriate stimulation of this pathway results from mutations that cause 1) receptor activation in the absence of hormone, 2) GTP/GDP exchange in the absence of activated receptor, or 3) inhibition of GTPase activity.

With the discovery of its role in isolated somatotrope tumors,  $G_{s\alpha}$  became an excellent candidate gene for MAS, and mutations encoding substitution of Arg201 with either Cys or His were soon found in affected tissues from many MAS patients.<sup>16-19</sup> Mutant alleles were detected in variable abundance in different affected tissues from the same patient, including abnormal nonendocrine tissues, consistent with Happle's somatic mosaic model. Some types of cells harboring the  $G_{s\alpha}$  mutation have increased proliferation, but others may have impaired growth. For example, even severely affected MAS patients typically have little or no evidence of mutation in DNA prepared from blood leukocytes, a fact that precludes straightforward molecular diagnosis. In only one case in which affected tissue was available for analysis has there been a failure to find the Arg201 mutation.<sup>20</sup> Mutations of Gln227 have not been found in MAS, possibly because it has a more powerfully activating, lethal effect on embryonic cells even when expressed in the mosaic state. Although most MAS patients manifest only classic features of the syndrome, a subset of patients with hepatobiliary abnormalities, cardiovascular disease, and early death has been described.<sup>18</sup>

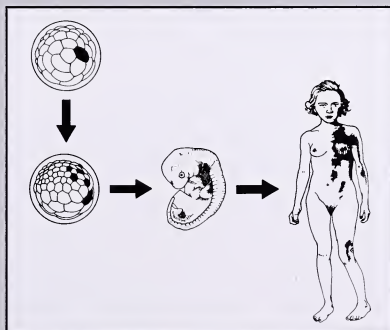
Severe disease may be associated with an earlier mutational event that leads to a widespread

distribution of mutant cells in the embryo, while incomplete forms of MAS may result from a mutational event that occurs later in embryologic development. A focal somatic mutation of  $G_{s\alpha}$  gene during adulthood serves as the basis of diseases that affect only a single tissue, such as somatotrope or thyroid adenomas<sup>5,8-10</sup> and monostotic fibrous dysplasia.<sup>21</sup> Studying the pathophysiologic consequences of constitutive activation of  $G_s$  in bone and other MAS tissues may provide insight into the role that  $G_s$  normally plays in cellular development and differentiated function. Understanding the aberrant behavior of the mutant cells that produce lesions of fibrous dysplasia is certainly needed to develop better therapy for this painful and disabling condition.

### OTHER ACTIVATING G PROTEIN MUTATIONS

Mutations that block GTPase activity of the  $\alpha$  subunit of  $G_2$  have been described in a very small number of ovarian and adrenocortical tumors,<sup>8</sup> and their general significance remains to be proven. A rare condition characterized by a combination of gonadotropin-independent male precocious puberty (gain of function) and pseudohypoparathyroidism type Ia (loss of function) has been shown to be due to a unique mutation of  $G_{s\alpha}$  that allows spontaneous release of GDP in the absence of receptor (Figure 2).<sup>22</sup> At testis temperature (33°C), the mutant protein is constitutively active; however, at normal body temperature (37°C), it is rapidly degraded, thus explaining the paradoxical phenotype.

Figure 3  
Somatic Mutation in Early Embryogenesis  
Produces Mosaicism in McCune-Albright  
Syndrome



Adapted from an illustration by Frank Netter. In: Netter FH. *The Endocrine System*. Vol 4. Summit, NJ: CIBA, 1965.



## ACTIVATING RECEPTOR MUTATIONS

In the course of studying the structure of adrenergic receptors, it was observed<sup>23</sup> that substitution of a single Ala residue located at the junction of i3 and TM6 promoted activation of the receptor even in the absence of agonist. Furthermore, the mutant receptor was oncogenic when expressed in rodent fibroblasts.<sup>24</sup> The discovery that artificial mutagenesis could be used to generate receptors with unregulated activity raised the possibility that a naturally occurring gene mutation that led to constitutive activation of a GPCR could serve as a mechanism of human disease.

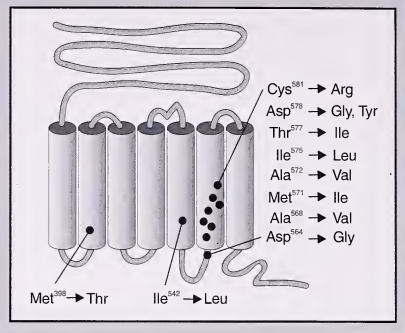
## ACTIVATING MUTATIONS OF THE LUTEINIZING HORMONE RECEPTOR IN TESTOTOXICOSIS

Testotoxicosis, also known as familial male precocious puberty, is a gonadotropin-independent disorder that is inherited in an autosomal dominant, male-limited pattern.<sup>25</sup> Testosterone secretion and Leydig cell hyperplasia occur in the context of prepubertal levels of luteinizing hormone (LH), and the onset of puberty in affected boys usually occurs by 4 years of age. It was hypothesized that testotoxicosis was due to a mutant LH receptor (LHR) that could be activated in the presence of little or no agonist, and a heterozygous mutation that results in substitution of Asp578 in TM6 with Gly was first found in affected individuals from 9 different kindreds.<sup>26,27</sup>

To assess the functional effect of the Asp578→Gly mutation, wild-type and mutated human LHR were transiently expressed in COS-7 cells.<sup>26</sup> In contrast to the silent wild-type LHR, the mutant LHR produces a 4.5-fold increase in basal cAMP production in COS-7 cells, indicating that it is constitutively active. Agonist-independent stimulation of cAMP production represents about 40% of the maximal stimulation produced by the agonist human chorionic gonadotropin (hCG), and is not simply due to increased receptor expression.<sup>28,29</sup> The mutant receptor is also capable of responding to increasing concentrations of hCG, with a median effective concentration (EC<sub>50</sub>) and maximal hCG-stimulated cAMP production similar to that of the wild-type receptor. The mutation has no effect on agonist binding affinity, which is known to be determined primarily by sequences in the large N-terminal domain.<sup>28,29</sup>

The Asp578→Gly LHR mutation is the most common cause of familial testotoxicosis, and it has also been detected in sporadic cases of gonadotropin-independent, male precocious puberty.<sup>28,30</sup> Different mutations of the LHR, mostly clustered in TM6, have been found in other patients.<sup>27,29-33</sup> The location of activating LHR mutations is shown in Figure 4.

Figure 4  
Activating Mutations of the Luteinizing Hormone Receptor in Testotoxicosis



Some LHR mutations produce biochemical phenotypes similar to that of the Asp578→Gly substitution, but others do not. For example, an LHR mutation encoding substitution of Asp578 with Tyr promotes much higher basal cAMP accumulation in transfected cells than that produced by the other mutations.<sup>30,34</sup> This mutation has been found in 3 unrelated boys with unusually early signs of puberty, suggesting that their clinical phenotype is related to the strongly activating nature of the Asp578→Tyr substitution.

Dominant mutations that lead to constitutive activation of the LHR-mediated cAMP signaling pathway can explain the pathophysiology of gonadotropin-independent precocious puberty in males. LHR-mediated effects, including testosterone production, are known to involve increased production of cellular cAMP.<sup>35</sup> Intracellular cAMP accumulation triggered by unoccupied mutant receptors appears sufficient to cause Leydig cell hyperfunction and hyperplasia, although the delay in phenotypic expression must be related to other developmental events. LH alone is adequate to trigger steroidogenesis in Leydig cells, but both LH and follicle-stimulating hormone (FSH) are required to activate ovarian steroidogenesis, a fact that explains why females carrying the mutant allele do not exhibit precocious puberty.

In contrast to G proteins, the actual 3-dimensional structure of a GPCR has not yet been defined. Activating LHR mutations may provide insight into structural features involved in receptor activation. The location of most of the mutations is consistent with earlier data, which show that residues at the base of TM6 and in the adjacent C-terminal portion of i3 play a critical role in G protein coupling. In the inactive receptor state, the conformation of TM6

may be restricted by a set of interhelical bonds. One can imagine that hormone binding serves to break these constraints, thus allowing key residues on the cytoplasmic face to become exposed. Substitution of certain residues in TM6 may partially mimic agonist occupancy by weakening or eliminating interhelical bonds. Although some GPCR substitutions appear to act by increasing the proportion of receptors in the active conformation, it is possible that other activating substitutions will be found to act primarily by increasing the affinity of the isomerized receptor for G protein or by interfering with normal desensitization mechanisms.

## ACTIVATING RECEPTOR MUTATIONS IN OTHER DISEASES

Knowledge that the growth and function of thyroid follicular cells are positively regulated by cAMP inspired the successful search for activating thyroid-stimulating hormone receptor (TSHR) mutations in hyperfunctional thyroid adenomas. Several different somatic TSHR gene mutations have been identified in sporadic adenomas, and autosomal dominant thyroid hyperplasia has been shown to represent the result of germline mutations in the TSHR gene.<sup>10,36-38</sup> As with LHR mutations in testotoxicosis, many of the TSHR mutations are clustered in TM6, but substitutions found in e1, e2, TM3, and TM7 indicate that other regions of the TSHR must also participate in stabilizing the inactive receptor conformation.

The parathyroid and kidney help maintain extracellular  $\text{Ca}^{2+}$  concentrations within a narrow physiologic range by promptly responding to increased  $\text{Ca}^{2+}$  with decreased parathyroid hormone (PTH) secretion and decreased  $\text{Ca}^{2+}$  reabsorption, respectively. This process has recently been shown to be mediated by a G protein-coupled  $\text{Ca}^{2+}$  receptor. Heterozygous mutations encoding 2 different substitutions in this receptor have been found in affected members of 2 kindreds with autosomal dominant hypocalcemia, indicating that increased receptor sensitivity to extracellular  $\text{Ca}^{2+}$  or constitutive receptor activity is responsible for this rare disorder.<sup>39,40</sup>

Jansen's disease, or metaphyseal chondrodysplasia, is an uncommon form of dwarfism often associated with PTH-independent hypercalcemia and hypophosphatemia. It has been shown to be due to constitutively activating mutations of the PTH receptor that lead to abnormal formation of endochondral bone and inappropriate signaling in the kidney.<sup>41,42</sup>

## FUTURE PROSPECTS

Therapy for patients with activating mutations of  $\text{G}_s\alpha$  or  $\text{G}_{s\alpha}$ -coupled receptors is generally directed at blocking the downstream effects wrought by the

overactive cAMP signaling cascade or ablation of the abnormally functioning tissue. Because somatotrope adenomas contain somatostatin receptors linked to inhibition of adenylyl cyclase activity, treatment with a somatostatin analogue can be beneficial. The realization that some types of receptor antagonists have the ability to preferentially bind and stabilize the inactive conformation of a receptor (so-called negative antagonists) raises the possibility that such drugs could someday be used to treat diseases due to agonist-independent receptor activity.<sup>43</sup>

Is it possible to predict other diseases that might be due to activating mutations in G protein-coupled pathways? In several of the syndromes discussed above, hypotheses guided by knowledge of the biochemistry, pathophysiology, and genetics of a disease have led to the discovery of the causative gene mutations; however, other searches have been less fruitful.<sup>44,45</sup> It has been suggested that activated GPCR and  $\text{G}\alpha$  subunit genes with proven oncogenic potential in cultured rodent fibroblasts<sup>24,46</sup> might be detected in some types of human malignancy. The phenotypes of transgenic mice expressing activated receptors or G protein subunits may also provide clues to human disease. Finally, it is important to consider that variation in the genes that encode other components in the G protein signaling cascade, including the  $\beta$  and  $\gamma$  subunits, effectors, and proteins involved in receptor desensitization, may also be found to play a role in some forms of endocrine disease.

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## From the Endocrine Society Meeting, June 13, 1996

# GH Axis – Child and Adolescent: A Review of the Clinical Oral Session

Eight papers were presented. The first was entitled, *IUGR and Postnatal Growth Failure in a Patient Homozygous for a Partial IGF-1 Gene Deletion* (OR 46-1).<sup>1</sup> The first case of a partial insulin-like growth factor 1 (IGF-1) gene deletion was described in a human. The patient was a 15-year-old boy with a birth weight of 1.37 kg at 37 weeks gestation and a height standard deviation score (SDS) of -6.9 at 15 years of age. He was resistant to exogenous growth hormone (GH), had sensorineural deafness and moderate mental retardation. Serum GH was elevated, and IGF-1 was practically nonexistent. Partial deletion of the IGF-1 gene is compatible with life. IGF-1 is important in prenatal and postnatal growth and possibly in central nervous system development.

The second was entitled, *Dwarfism of Sindh: A Novel Form of Familial Isolated GHD Linked to the Locus for GHRHR* (OR 46-2).<sup>2</sup> Eighteen dwarfs in Pakistan inherited an autosomal recessive GH-releasing hormone receptor (GHRHR) defect, with phenotypes resembling GH deficiency (GHD) or GH insensitivity. GH, IGF-1, IGF-binding protein-3 were low and failed to increase with GHRH or other pharmacologic stimuli for GH release. An inactivating mutation in the GHRHR appears likely on the basis of LOD scores.

The third paper (OR 46-3) dealt with the correlation of hormonal circadian rhythms with types 1 and 3 procollagens. PICP (type 1) increased

markedly, following GH pulsations, and decreased markedly following cortisol elevation.<sup>3</sup> PHINP (type III) did not change. The findings suggested that PICP levels in the morning may be low due to morning cortisol elevation, and time standardization is important when evaluating this test. By inference, interpretation of PICP levels may be hazardous because of marked fluctuations over brief periods.

The fourth paper (OR 46-4) dealt with intranasal use of GHR peptide as a therapeutic agent. GHR peptide in short, GH-sufficient children produced a modest average increase of 2.1 cm/y during 9 to 10 months of treatment.<sup>4</sup> The emphasis was not on growth, but a fall in the GH released over time to intranasal hexarelin, which occurred without a fall in the initial IGF-1 increased levels. The effective intranasal dose for GH release was 20 times the intravenous dose required. Hexarelin was given three times daily.

The fifth presentation was entitled, *Contrasted Doses of GH to GHD Patients in Respect to Achieving Respectable Adult Heights* (OR 46-5).<sup>5</sup> Doses of 0.06 to 0.19 mg/kg/wk given to GHD patients with spontaneous puberty produced no gain in height SDS during puberty. Doses of 0.3 mg/kg/wk continued through puberty advanced the mean SDS from -2.1 ± 1.4 at initiation of puberty to 0.9 ± 1.2 at completion of puberty. The larger dose more closely simulates the secretion rate of



GH during puberty, when GH release in the normal adolescent is stimulated by sex steroid secretion.

The sixth presentation was entitled, *Catch up Growth and Height Achievement in Older, Late-Treated GHD Patients* (OR 46-6). It pertained to GHD children >15 years of age who were minimally or not sexually developed when treatment was instituted.<sup>6</sup> Their mean bone age was  $12.2 \pm 1.8$  years. Year 1, 2, and 3 growth rates with treatment were  $8.5 \pm 3.1$  cm,  $7.2 \pm 2.3$  cm, and  $6.0 \pm 2.0$  cm, respectively. The conclusions were that over the 3 years, improvement of height age ( $3.2 \pm 1.2$  years), height SDS ( $2.3 \pm 1.1$  SD), and Bayley-Pinneau predicted height ( $0.9 \pm 1.4$  SD) were observed. Two patients over 20 years of age, who were sexually infantile, responded similarly.

The seventh paper was from a collaborative European study, entitled *Four Years of GH Therapy in 3 Dosage Regimens in 216 Children With ISS* (OR 46-7).<sup>7</sup> The conclusions were that GH at  $3.0$  or  $4.5$  IU/m<sup>2</sup> ( $1.0$  to  $1.5$  mg/m<sup>2</sup>) resulted in a doubling of the height velocity during the first year. Increasing the dosage after the first year ( $3.0$  to  $4.5$  IU/m<sup>2</sup>) reduced the waning growth effect. Growth and final height prognosis improved during 4 years of GH therapy. This was better with  $4.5$  IU/m<sup>2</sup> than with  $3$  IU/m<sup>2</sup>.

The eighth presentation, a report from European collaborators, dealt with long-term response to rhGH treatment in Turner syndrome (OR 46-8). One hundred ninety patients were studied. The Europeans concluded that a 5.0-cm increment (corrected) was added with GH treatment, with wide individual variation. A significant discussion ensued regarding the effect and necessity of beginning GH therapy earlier than the  $\geq 9$  years of age (average) for patients in the reported study.

Robert M. Blizzard, MD

1. Woods KA, et al. Department of Endocrinology, St. Bartholomew's Hospital Medical College, London.
2. Maheshwari H, et al. Department of Medicine & Pediatrics, Northwestern University Medical School, Chicago.
3. Fiallo RV, et al. Department of Pediatrics, New York Hospital-Cornell Medical Center, New York.
4. Laron Z, et al. Department of Pediatric Endocrinology, Tel Aviv University.
5. Blethen SL, et al. Department of Pediatrics, SUNY, Stony Brook, New York.
6. Brasel JA, et al. Harbor UCLA Medical Center, Torrance, California.
7. Mombarg LTM, et al. Department of Pediatrics, University Hospital, Leiden.
8. Van der Boeck J, et al. Department of Pediatrics, Academic Hospital, Leiden.

#### Abstracts From the Literature

### Short Stature Caused by a Mutant Growth Hormone

The authors studied a 4.9-year-old boy with short stature (height, -6.1 SD below mean for age and sex) whose growth was normal in utero. Basal and stimulated secretion of immunoreactive growth hormone (GH) was normal but levels of bioactive GH were subnormal. He responded to the administration of GH with an increase in growth rate. Isoelectric focusing was performed, and 2 GH peaks were detected in the proband in comparison to 1 peak in normal subjects. Examination of the *GH-1* gene revealed a heterozygous mutation (guanine to cytosine transversion) of 1 GH gene allele at codon 77 in exon 4, with substitution of cysteine for arginine. This mutation is near a controlling point for the binding of GH to its receptor. Thus, the patient had 2 species of GH, 1 wild-type and 1 mutated form. A similar heterozygous mutation was found in the father, who was of normal height but who had 1 serum GH peak by isoelectric focusing. Further analysis of the mutated GH expressed in *Escherichia coli* revealed that it had normal immunoreactivity compared with wild-type GH, but that it bound to the extracellular domain of the GH receptor (ie, the GH-binding protein) 6-fold more avidly than did native GH. Since this mutated GH did not stimulate intracellular signaling pathways in IM-9 cells, which have GH receptors, it inhibited the biologic effects of wild-type GH in this system. The investigators suggest that the mutated form of GH impaired growth by antagonizing the effects of the native GH molecule, which was also synthesized and secreted by the patient. The reason why

the father with the same heterozygous mutation in the *GH-1* gene did not express this abnormal allele was unexplained.

Takahashi Y, et al. *N Engl J Med* 1996;334:432-436.

**Editor's comment:** In the last few months there has been great interest in children with idiopathic short stature (ISS). Partial GH insensitivity among patients with ISS was described earlier (*J Pediatr* 1995;127:244-250, published in abstract form in *GGH Vol 11*[4]:8). This was followed by the description of specific mutations of the GH receptor gene associated with ISS (*N Engl J Med* 1995;333:1093-1098, published in abstract form in *GGH Vol 12*[1]:14 & 15). Now, Takahashi et al describe a mutation in the GH gene itself that produced an abnormal GH and was clinically associated with short stature in the affected individual.

These papers provide data heralding a new subset of patients in whom GH gene mutations or GH receptor abnormalities explain the bioinactivity of GH. The prevalence of these abnormalities in children with ISS is unknown. Moreover, the features that clinicians should follow to identify GH insensitivity or bioinactivity also are not clarified. The diagnosis of these conditions continues to be based upon esoteric, highly sophisticated biochemical assessments.

Fima Lifshitz, MD



**2nd Editor's comment:** Long sought but heretofore undiscovered, the elusive bioinactive GH molecule has now been identified in 1 patient. Previously, many patients have been suspected of having a biologically inactive, but immunologically active, GH (Kowarski et al, 1978; Valenti et al, 1985; Hayek et al, 1978; Bright et al, 1983; and others). However, with the technology of the 1970s and 1980s, it was not possible to prove that such a syndrome exists. Isoelectric focusing, studies of gene structure, and the interest and expertise of these authors have made

the suspected syndrome a fact. They clearly demonstrated that the proband synthesized and secreted an atypical form of GH that was able to bind with high affinity to the extracellular domain of the GH receptor, was unable to initiate signal transduction, and inhibited the biologic effects of native GH. The failure of the father with the same heterozygous mutation to express this phenotype demands further consideration.

Allen W. Root, MD

## Effects of Recombinant Human Growth Hormone (rhGH) Treatment in Intrauterine Growth-Retarded Preterm Newborn Infants on Growth, Body Composition and Energy Expenditure

The investigators from Amsterdam administered rhGH (1.0 IU/kg or 0.33 mg/kg/d) to 7 preterm infants (mean gestational age, 30.4 weeks) with IUGR (mean birth weight, 938 g) beginning at 7 days of age and continuing until achieved weight was 2,000 g (34 to 68 days of treatment). When compared with an untreated control group of IUGR preterm infants, there was no effect of rhGH on: time to doubling of birthweight; increments in body weight, length, and head circumference; ponderal index; serum glucose or insulin values; skin-fold thicknesses; total body water; or energy expenditure. The authors concluded that administration of rhGH had no effect on growth or energy metabolism in preterm infants with IUGR.

van Toledo-Eppinga L, et al. *Acta Paediatr* 1996;85:476-481.

**Editor's comment:** Despite some problems with the applicability to IUGR preterm infants of the utilized methodology, the data are of interest because they indicate that even exceedingly high doses of rhGH cannot positively affect the growth or metabolism of such children. Whether such therapy can have adverse effects is unknown at present. The findings also indicate the need to search for growth factors other than rhGH (perhaps insulin-like growth factor 2, insulin, etc) that may be of benefit to IUGR preterm infants. The utility of rhGH in preterm infants with appropriate growth for gestational age has yet to be assessed.

Allen W. Root, MD

I. Wollmann H, et al. *Acta Paediatr* 1996;85:398-400.

## Prenatal Diagnosis of 45,X/46,XX Mosaicism and 45,X: Implications for Postnatal Outcome

Prenatal diagnosis of chromosomal abnormalities is available for families who have an option whether to continue the pregnancy. Twelve patients with 45,X/46,XX mosaicism were diagnosed prenatally by amniocentesis and subsequently evaluated at 3 months to 10 years of age. All have had normal linear growth. Four had anomalies, including esotropia and ptosis (1); labial fusion (1); atrial septal defect (1); and urogenital sinus, dysplastic kidneys, and hydrometrocolpos (1). The patient with ophthalmologic abnormalities is mentally delayed. None would have warranted karyotyping for clinical suspicion of Turner syndrome. These 12 were compared with 41 45,X/46,XX patients diagnosed postnatally. The prevalence of 45,X/46,XX mosaicism is 10-fold higher among amniocenteses than in series of postnatally diagnosed individuals with Turner Syndrome, which suggests that most individuals with this karyotype escape detection and that an ascertainment bias exists toward those with clinically evident abnormalities. The authors note that the phenomenon of a milder phenotype for the prenatal group is similar to that observed for 45,X/46,XY individuals diagnosed prenatally. The

authors emphasize that prenatal counseling for 45,X/46,XX in the absence of such ultrasound abnormalities as hydrops fetalis should take into account the expectation of a milder phenotype than that of patients ascertained postnatally. The same does not hold true for 45,X diagnosed prenatally.

Koeberl DD, et al. *Am J Hum Genet* 1995;57:661-666.

**Editor's comment:** This presentation provides important information for genetic counseling. One should not be too discouraging when discussing the expectations of a fetus when a 45,X/46,XX karyotype or a 45,X/46,XY karyotype is reported. Many of these 45,X/46,XX children will be phenotypically normal and possibly may end up with a 46,XX karyotype. The 45,X cell line may sometimes disappear, although that was not investigated in this study. This comment is made because the prevalence of 45,X/46,XX mosaicism is 10-fold higher among amniocenteses data than in series of postnatally diagnosed individuals with Turner syndrome.

Judith G. Hall, MD

## Behavioral Phenotypes in Dysmorphic Syndromes

Syndromes with congenital anomalies usually are diagnosed by their physical features or a particular combination of features that are observed on clinical grounds. Recently, objective means of defining and measuring behavior such as specific patterns of speech and language, types of attention deficits, particular social impairments, and other behavioral disturbances such as self-injury, skin scratching, and lip biting have been developed. These even have been quantified. Thus, it now becomes possible to define the specific behavior phenotypes in a number of syndromes. For instance, mimicking is common in Down syndrome. A discrepancy between performance and verbal skill also is typical of Turner syndrome. Impaired speech and language development often is found in Klinefelter syndrome. Learning and language difficulties, impaired social relations, and crimes against property often are found in individuals with the XYY syndrome. In the fragile X mental retardation syndrome, visuo-spatial skills are impaired such that there is difficulty in climbing stairs and problem solving for sequential events. Autistic and ritualistic disturbances also are frequent. In tuberous sclerosis, autism, hyperactivity, and hypersarhythmic salaam attacks are seen. In Williams syndrome, superior vocal skills are observed, resulting in "cocktail party" chatter. Patients with this syndrome also have hyperacusis. In Prader-Willi syndrome, hypotonia, hyperphagia, and tantrums are typical; and in Angelman syndrome, a happy disposition with paroxysmal laughter and a jerky ataxic gait usually are seen. In Rett syndrome, loss of mental abilities together with hand-wringing are seen. In Sotos

syndrome, hyperactivity, clumsiness, and poorly articulated speech are observed. The authors urge better description of behavior in future clinical reports.

Turk J, Hill P. *Clin Dysmorphol* 1995;4:105-115.

**Editor's comment:** *It is clear that defining the behavior seen in syndromes will help to make specific diagnoses. The opportunity to record movement and behavior using video cameras now exists. Just as with physical features, it is sometimes hard to describe accurately types of movements and various facial expressions. Recording and studying behavior will be important for the future in order to delineate the mechanisms involved in a particular disorder.*

*Because of the lack of specificity and quantification in the past, it was often hard to describe the behavioral characteristics found in a specific syndrome. Abnormal respiration is another type of behavior, and is characteristic in the Jooebert syndrome. Our ability to define behavioral characteristics will increase with time. I expect there will be many "behavior" syndromes with normal physical features—after all, half the human genes have to do with the brain.*

*In addition, the authors have very thoroughly reviewed the historical and behavioral perspectives of various syndromes. Geneticists, pediatric endocrinologists, psychologists, and nurses dealing with syndromes should benefit significantly by reading the complete article.*

Judith G. Hall, MD

## Autoantibodies to the Extracellular Domain of the Calcium Sensing Receptor in Patients With Acquired Hypoparathyroidism

The autoimmune pathogenesis of acquired hypoparathyroidism has been difficult to document with certainty. Earlier studies reported the presence in sera from patients with acquired hypoparathyroidism of antibodies to parathyroid tissue identified by indirect immunofluorescence. Antibodies have also been observed that inhibit the secretion of parathyroid hormone, or that are cytotoxic to parathyroid cells, but such studies have been difficult to replicate. The present investigators hypothesized that patients with this disorder may have antibodies to the G protein-associated calcium sensing receptor, which is expressed on the cell membrane of parathyroid cells.

In preliminary studies, antibodies to extracts of human parathyroid glands were detected by immunoblot analysis in only 5 of 25 (20%) patients with acquired hypoparathyroidism. Since the antigen appeared to be of the same size as the calcium sensing receptor (120 to 140 kd), further studies utilizing the membrane calcium sensing receptor expressed in transfected cells were undertaken. In 8 of 25 (32%) patients (which included all those previously positive by immunoblot

analysis of human parathyroid tissue), antibodies to this receptor were detected by immunoblot. When the calcium sensing receptor was differentially expressed as its extracellular domain and as its transmembrane-intracellular domain, 14 of 25 (56%) patients demonstrated antibodies to the extracellular portion of the receptor and none to the transmembrane-intracellular domain. Patients with both idiopathic acquired hypoparathyroidism as well as those with type I autoimmune polyglandular syndrome demonstrated antibodies to this segment of the calcium sensing receptor. In none of 50 patients with a variety of other autoimmune diseases or in normal controls were antibodies to this antigen detected.

The authors concluded that many patients with acquired hypoparathyroidism have antibodies to the extracellular portion of the calcium sensing receptor. They speculate that some patients in whom these antibodies were not detected may have had the disease for prolonged periods, leading to loss of the autoantigen needed for stimulation.

Li Y, et al. *J Clin Invest* 1996;97:910-914.

**Editor's comment:** This report adds further data supporting the autoimmune etiology of acquired hypoparathyroidism in the majority of patients. The relationship of antibodies to the extracellular domain of the calcium sensing receptor in relation to the etiopathogenesis of acquired hypoparathyroidism is uncertain. In preliminary studies, the authors report that these antisera did not affect intracellular calcium levels *in vitro* in cells transfected with this receptor. These data indicate the need to study further the biologic activity of these antibodies and to search for other antigens that may be of pathophysiologic importance in this disorder.

Allen W. Root, MD

**2nd Editor's comment:** Exactly 30 years ago, Walter David, Darwin Chee, and I first reported the presence of parathyroid

antibodies in the sera of hypoparathyroid patients (Clin Exp Immunol 1966;1:119), as Li et al pointed out in their excellent article. Neufeld, Maclaren, and I then pursued over 15 years the theory that acquired hypoparathyroidism often was of autoimmune origin, but we and others had great difficulty in confirming our hypothesis in the laboratory. Li, Maclaren, and colleagues now have confirmed that autoantibodies exist against a specific component of the parathyroid cells. Observing this unraveling of questions and the near solving of the hypothesis over 30 years has been exciting and rewarding to me, and one of the pleasures and blessings of being given the opportunity to live and continue to be professionally active over such an extended period.

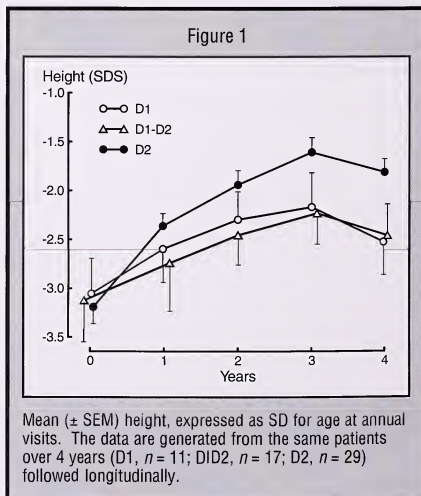
Robert M. Blizzard, MD

## Follow-Up of Three Years of Treatment With Growth Hormone and of One Post-Treatment Year, in Children With Severe Growth Retardation of Intrauterine Onset

Job et al report follow-up data on their original randomized double-blind study of 2 doses of growth hormone (GH)—(0.4 IU/kg/wk (dose D1) or 1.2 IU/kg/wk (dose D2) (*J Clin Endocrinol Metab* 1994;78:1454-1460)—in prepubertal children with very short stature of intrauterine onset. Previously, they reported that growth velocity increased in intrauterine growth retarded (IUGR) children treated with GH in a dose-dependent manner. At the end of 2 years of GH treatment, subjects receiving the low dose of GH (D1) were randomized either to continue the same dose or be switched to the higher dose (D1D2) and treated for an additional year. Finally, a follow-up year of no GH treatment was added to their study. Seventy-eight subjects were studied. Both birth length and birth weight had to be  $-2$  SD or more below the mean for gestational age; height at admission had to be  $-2$  SD or more below the mean according to the usual French standards; bone age had to be either retarded or equal to the chronologic age; and the growth velocity for the previous 12 months could not exceed the mean for age. In addition, all patients had to be prepubertal. Height and sexual development were assessed every 3 months during GH treatment and every 6 months during the posttreatment year at 10 different centers in France and Belgium. In addition to careful height and weight measurements and assessment of sexual development, bone age was determined by the method of Gruelich and Pyle every 6 months. Sixty-six children remained in the study at follow-up.

Average age at the onset of the study in 1988 was  $8.1 \pm 0.2$  years. The mean annual height velocities were greatest during the initial year of GH treatment and subsequently declined. At the end of 3 years of treatment, the height reached  $-2.37$  SD in D1,  $-2.17$  in D1D2, and  $-1.58$  in D2 (Figure 1). The total height gain was  $0.77 \pm 0.1$  SD in D1,  $0.93 \pm 0.15$  SD in D1D2, and  $1.61 \pm 0.08$  SD in D2. The percentage of children whose height was within the normal range for age was 46.7% in D1, 52.2% in D1D2, and 70% in D2.

During the follow-up year without treatment, growth deceleration was observed in most patients, with mean growth velocity falling below  $-1$  SD. The mean loss in height was approximately 0.25 SD for age. Skeletal maturation over 36 months of GH treatment was not significantly different among the 3 groups. Mean bone age, however, remained retarded in all 3 groups at the end of the fourth year of study. There were no significant differences among the 3 groups in the frequency of occurrence of puberty or in age at its onset; the rate of sexual maturation after its onset did not differ among the groups.





The authors state that their data confirm that GH treatment can accelerate the growth of IUGR short children beyond 2 years of treatment despite "the waning effect" of GH and that this growth is accompanied by some degree of acceleration in bone maturation. The authors note that the strengths of their study include: (1) the cohort, which excluded familial short stature but did include 6 cases of Silver-Russell-type dwarfism, was homogeneous; (2) puberty began within the normal age range; and (3) they included the growth velocity after discontinuation of GH treatment. There are no data, however, on final heights.

Job JC, et al. *Pediatr Res* 1996;39:354-359.

**Editor's comment:** This is a very interesting paper. Job et al have performed an evaluation of long-term use of GH in IUGR short children. It would be of interest to have more

information with regard to the range of bone age retardation in the patients when initially seen. With mean heights at the end of the study averaging from  $-1.8 \pm 0.2$  SD to  $-2.5 \pm 0.4$  SD and bone age being delayed approximately 1 year or more, it is unclear whether a significant number of these children also have constitutional delay of growth and adolescence. In addition, the inclusion of children with Russell-Silver syndrome may have adversely affected the growth response data. However, the authors are to be congratulated in carrying out such a long-term study and including a year of follow-up. It would have been interesting to have included a control population of similarly height-challenged IUGR patients who were not treated and were of similar age. We would hope Job and colleagues will continue their studies and report final heights in these patients in the next few years.

William L. Clarke, MD

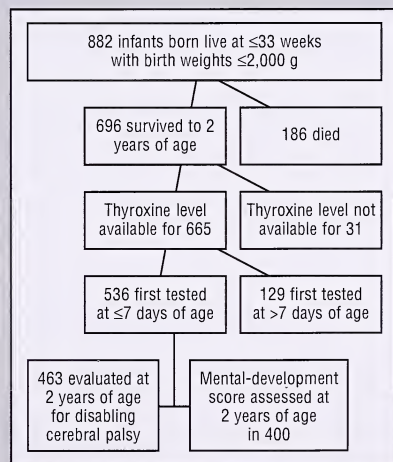
## The Relation of Transient Hypothyroxinemia in Preterm Infants to Neurologic Development at Two Years of Age

Taking advantage of the prospective design of the Central New Jersey Neonatal Brain Hemorrhage Study, this retrospective study was performed in a historical cohort. The authors chose those infants who were born at 33 weeks of gestation or earlier, who had undergone screening for congenital hypothyroidism within the first 7 days of life, and who survived until the age of 2 years or beyond ( $n=536$ ; Figure 1). The levels of thyroxine were retrieved from the newborn screening program and were expressed as a SD score (SDS) to correct for the daily interassay variation. Severe hypothyroxinemia was defined as a blood thyroxine value more than 2.6 SD below the mean for New Jersey newborns. None of the infants had congenital hypothyroidism.

Neurologic and developmental outcomes were assessed at 2 years of age by means of the Bayley Psychomotor Developmental Index and the Bayley Mental Developmental Index or the Stanford-Binet Intelligence Scales for Children. Emphasis was placed on the presence of disabling cerebral palsy and/or low mental developmental scores. Twenty-two prenatal, perinatal, and early neonatal variables were analyzed in order to adjust for any association between hypothyroxinemia and a given neurodevelopmental outcome. Infants with severe hypothyroxinemia had a risk of disabling cerebral palsy that, depending on the extent of adjustment for covariates, was 4.4 to 17.6 times that of the infants with normal thyroxine concentrations. The mental development scores at 2 years of age were 8 to 18 points lower in infants who had had severe hypothyroxinemia than in those with normal thyroxine levels. The authors conclude that severe hypothyroxinemia in preterm infants may be an important cause of problems in neurologic and mental development detected by 2 years of age.

Reuss ML, et al. *N Engl J Med* 1996;334:821-827.

Figure 1  
Enrollment and Assessment of the  
Study Subjects



The study enrolled 882 infants born at or before 33 weeks of gestation who were therefore at risk for hypothyroxinemia. The infants were drawn from the 1,105 newborns with birth weights of 2,000 g or less who were enrolled in the population-based Central New Jersey Neonatal Brain Hemorrhage Study.



**Editor's comment:** This paper is very important as it provides data indicating that transient hypothyroxinemia without hyperthyrotropinemia in preterm infants is not benign. The study by Reuss et al rings a bell of alarm and prompts us to approach these infants more carefully. The mechanism of transient hypothyroxinemia in preterm infants, however, has not been elucidated. It may involve either a metabolic adaptation to nonthyroidal illness or an incomplete maturation of the hypothalamic-pituitary-thyroid axis as discussed by Vulsma and Kok<sup>1</sup> in the editorial comments that accompanied the paper.

The traditional belief that the fetus does not need thyroxine for intrauterine development was based on the assumption that negligible amounts of thyroxine from the mother crossed the placenta. This belief was first challenged when significant passage of thyroxine from the mother to the fetus was identified, and it is now being challenged again with the findings of Reuss et al of poor mental and developmental outcomes of preterm infants displaying transient hypothyroxinemia. Treatment of these infants aiming to correct the subnormal levels of thyroxine as soon as detected will be the next step, but this should be undertaken only in a controlled study.

Fima Lifshitz, MD

1. Vulsma T, Kok JH. *N Engl J Med* 1996;334:857-858.

**2nd Editor's comment:** Having just reviewed this abstract and Dr. Lifshitz's editorial comment, I attended The Endocrine Society meeting in San Francisco and read an abstract by Dr. M.K. Hunter et al entitled, (Program, 10th IC of Endocrinology, Vol II: June 14 and 15, 1996, OR 48-4, page 7113). Follow-up of Newborns With Low T<sub>4</sub> and Non-Elevated TSH Concentrations. The content of the abstract was related to the article by Reuss et al. Therefore, the important comments and data follow:

Over a 20-year period, the Northwest Regional Screening Program screened 1,747,805 newborn infants. Follow-up of infants with low thyroxine levels without thyrotropin (TSH) elevation led to the diagnosis of hypothyroidism in 60, including 25 infants with delay in TSH rise (1:67,226 infants), 9 infants with mild hypothyroidism (TSH <25 IU/L), and 26 infants with hypopituitary hypothyroidism (1:67,223), in addition to 4,334 infants with thyroid-binding globulin deficiency (1:4,027). Follow-up was scheduled at 1 year of age.

These data indicate that follow-up of infants (preterm or term) with low thyroxine and normal TSH levels is important.

Robert M. Blizzard, MD

## Insulin-Like Growth Factor Binding Protein-3 Generation: An Index of Growth Hormone (GH) Insensitivity

Eleven children with possible growth hormone (GH) insensitivity (GHI) and 8 children with proven GH deficiency (GHD) were studied with an insulin-like growth factor (IGF) generation test in which IGF-1, IGFBP-3, and GHBP were measured before starting a 4-day course of subcutaneous GH at 0.1 U/kg/d, and 12 hours after the last GH injection. GHI was defined based on short stature for target height (-2 SD for mid-parental height), a high basal GH (>10 mU/L), and/or high peak GH (>40 mU/L) on a standard GH provocation test. GHD was defined based on a peak GH response to arginine ≤10 mU/L. The 2 groups were comparable in terms of their age, body mass index, height, and growth velocity. The change in these parameters was analyzed as an absolute increment, as an increment in SDS, and as a percentage change. None of the children fulfilled the Pharmacia Study Group IGF generation test criteria for the diagnosis of Laron syndrome; ie, IGF-1 increment <15 µg/L and IGFBP-3 increment <0.4 mg/L. The results of ΔIGF-1 and ΔGHBP in the generation test did not show statistical differences (ie, could not discriminate) between the GHI and the GHD patients regardless of whether the results were analyzed as absolute change, as percentage increment, or SDS increment. However, the results of IGFBP-3 showed statistical differences between the 2 groups of patients when comparing the poststimulation peaks by increment as well as the percentage increments. Significant *inverse*

correlations were found between peak GH obtained during provocative tests. Both the IGF-1 and IGFBP-3 rose in the IGF generation test. Percentage increase of IGFBP-3 was identified as the most significant parameter to predict GH peak by stepwise multiple regression analysis.

The authors speculate that some children may have selective resistance in either the GH-IGF-1 axis or the GH-IGFBP-3 axis, given the varied combination of responses found in the study. IGF-1 generation per se was inadequate as an index of partial GHI and should be used in conjunction with IGFBP-3 generation.

Thalange NKS, et al. *Pediatr Res* 1996;39:849-855

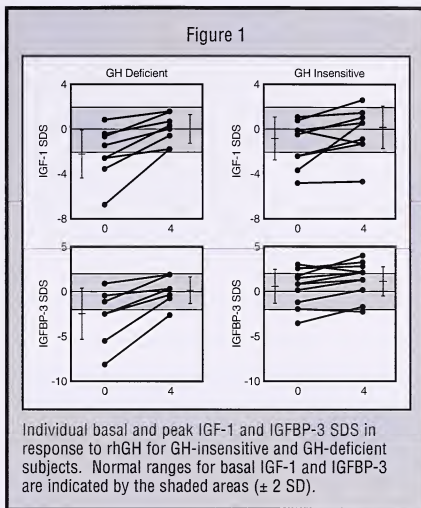
**Editor's comment:** Bioinactive GH secondary to mutations of the GH gene<sup>1</sup> and GH receptor mutations associated with clinical pictures of partial GHI<sup>2,3</sup> (see GGH vols 11:4 and 12:1, respectively) have been recently identified. Although these newly described and documented entities definitely are helping us understand the different pathophysiologic mechanisms of short stature, accurate diagnosis can be made only with sophisticated technology. Their clinical recognition continues to be elusive. This paper by Thalange et al attempts to identify easily available biochemical markers in the context of dynamic testing, which may yield a diagnostic clue in identifying patients

with GHI. Although their results support the IGFBP-3 generation test as a suitable tool to include in the diagnostic approach, its degree of uncertainty is still considerable. The number of patients included in this report is small, and much heterogeneity was found in the basal levels of both IGF-1 and IGFBP-3. Selective blocks of IGF-1 versus IGFBP-3 generations have been proposed to explain some differences. This concept permits the suggestion that post-receptor defects currently unexplored may constitute another etiologic category of short children identified as having idiopathic short stature.

Fima Lifshitz, MD

1. Takahashi Y, et al. *N Engl J Med* 1996;334:432-436.
2. Attie KM, et al. *J Pediatr* 1995;127:244-250.
3. Goddard AD, et al. *N Engl J Med* 1995;333:1093-1098.

**2nd Editor's comment:** The authors are reputable investigators who have attempted to clarify the confusion that exists about GHI. As so often in the past when trying to elucidate the presence of partial GHD, their work has not provided us with a tool to clinically detect partial GHI. For one or many reasons, a majority of the GHD and GHI patients did not have expected biochemical baseline results, and the responses to rhGH were very variable (Figure 1). This study would have been enhanced if the purported GHI individuals had been limited to nondysmorphic, idiopathically short persons (4 of the alleged GHI patients had syndromes); if bone ages had been included in the auxologic data to permit readers to better formulate their own impressions regarding the inclusion of short children in the GHI category; and if the patients with sexual development were specified with respect to their stage of pubertal development. Regardless of the deficiencies in the



study and/or presentation, the authors are to be commended for their attempt to solve a complex problem: how to diagnose partial GHI.

The authors have been invited to respond to these remarks, which are intended to be constructive, by writing a letter to the Editor of GROWTH, Genetics & Hormones for publication in a future issue.

Robert M. Blizzard, MD

## Testicular and Ovarian Resistance to Luteinizing Hormone Caused by Inactivating Mutations of the Luteinizing Hormone Receptor Gene

The investigators report 2 families with different homozygous mutations of the luteinizing hormone receptor (LHR) gene. Both mutations lead to inactivation of the LHR but each produces a different phenotype. In the first family, 3 phenotypically female siblings, 15, 23, and 32 years of age, had XY karyotypes; absence of thelarche but normal pubarche; inguinal gonads; and absence of müllerian duct structures. A cytosine to thymine transition was identified at nucleotide 1660, leading to substitution at amino acid 554 of a stop codon (TGA) for arginine (CGA) within the third intracytoplasmic loop of the LHR. Thus, a truncated and nonfunctional LHR resulted. The same mutation was present in a 22-year-old XX sibling who had normal secondary sexual development, 1 episode of vaginal bleeding, and then prolonged amenorrhea. She had a small uterus, cystic ovaries, and elevated luteinizing hormone (LH) but normal follicle-stimulating hormone (FSH) levels.

In the second family, a male child with micropenis had a cytosine to adenine transversion at nucleotide 1847, leading to alteration of amino acid 616 from serine (TCT) to tyrosine (TAT) within the seventh transmembrane domain of the LHR. There was no testosterone secretory response to human chorionic gonadotropin, but normal adrenocortical response to corticotropin. Expression of the mutated form of the LHR in COS-7 cells revealed that it did not bind LH or transmit an intracellular signal.

Latronico AC, et al. *N Engl J Med* 1996;334:507-512.

**Editor's comment:** The association of a homozygous inactivating mutation of the LHR with male pseudohermaphroditism has been anticipated and, indeed, previously reported<sup>1</sup> and abstracted in GROWTH, Genetics & Hormones 1996;12(2):24.

*Of interest is the phenotype of an XX individual who is homozygous for the same mutation. This woman had secondary amenorrhea but no other obvious clinical manifestation of this mutation. This observation is instructive because it indicates that (1) a functional LHR is not necessary for pubertal ovarian function; (2) normal female puberty through menarche can be guided by FSH alone; (3) adrenal androgens alone are sufficient for normal sexual hair growth in the female (thus confirming other data); and (4) the heterozygous loss of 1 functional LHR is of no clinical or reproductive consequence. (The parents of the affected children were not studied but had 14 children.)*

*A mutation in the seventh transmembrane domain of the LHR in the child in family 2 prevents movement from the endoplasmic reticulum to the plasma membrane surface and thus binding of LH to its receptor. Since the affected subject with this defect had micropenis rather than ambiguous genitalia, presumably functional LHR was expressed on the fetal Leydig cell membrane in the first trimester of gestation, but not thereafter. (See GROWTH, Genetics & Hormones 1996;12[2]:24—2nd editor's comment.)*

Allen W. Root, MD

1. Kremer H, et al. *Nature Genet* 1995;9:160-164.

## Teratogen Update: Diethylstilbestrol

Diethylstilbestrol (DES) teratogenicity occurred over a period of about 3 decades when it was used to avert miscarriage. Female fetuses who had a significant exposure to DES and other synthetic estrogens (now collectively referred to as DES) are now known to be at risk for carcinogenic and teratogenic effects. DES-exposed daughters have an increased risk for developing clear cell adenocarcinomas of the vagina and cervix and structural abnormalities of the genital tract that predispose to vaginal adenosis and other vaginal epithelial changes. Some male fetuses exposed to DES have structural abnormalities of the genital tract, but as yet no increase in cancer has been reported. Fertility and sexual function in these men appear to be normal. Girls exposed in utero to DES also have a somewhat higher risk of breast cancer than women who were not exposed. There is no evidence that grandchildren of DES-exposed daughters and sons have any abnormalities. It would appear that the epidemic of clear cell adenocarcinoma is over. It is not entirely clear whether there may be problems in intrauterine DES-exposed individuals who now are over the age of 50. Carcinomas developed in only a small proportion of this population. It appears that the mechanism by which DES caused these problems has to do with interfering with the "natural regression" of certain tissues in embryonic and fetal life.

Mittendorf R. Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. *Teratology* 1995;51:435-445.

Wilcox AJ, et al. Fertility in men exposed prenatally to diethylstilbestrol. *N Engl J Med* 1995;332:1411-1416.

**Editor's comment:** *These papers are helpful for reassuring at-risk individuals. The sad part of the whole DES story is that there was no beneficial effect in maintaining pregnancies and, consequently, a large number of children were exposed unnecessarily to DES. We must remind ourselves to be sure before prescribing a therapeutic agent that there is in fact a demonstrated therapeutic effect. We then must weigh the potential positive effect against the possible negative effects. Today we would like to think that clinical studies ensure that all therapies actually do what they are meant to do. However, possible long-term adverse effects are hard to predict, and a judicious approach to any therapy is obligatory under the Hippocratic oath to do no harm. Fortunately, future research funded by National Cancer Institute will permit monitoring of the DES-exposed population to determine whether any other abnormalities become apparent.*

*Those who wish to read a very complete and extensive review of the DES story are referred to Mittendorf's article. Wilcox's article is more limited in scope, as it is confined to findings in males; nevertheless, it is an important report.*

Judith G. Hall, MD

## Teratogenicity of High Vitamin A Intake

In general, vitamins are thought to be essential for embryogenesis and necessary for health in the fetus, infant, child, and adult. However, fat-soluble vitamins have been recognized to cause toxicity and, potentially, teratogenicity when taken in large doses. Vitamin A is available in many forms as part of supplementary vitamin capsules. It also is present in the diet, coming from certain vegetables and animal sources, including dairy products, liver, and fortified foods. Currently,

the recommended daily allowance of vitamin A for women is 800 retinol equivalents, which corresponds to 2,700 IU. Vitamin A has been found to be teratogenic in humans, and recently there has been an epidemic of teratogenicity because of isotretinoin used to treat severe acne. The malformations that can be seen in retinoic acid embryopathy include craniofacial, cardiac, thymic, and central nervous system abnormalities (Table 1).



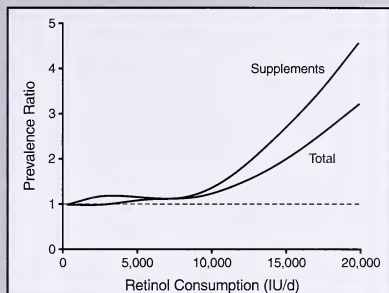
Table 1  
Birth Defects According to Category With  
Retinoic Acid Embryopathy

| Type of Defect   | No. |
|--|-----|
| Cranial neural crest   |     |
| Craniofacial, central nervous system<br>(except neural tube), and thymic | 69  |
| Heart  | 52  |
| Total  | 121 |
| Neural tube  | 48  |
| Musculoskeletal and Urogenital   |     |
| Musculoskeletal  | 58  |
| Urogenital   | 42  |
| Total  | 100 |
| Other  |     |
| Gastrointestinal   | 24  |
| Nongastrointestinal  | 46  |
| Total  | 70  |
| Total  | 339 |

Rothman et al have interviewed 22,748 women concerning their diet and illnesses during the first trimester of pregnancy. All sources of retinol intake were tabulated and an association was made with various types of birth defects. There is a major concern regarding supplementary vitamin A but not the beta carotene of the dietary form of vitamin A. A relationship was found between high vitamin A consumption during early pregnancy and the occurrence of a variety of birth defects. The data appeared to indicate a teratogenic effect of vitamin A intake not far above the currently recommended dose. Consuming more than 10,000 IU per day was found to be associated with an increased incidence of birth defects when the high levels of vitamin A were taken before the seventh week of gestation (Figure 1). It was estimated that 1.4% of the women in the study averaged more than 10,000 IU of vitamin A per day.

Table 1 and Figure 1 reprinted by permission of *The New England Journal of Medicine*; Rothman K J, et al. *N Engl J Med* 1995;333: 1369-1373.

Figure 1  
Retinol Consumption (IU/d)



Estimated prevalence ratio for birth defects related to the cranial neural crest, according to retinol intake during the first trimester of pregnancy.

**Editor's comment:** This finding is of great concern because the general public thinks that vitamins are benign and if "a little is good, a lot is better." The study points out there is a fine line between enough and too much. Of particular concern are the additive effects of multivitamins, prenatal vitamins, and fortified foods. Care should be taken by pregnant women or women who wish to become pregnant to limit vitamin A supplementation. In view of the fact that we wish pregnant women to be sure to take sufficient folic acid prior to becoming pregnant and in early pregnancy, the situation can be confusing. It is quite clear that vitamin A can be teratogenic and can be related to other problems besides the classic picture of retinoic acid embryopathy.

Judith G. Hall, MD

## Growth and Physical Outcome of Children Conceived by in Vitro Fertilization

The authors report the status at 2 years of age of 289 Australian children from Victoria who were conceived by in vitro fertilization (IVF). The birth weights of singleton IVF and naturally conceived control infants were similar (IVF: 3,196 g; control: 3,294 g), while the birth weights and gestational ages of IVF twins were slightly greater than those of control twins (IVF: 2,297 g, 35.0 weeks; control: 2,053 g, 33.7 weeks). At 2 years of age, the weight and head circumference percentiles of the entire group of IVF and control children were similar (IVF: 56.3 g; 63.4 cm, respectively; control: 56.2 g; 65.7 cm, respectively). Length percentile of the IVF children was significantly ( $P=0.004$ ) greater than that of the control children (57.7 cm versus 49.9 cm), the reason for which was not apparent. There was no significant difference between

IVF and naturally conceived children with respect to: congenital malformations, subsequent hospitalizations and operations, or neurologic status. The investigators concluded that IVF had no adverse effect on growth, general health, and development at 2 years of age.

Saunders K, et al. *Pediatrics* 1996;97:688-692.

**Editor's comment:** More than 34,000 children have been delivered by assisted reproductive techniques. It is encouraging to note that these interventional methods have produced predominantly normal offspring.

Allen W. Root, MD



Specification of Pituitary Cell Lineages by the Lim Homeobox Gene *Lhx3*

*Lhx3* is a mouse LIM homeobox gene (one associated with morphologic development) that is expressed in the pituitary, hindbrain, spinal cord, and pineal gland. In order to determine the role of this gene in pituitary differentiation, the authors established a model in which this gene has been disrupted and rendered inactive, ie, "knocked out." Animals heterozygous for this mutation are normal and fertile. Although animals homozygous (*Lhx3*<sup>-/-</sup>) for this recessive mutation are of normal size at birth, they are either stillborn or die within 24 hours, possibly because of abnormalities of respiratory control or adrenocortical function. In *Lhx3*<sup>-/-</sup> animals, the pituitary gland fails to form and differentiate properly. Thus, by embryonic day 10.5, the mutant Rathke's pouch differed from the intact, wild-type animal; its opening to the oral cavity was wider and its lining multilayered. As development progressed, Rathke's pouch failed to pinch off from the oral cavity or to develop

the histologic appearance of the normal anterior pituitary. The transcription factor *Pit-1* failed to appear in the mutant pituitary anlagen, and thus development of somatotropes, lactotropes, and thyrotropes was disrupted, as was synthesis of growth hormone, prolactin, and  $\beta$ -thyrotropin. In addition, the gonadotropes failed to differentiate in the homozygous (*Lhx3*<sup>-/-</sup>) mutant animals. Pro-opiomelanocortin (POMC) was detectable in the hypothalamus (the floor of diencephalon) and in a few cells of the mutant pituitary, suggesting that primary corticotrope differentiation was not dependent on *Lhx3*. Inasmuch as proliferation of corticotropes was limited in the mutant animals, this gene may be necessary for further development of this cell line. The authors conclude that *Lhx3* is a critical homeobox gene for anterior pituitary development.

Sheng HZ, et al. *Science* 1996;272:1004-1007.

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**Editor's comment:** These investigators elegantly describe the consequences of a homozygous mutation in *Lhx3* in mice. The human counterpart of this disorder awaits detection, but may be found in patients with pharyngeal pituitaries. Another step in the genetic control of pituitary formation and differentiation has now been identified.

*Lhx3* maintains expression of *Rpx*, a homeobox gene necessary for the early stages of pituitary development, and regulates the expression of *Pit-1*, which in turn stimulates the differentiation of a common cellular precursor into thyrotropes and somatomammotropes, the latter further differentiate into somatotropes under the direction of growth hormone-releasing hormone.

Allan W. Root, MD

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# GROWTH

## Genetics & Hormones

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### Recombinant Human Growth Hormone Therapy for Children With Chronic Renal Insufficiency: An Update 1996

**Richard N. Fine, MD**

*Professor and Chairman*

*Department of Pediatrics*

*State University of New York at Stony Brook*

*Stony Brook, New York*

Growth retardation as a clinical consequence of uremia, or chronic renal insufficiency (CRI), was identified in the 19th century.<sup>1</sup> Minimal medical attention was devoted to this facet of CRI until the emergence of the dual therapeutic modalities of dialysis and renal transplantation in the 1960s to prolong the lives and effect rehabilitation in a previously uniformly fatal disease process.

Vigorous investigative efforts indicated that the etiology of the growth retardation in children with CRI was multifactorial: (1) age at onset; (2) primary renal disease; (3) fluid and electrolyte abnormalities, especially acidosis; (4) renal osteodystrophy; (5) inadequate caloric intake; and (6) perturbations of growth factors.<sup>2</sup> The last factor has gained increasing significance since the seminal demonstration by Mehls et al.<sup>3</sup> in the early 1980s that rhGH improved the growth velocity of growth-retarded uremic rats.

#### **CME CERTIFICATION:**

##### **An Important Change Coming for GGH**

With the coming of the new year, we have an exciting announcement regarding a change in the status of *GGH*! Beginning with Volume 13, Number 1, *GGH* will be designated by the University of Virginia School of Medicine as a continuing medical education activity certified for Category 1 credit of the Physician's Recognition Award of the American Medical Association. We hope that many of you will find this change will be of value and interest.

Initial clinical studies in children with CRI (glomerular filtration rate between 5 and 75 mL/min/1.73 m<sup>2</sup>) demonstrating the salutary effect of rhGH in improving growth velocity were published in the late 1980s.<sup>4,5</sup> These were followed by short-term (6 months)<sup>6</sup> and long-term (2 years) multicenter, randomized, placebo-controlled studies that validated the safety and efficacy of rhGH in children with CRI. The latter study led the Food and Drug Administration (FDA) to approve, in 1994, the use of rhGH for children with CRI and end-stage renal disease (ESRD) (glomerular filtration rate <5 mL/min/1.73 m<sup>2</sup> and/or the clinical need to initiate dialysis) prior to renal transplantation.

This review will focus on the following pertinent issues regarding the use of rhGH in this patient population: (1) What is the long-term (>5 years) outcome of rhGH use in children with CRI? (2) Is rhGH safe and effective in infants and very young children (<2½ years) with CRI? (3) What is the optimal approach to the clinical management of patients who reach their target height (50th centile for midparental height) while receiving rhGH? (4) Does rhGH accentuate the glucose intolerance associated with CRI? (5) Are there osseous complications associated with the use of rhGH in children with CRI? (6) What factors are responsible for suboptimal responses to rhGH? (7) Should growth-retarded children undergoing dialysis receive rhGH? (8) What is the mechanism for the beneficial effect of rhGH in this patient population?

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## LONG-TERM (>5 YEARS) RESULTS

Of the 11 patients included in the initial pilot study, 6 were treated for >5 years and 1 patient is still receiving rhGH after >8 years (Figure 1).<sup>4,5</sup> The magnitude of improvement in growth velocity was not sustained at the same level that was obtained during the initial year of rhGH treatment; however, continued improvement in standardized height was noted during long-term treatment (Figure 2).

Twenty patients (aged 0.7 to 11.3 years) in the multicenter, randomized, placebo-controlled study have been treated with rhGH for >5 years.<sup>7</sup> Growth velocity following initiation of rhGH treatment waned with succeeding years of therapy (Figure 3); however, as with the patients in the pilot study, long-term treatment was associated with a continued beneficial effect on standardized height.

The only significant side effect associated with long-term rhGH treatment was one case of avascular necrosis (AVN). Although there was a decline in the mean renal function during the 5 years of rhGH treatment, it is apparent that there was no acceleration in the magnitude of decline as a consequence of rhGH treatment (Figure 4).

Therefore, the data to date indicate a continued salutary effect of long-term rhGH treatment in children with CRI with minimal adverse events and/or side effects.

Figure 1  
Recombinant Human Growth Hormone Treatment in Chronic Renal Failure

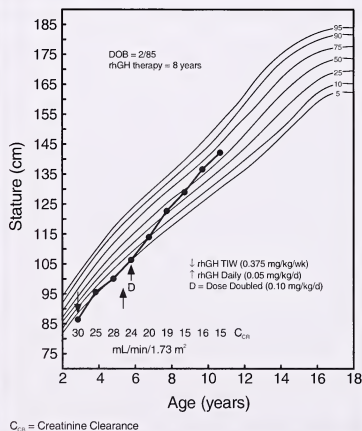
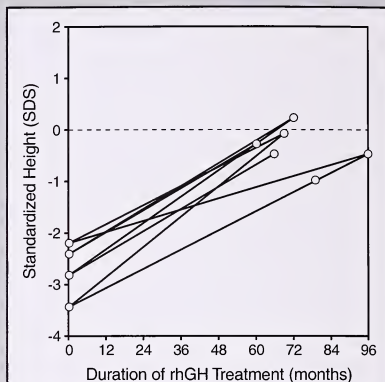


Figure 2  
Long-Term (>5 Years) Recombinant Human Growth Hormone Treatment in Chronic Renal Insufficiency: Change in Standardized Height



## TREATMENT OF INFANTS (<2 ½ YEARS OF AGE)

Of the 125 patients with CRI included in the multicenter, randomized, placebo-controlled study,<sup>6</sup> 30 (24%) were <2½ years of age at the initiation of rhGH treatment.<sup>8</sup> The first-year growth rate was  $14.1 \pm 2.6$  cm/y in the rhGH-treated group ( $n=19$ ) compared with  $9.3 \pm 1.5$  cm/y in the placebo-treated group ( $n=11$ ,  $P<0.00005$ ). Significant improvement in growth velocity in the rhGH-treated group compared with the placebo-treated group persisted during the second year of the study, albeit to a lesser magnitude ( $P<0.025$ ).

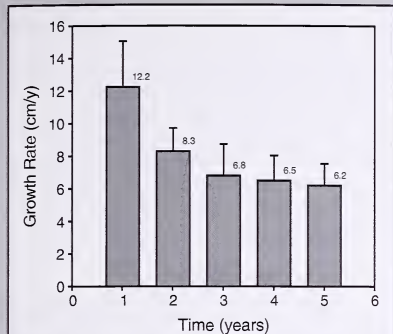
At 2 years, the height SDS was  $+2.0 \pm 0.7$  in the rhGH-treated group compared with  $-0.2 \pm 1.1$  in the placebo-treated group ( $P<0.00005$ ). No specific adverse events were identified in these young patients receiving rhGH.

These data are consistent with the interpretation that rhGH is safe and effective in young children <2½ years of age with growth retardation secondary to CRI. The youngest patient included in this study was 0.7 years.

It is imperative that potential contributing factors to growth retardation in infants with CRI be corrected before the initiation of rhGH in this age group. Fluid and electrolyte imbalances and acidosis should be normalized; optimal nutritional intake should be ensured with the potential reliance upon nasogastric



Figure 3  
Recombinant Human Growth Hormone  
Treatment in Chronic Renal Insufficiency:  
5 Year Annual Growth Rate



tube feeding; and renal osteodystrophy should be minimized prior to initiating rhGH treatment.

#### TREATMENT OPTIONS ONCE TARGET HEIGHT IS REACHED

Once the target height (50th percentile for midparental height) is reached, there are potentially 3 options available to sustain optimal standardized height (target height): (1) continue administration of rhGH at the current dosage schedule; (2) discontinue rhGH and observe for fluctuation in standardized height, reinstituting rhGH if the standardized height falls below an arbitrary level (<25th centile for midparental height); and (3) continue administration of rhGH at an arbitrary lower dosage.

Data from the multicenter study<sup>9</sup> would tend to support the second option. Twenty-two children were "paused," ie, rhGH was temporarily discontinued, because the patients' target height was reached. Six of the 22 children remained paused at the time of the report for a mean  $\pm$  SD duration of  $25.5 \pm 26.9$  months; however, 16 of the 22 children required resumption of rhGH after pausing for a mean  $\pm$  SD of  $9.0 \pm 4.6$  months because of a reduction in standardized height. During the pause, the latter group of patients grew only a mean  $\pm$  SD of  $2.7 \pm 1.7$  cm/y, following resumption of rhGH, the growth velocity increased to  $7.2 \pm 1.7$  cm/y. Although 73% (16 of 22) of children who were paused following attainment of target height required reinstitution of rhGH, it appears that discontinuation of rhGH once target height is reached is the optimal

strategy, since 27% of the children had sufficient subsequent growth velocity to sustain their target height without additional rhGH therapy.

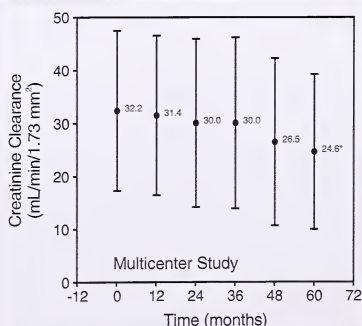
#### GLUCOSE INTOLERANCE

In the long-term (>5 years) follow-up of 20 growth-retarded children with CRI treated with rhGH in the multicenter study,<sup>7</sup> there was no significant change in the mean fasting glucose or 2 hour postprandial glucose values compared with baseline at any time interval following initiation of rhGH treatment.

The fasting plasma insulin levels were significantly increased compared with baseline levels at each interval following initiation of rhGH treatment. Similarly, the 2 hour postprandial plasma insulin levels were significantly increased compared with baseline levels at 24, 48, and 60 months of rhGH treatment. The mean  $\pm$  SD HbA<sub>1c</sub> level was  $5.3 \pm 0.9\%$  at baseline and  $6.1 \pm 0.9\%$  at 5 years ( $P=0.0003$ ). To date, there have been no clinical consequences associated with the hyperinsulinemia in patients with CRI treated with rhGH.

Saenger et al<sup>10</sup> evaluated carbohydrate metabolism by fasting and postprandial glucose, insulin, and HbA<sub>1c</sub> levels in patients with either CRI, GHD, Turner syndrome, or ISS who were treated with rhGH for 5 years. Some of the children with CRI were probably included in the long-term multicenter study report. Mean fasting and postprandial glucose values remained unchanged throughout the 5-year term in all 4 groups. Mean fasting and postprandial

Figure 4  
Recombinant Human Growth Hormone  
Treatment in Chronic Renal Insufficiency:  
5 Year Annual Creatinine Clearance Data



\* $P=0.04$  compared with baseline



insulin values rose yet remained within the normal range at 5 years. The mean HbA<sub>1c</sub> levels in the CRI patients were slightly elevated to 6.3% at 5 years. This comparative study indicated that carbohydrate metabolism was not adversely impacted by rhGH in CRI patients compared with other groups of patients treated with rhGH.

## OSSEOUS COMPLICATIONS

Data from the multicenter study indicated no significant difference in radiographic osteodystrophy scores or in serum calcium, phosphorus, or parathyroid hormone (PTH) levels between the rhGH and control groups.<sup>11</sup> However, the increment in growth velocity in response to rhGH treatment was blunted in the presence of secondary hyperparathyroidism.<sup>12</sup>

Slipped capital femoral epiphyses (SCFE) were noted in 2 of the 125 patients included in the multicenter study; 1 child was treated with rhGH for 3 years and 1 for only 3 months at the time of diagnosis. Both patients had severe renal osteodystrophy.

Watkins et al<sup>13</sup> noted AVN in 6 of 17 children with CRI receiving rhGH; 3 had AVN prior to rhGH treatment and 3 had no previous radiographs. Similarly, Mehls et al<sup>14</sup> reported 2 cases of AVN in 103 prepubertal patients with CRI treated with rhGH. Boechat et al<sup>15</sup> reviewed the radiographs of 205 children included in multicenter studies of rhGH therapy in CRI and detected 15 cases of AVN; 8 had AVN prior to rhGH and 7 had no prior radiographs.

Consequently, the risk of SCFE and/or AVN in children with CRI receiving rhGH remains equivocal. Nonetheless, it is prudent to obtain radiographs of the osseous structures prior to initiating rhGH in children with CRI and to repeat the radiologic studies if clinical symptoms ensue.

Similarly, it is advisable to correct the radiologic and/or serologic abnormalities of renal osteodystrophy prior to initiating rhGH treatment. Persistent renal osteodystrophy may blunt the response to rhGH.

## SUBOPTIMAL RESPONSE TO rhGH

There are few data detailing the incidence of suboptimal responses to rhGH in children with CRI during the initial year of treatment. Almost without exception all previous reports have noted a decline in the growth velocity during the second and all succeeding years of rhGH treatment. Despite this decline, continued improvement in standardized height (Figure 2, page 50) has occurred.

Potential causes of suboptimal responses either initially or subsequently include the following: (1) failure to correct other contributory causes of growth retardation in children with CRI, ie acidosis, fluid and

electrolyte abnormalities, and inadequate caloric intake; (2) persistent renal osteodystrophy, as evidenced by an inverse correlation between PTH level and growth velocity; (3) level of renal functional impairment, ie, ESRD patients may have a decreased response compared with CRI patients; and (4) noncompliance. Serial measurements of IGF-1 may be helpful in detecting the latter. Failure to demonstrate an increase in the IGF-1 level in response to presumed rhGH administration may indicate noncompliance.

In a few instances, upward adjustment of the rhGH dosage has proven effective in improving growth velocity.<sup>16</sup> However, prior to contemplating adjustments in dosage, it is imperative that the other potential causes of suboptimal response be investigated and corrected.

## rhGH IN DIALYSIS PATIENTS

There are limited data detailing the use of rhGH in growth-retarded children undergoing dialysis. Unfortunately, no controlled studies have been undertaken; however, Phase I studies have uniformly demonstrated a significant improvement in growth velocity during the initial year of rhGH treatment compared with that obtained during the year prior to treatment.<sup>14,17,18</sup> These studies primarily included patients undergoing peritoneal dialysis.

During the second year of rhGH treatment, growth velocity diminished substantially to a level comparable to that seen during the year prior to treatment.<sup>14,18</sup> Reasons for this marked reduction in response were not apparent; however, it has been suggested that progressive renal osteodystrophy, persistent acidosis, reductions in residual renal function, and noncompliance may all be contributory.

Limited data are available utilizing rhGH in children undergoing hemodialysis.<sup>19</sup> Comparison of

### In Future Issues

#### Status of and Indications for Leg Lengthening Procedures

Deborah Stanitski, MD

#### The Neuroendocrinology of Stress in Its Relation to Alterations in Growth

George Chrousos, MD

#### The Pathophysiology of Growth Failure in Renal Disease

David Powell, MD

#### Update Article Pertaining to the Genes of Growth Factors and Hormones

Victor McKusick, MD

results obtained in children undergoing hemodialysis, continuous ambulatory peritoneal dialysis (CAPD), or automated peritoneal dialysis (APD) seem to indicate similar efficacy.

At least one report has indicated that the response to rhGH in children undergoing dialysis is less than that achieved in children with CRI.<sup>20</sup> However, others have not substantiated this finding.<sup>6</sup>

The lack of uniform, long-term, continued improvement of growth velocity and/or standardized height in children undergoing dialysis who are receiving rhGH has led to questioning of the justification for rhGH treatment in the pediatric dialysis population. This is an important issue since approximately 50% of the pediatric patients undergoing dialysis in North America have a SDS exceeding -1.88.<sup>21</sup>

### MECHANISM OF rhGH EFFICACY IN CRI

The current hypothesis proposes that rhGH increases the level of bioavailable (free) IGF-1, which subsequently stimulates bone growth. Endogenous growth hormone (GH) levels are elevated in CRI primarily as a result of reduced renal clearance, since GH secretory rates are normal.<sup>22</sup> Despite elevated GH levels, IGF-1 secretory rate by the liver is reduced, possibly as a result of a reduction in the number of hepatic GH receptors. This is reflected by a reduction in GH-binding protein levels in uremia. Furthermore, IGFBP levels, primarily IGFBP-2 and possibly IGFBP-3, are elevated, which reduces the levels of available free IGF. rhGH increases the IGF levels to a greater extent than it increases the IGFBP levels, thereby effectively increasing levels of free IGF to enhance bone growth.

### SUMMARY

1. Long-term (>5 years) rhGH treatment in children with CRI produces sustained improvement in standardized height.
2. rhGH treatment of infants (<2½ years of age) with CRI is as effective at improving growth velocity as it is in older children with CRI.
3. Once target height (50th centile for midparental height) is reached, the optimal approach is to pause rhGH treatment and observe the patient. If standardized height declines significantly, ie, -2 SDs, rhGH is effective when reinitiated.
4. Neither short-term nor long-term rhGH treatment in children with CRI adversely impacts on glucose tolerance.
5. The presence of renal osteodystrophy may blunt the impact of rhGH and increase the risk of SCFE and/or AVN in children with CRI. Pretreatment

radiologic evaluation and radiologic surveillance of clinical symptoms is indicated.

6. Suboptimal response to rhGH in children with CRI is rare and may indicate the need for upward dosage adjustment. However, prior to this, potential causes of growth failure in CRI should be corrected.
7. rhGH is effective during the initial year of treatment. Growth velocity may be blunted during subsequent years of treatment. The precise mechanism of this blunting has not been delineated.
8. rhGH has been shown to improve growth velocity in patients undergoing either peritoneal dialysis or hemodialysis; however, long-term data are lacking and the response may be less than that achieved in patients with CRI.
9. rhGH is probably effective in infants, children, and adolescents with CRI because it increases the levels of bioavailable (free) IGF.

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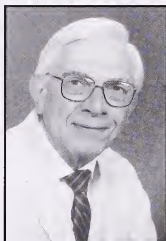
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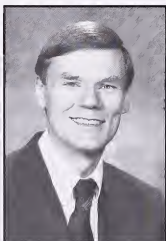
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**William A. Horton, MD**, is Director of the Research Unit at the Portland Shriners Hospital and Professor of Molecular and Medical Genetics at Oregon Health Sciences University. Before moving to Portland in 1993, he was head of medical genetics at the University of Texas Medical School in Houston for 10 years. He has enjoyed a long-standing interest in normal and abnormal skeletal development and growth, from both the clinical and laboratory perspectives, and has published more than 200 papers in these fields. Recently, he has focused his attention largely on studies of the molecular pathogenesis of human chondrodysplasias and has used experimental models such as genetically engineered mice. He has served on the Editorial Board of *GROWTH, Genetics, & Hormones* since 1989.





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**Allen W. Root, MD**, is Professor of Pediatrics (1973-Present) and of Biochemistry and Molecular Biology (1986-Present) at the University of South Florida College of Medicine in Tampa. His clinical and research interests have been broadly based and include disorders of growth, sexual differentiation and maturation, mineral metabolism, and thyroid function. In addition to *GROWTH, Genetics & Hormones*, Dr. Root has served as a member of the editorial boards for the *Journal of Pediatrics*; *Journal of Clinical Endocrinology and Metabolism*; *Journal of Pediatric Endocrinology and Metabolism*; *Journal of Adolescent Health Care*; *Adolescent and Pediatric Gynecology*; and *Pediatrics in Review*. Dr. Root has served on the Editorial Board of *GROWTH, Genetics, & Hormones* since 1992.



#### Abstracts From the Literature

### Nonsense Mutation in the Human Growth Hormone-Releasing Hormone Receptor Causes Growth Failure Analogous to the Little (*lit*) Mouse

Growth hormone-releasing hormone (GHRH) was identified in 1982 as a potent stimulus for GH-secretion. The synthesis and secretion of GH in the anterior pituitary is regulated by the hypothalamus through GHRH and somatostatin. GHRH stimulates the secretion of GH while somatostatin inhibits its secretion. These hormones bind to the GHRH receptors (GHRHRs) and control the synthesis and secretion of GH.

Wajnrajch and coworkers have identified a nonsense mutation within the GHRHR in a family with proportionate short stature. Although the clinical and laboratory features resulting from this mutation mimic those of GH-deficient individuals, these patients are capable of making GH but not releasing it. Their short stature is due to a nonfunctioning GHRHR caused by the mutation. This mutation is responsible for unresponsiveness to exogenous GHRH and consequent growth failure. The *GHRHR* gene was identified in 1992 and was mapped to chromosome 6 in mice. Human *GHRHR* shows strong sequence homology to the murine gene. In humans, the gene is mapped to chromosome 7p15.

The authors reported nonsense mutations in the *GHRHR* gene in 2 members of a consanguineous family. Mutations of *GHRHR* gene must be considered as a cause for clinical features suggestive of GH deficiency in cases in which the GH gene (*GH1*) itself is normal. The mutation is a Glu72Stop mutation and its position is close to the *lit* mutation (Asp60Gly) identified in 1992 and mapped to chromosome 6 in mice. The mutation occurs in the same highly conserved region of the extracellular domain.

In this report, Wajnrajch et al have identified a nonsense mutation in the GHRHR in humans for the first time. The Glu72Stop mutation produces a severely truncated GHRHR that lacks the G-protein sites. This produces a disruption of hormonal signals. Patients with this mutation respond to exogenous GH but not to GHRH. The identification of this mutation causing GHRH dysfunction suggests that both the GH-releasing peptide (not GHRH) and nonpeptidyl benzazapines might be useful therapeutic agents in these disorders because both stimulate GH release independent of the GHRHR.

The GHRHR may play a role in prolactin synthesis in the mouse, as evidenced by reduced levels in the *lit* mouse. However, the baseline and thyroid-releasing hormone-stimulated prolactin levels were tested and were normal in this family.

Receptor-activating mutations also should be looked for in GH excess diseases such as acromegaly. Activating mutations in the stimulatory G-protein  $\alpha$  subunit, to which the GHRHR is functionally coupled, is seen in some acromegalic patients. Activating mutations in related G-protein coupled receptors occur in human diseases, including one in the LH-R in one type of male precocious puberty (testotoxicosis), one in the TSH-R in congenital persistent thyrotoxicosis, one in the PTH-R in metaphyseal chondrodysplasia, and one in the calcium-sensing receptor in dominant hypocalcemia.

Wajnrajch MP et al. *Nature Genet* 1996;12:88-90.  
Mayo KE. *Nature Genet* 1996;12:8-9.



**Editor's comment:** The discovery of mutations such as reported here, which help to define metabolic pathways, are very satisfying. In the future, additional mutations will enhance our knowledge regarding the diagnosis and treatment of syndromes with hormonal deficiency and excess.

Growth problems and short stature are a common pediatric problem. Mutation of intermediate processing steps such as GHRH binding do exist. It is as yet unclear how common this problem is, but it must be considered in all apparent GH-deficient children who do not respond to GHRH with GH release.

Judith G. Hall, MD

**2nd Editor's comment:** A second report has already been made.<sup>1</sup> A cluster of severe dwarfism has been described in

Pakistan. A total of 18 dwarfs was discovered in a kindred with high consanguinity. Inheritance is autosomal recessive, and the dwarfism severe (114 to 136 cm). Biochemical and endocrine evaluation was consistent with isolated GH deficiency (no GH response to GHRH, clonidine, L-dopa, or TRH). IGF-1 was extremely low (<10 ng/mL), as was IGFBP-3. Both responded well to GH. The GHRHR locus on chromosome 7p15 was highly linked to the dwarfism phenotype. It appears that this form of dwarfism is caused by an inactivating mutation in the GHRHR gene, and that this entity represents a human homologue of the little (lit/lit) mouse.

Robert M. Blizzard, MD

1. Maheshwari H, et al. *The Endocrine Society Program Book*. 1996; Abstract OR46-2:709.

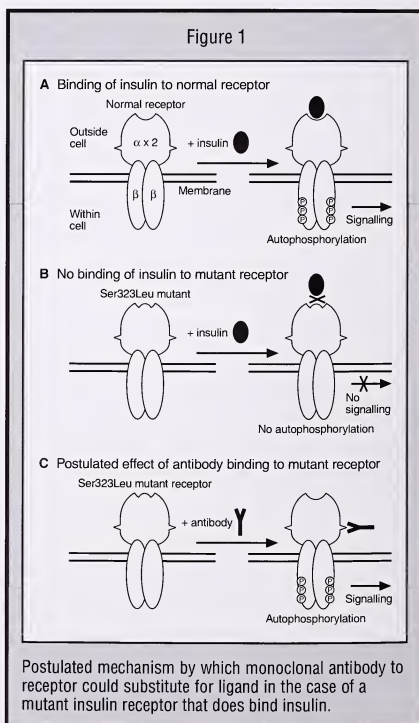
## Functional Activation of Mutant Human Insulin Receptor by Monoclonal Antibody

The investigators have identified a mutation (Ser323Leu) in the extracellular, ligand-binding domain of the insulin receptor that resulted in decreased binding of insulin and consequently severe insulin resistance (Rabson-Mendenhall syndrome). Although biologically inert, this mutant receptor is normally inserted into the insulin target cell membrane. The authors generated a monoclonal antibody to sequence 485-592 of the extracellular domain of the insulin receptor. They demonstrated that this antibody bound to and induced autophosphorylation not only in wild-type insulin receptor but also in the mutant insulin receptor expressed in Chinese hamster ovary cells. Cells transfected with the wild-type and mutant insulin receptors were also able to synthesize glycogen in response to this antibody. The authors suggest that it may be possible to treat patients with this form of insulin receptor defect with a stimulatory monoclonal insulin receptor antibody or to design drugs that bypass the defective ligand binding site (Figure 1).

Krook A, et al. *Lancet* 1996;347:1586-1590.

**Editor's comment:** Many genetic defects in cell membrane receptors lead to impaired synthesis, extreme shortening or abnormal folding of the translated protein, and hence failure of its insertion into the cell membrane. However, in those hormone resistance syndromes in which the receptor defect involves the extracellular domain and permits its translocation into the cell membrane, generation of receptor-stimulating antibodies may present a significant therapeutic option. In patients with insulin-resistant diabetes mellitus, IGF-1 has been utilized with success. However, concerns remain about the long-term consequences of the administration of this potent growth factor.

Allen W. Root, MD



## Putting the Brakes on Bone Growth

A fascinating story is emerging regarding the local control of linear bone growth. It has long been recognized that chondrocytes in skeletal growth plates progress through a complex differentiation process that involves proliferation and terminal differentiation (hypertrophy). Moreover, although a number of hormones and growth factors, most notably GH and IGF-1, are known to influence this progression, the local controls have remained poorly understood. Now, papers from 3 Boston research teams have defined a local negative feedback loop that serves as a brake on this process, controlling the rate of terminal chondrocyte differentiation (see Figure 1).

The feedback loop is simple; the proof of its existence was much more difficult. The loop has 2 major players: a signaling molecule called Indian hedgehog (Ihh) and parathyroid hormone-related protein (PTHrP). Ihh is 1 of at least 3 hedgehog proteins found in higher vertebrates that function as signal proteins, especially during early embryologic development. Hedgehog signals are thought to act through a receptor known as Patched (Ptc) and a transcription factor named Gli.

Through an elaborate series of experiments in developing chick limb buds and mouse embryos in which relevant genes were overexpressed and/or inactivated, the authors were able to determine the upstream and downstream relationships of loop components. First, they demonstrated that Ihh was produced by growth plate chondrocytes when they begin to terminally differentiate and that overexpression of Ihh suppressed terminal differentiation. Next, they showed that Ptc receptor and Gli transcription factor were expressed in perichondrial cells around the periphery of the growth plate.

Related experiments showed that PTHrP is synthesized by periarticular perichondrial cells and that PTHrP receptor, which is also a receptor for PTH, is expressed by proliferating chondrocytes just prior to terminal differentiation. Genetic inactivation of the receptor was associated with accelerated

chondrocyte terminal differentiation. Finally, the loop was closed when Ihh suppression of terminal differentiation was shown to depend on PTHrP.

The proposed model is shown in Figure 1. Briefly, as growth plate chondrocytes decide to terminally differentiate, they express high levels of PTHrP receptor. Once committed to this fate, they transiently express Ihh, which acts on the adjacent perichondrial cells through Ptc and Gli to directly or indirectly cause periarticular perichondrial cells to secrete PTHrP. PTHrP signals back to proliferating chondrocytes expressing PTHrP receptors, preventing them from progressing down the terminal differentiation pathway. Thus, the loop functions as a brake on terminal differentiation, essentially controlling the number of cells terminally differentiating at any given time.

Roush W. *Science*; 1996;273:579.

Vortkamp A, et al. *Science* 1996;273:613-622.

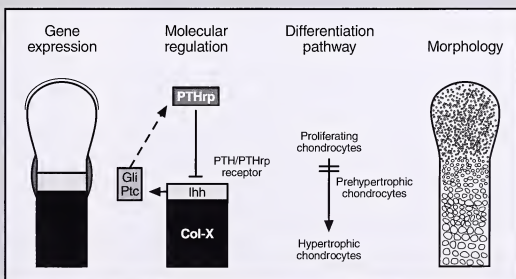
Lanske B, et al. *Science* 1996;273:663-666.

**Editor's comment:** This work provides a new context in which to consider control of bone growth. The greatest uncertainty is how Ihh signals to periarticular perichondrial cells that secrete PTHrP. Nevertheless, that PTHrP is required for Ihh inhibition of chondrocyte terminal differentiation is hard to dispute. Given the long distances in this model that PTHrP must diffuse through cartilage matrix, a recognized barrier to diffusion of many molecules—especially in larger bones such as those in humans—it is difficult to imagine how this feedback loop would be responsible for the fine-tuning of subtle events in the growth plate. As the authors imply, perhaps this loop is one of several locally acting mechanisms that control skeletal development and growth.

William A. Horton, MD

Figure 1  
Proposed Regulation of Cartilage Differentiation During Bone Growth

Ordinarily, growth plate chondrocytes become hypertrophic chondrocytes, briefly passing through a prehypertrophic stage during which they sequentially express the PTHrP/PTHrP receptor and Ihh genes. The Ihh signal is transmitted to the perichondrium, where it elicits expression of another set of genes, Gli and Ptc, which leads to expression of PTHrP in the periarticular perichondrium. PTHrP then signals back to its receptor in the prehypertrophic cells to block progression of more cells down the hypertrophic chondrocyte pathway, ie, it closes the negative feedback loop. As chondrocytes fully hypertrophy, Ihh expression ceases, which releases the "brake" imposed by the negative loop, allowing more cells to enter the hypertrophic pathway.



Reprinted with permission from Vortkamp A, et al. Regulation of Rate of Cartilage Differentiation by Indian Hedgehog and PTH-Related Protein. *Science* 1996;273: 613-666.

## Skeletal Overgrowth and Deafness in Mice Lacking Fibroblast Growth Factor Receptor 3

Molecular defects in fibroblast growth factor 3 receptor (FGFR3) have been found in patients with achondroplasia, hypochondroplasia, thanatophoric dwarfism, and Crouzon syndrome—dysplasias that adversely affect formation of endochondral bones (long bones, base of the skull, vertebrae). In mouse embryos, the gene for FGFR3 (*Fgfr3*) is expressed not only in cartilage but also in glial cells of the brain and spinal cord and in the cochlea. Colvin et al developed mice homozygous for absence of expressed FGFR3 by engineering a truncated *Fgfr3* that lacked the coding regions for its extracellular and transmembrane domains. Although *Fgfr3*<sup>-/-</sup> mice survived gestation and birth, 48% died within 21 days after delivery; however, some lived as long as 8 months. Kinking of the tail, kyphosis, scoliosis, increased femoral and humeral length and curvature, and abnormal rib formation developed in >75% to 100% of *Fgfr3*<sup>-/-</sup> mice. Histologic examination of the cartilage growth plate of the long bones revealed enlargement (+33% to 50%) of the hypertrophic zone in *Fgfr3*<sup>-/-</sup> mice. The authors attributed the skeletal abnormalities in *Fgfr3*<sup>-/-</sup> mice to disordered cartilage cell growth, development, turnover, and replacement by endochondral ossification and concluded that FGFR3 regulates these processes. Because the morphologic and histologic findings in *Fgfr3*<sup>-/-</sup> mice are the converse of those seen in patients with achondroplasia, the investigators suggest that this disorder is the result of constitutive activation of FGFR3 due to the mutation (Gly380Arg) in its transmembrane domain. In addition to the skeletal deformities noted above, abnormalities of cochlear formation and hearing were present in *Fgfr3*<sup>-/-</sup> mice. In these animals, the organ of Corti failed to differentiate and progress from the neonatal state. Thus, FGFR3 is also necessary for normal development of the organ of Corti and hearing.

Colvin JS, et al. *Nature Genet* 1996;12:390-397.

**Editor's comment:** The data presented in this elegant paper indicate that FGFR3 affects cartilage formation, maturation, and endochondral bone formation by regulating the size of the hypertrophic zone of growth plate cartilage, its invasion by blood vessels preparatory to ossification, and the turnover of cartilage cells. In achondroplasia, proximal long bones of the extremities (humerus, femur) are shortened, and the height of the hypertrophic zone of the cartilage growth plate is decreased. These findings are opposite to those present in *Fgfr3*<sup>-/-</sup> mice. The authors' suggestion that achondroplasia is the result of constitutive activation of FGFR3 is supported by data reported by Webster and Donoghue<sup>1</sup> and Naski et al.<sup>2</sup> These investigators transfected cells in cultures with FGFR3 with the mutations present in patients with achondroplasia (Gly380Arg, in the transmembrane domain) and in subjects with thanatophoric dysplasia (Arg248Cys, in the extracellular domain, and Lys650Glu, in the second tyrosine kinase region of the intracellular domain). In the absence of ligand (FGF1), there was proliferation of the transfected cells and dimerization and autophosphorylation of the FGFR3, indicative of the constitutive activation of the mutated FGFR3. (Interestingly, the Gly380Arg mutation leads to cellular proliferation in transfected cells, but apparently decreased proliferation of chondrocytes in vivo. This discrepancy requires explanation.) One mutation of FGFR2 present in some patients with Crouzon syndrome results in its constitutive activation as well.<sup>3</sup>

Allen W. Root, MD

1. Webster MK, Donoghue DJ. *EMBO* 1996;15(3):520-527.

2. Naski MC, et al. *Nature Genet* 1996;13:233-237.

3. Neilson KM, Friesel RE. *J Biol Chem* 1995;270:26037-26040.

## Molecular Definition of Breakpoints Associated With Human Xq Isochromosomes: Implications for Mechanisms of Formation

An isochromosome is a type of chromosomal aberration in which one of the arms is duplicated and the other arm is deleted; both the arms have the same set of genes but in a reverse sequence. Isochromosome for Xq is the most common structural abnormality observed in Turner syndrome, which results in a duplication of the long arms of X. About 15% of Turner syndrome patients have an i(Xq) in mosaic or nonmosaic form.

Wolff et al have brought to light a new mechanism for isochromosome formation. They studied 11 i(Xq)s derived from Turner syndrome patients using molecular techniques and found that the isochromosomes are not usually due to misdivision of the centromere as previously thought (Figure 1). Instead, they are formed after Xp breakage and a U-type reunion event in the pericentromeric region. Using fluorescent in situ hybridization (FISH) techniques, they have localized the

breakpoints in the band Xp11.2. The data support the hypothesis that structurally dicentric i(Xq)s initially contain 2 functional centromeres, resulting in the loss of the i(Xq) in some cells during the early divisions of the zygote. According to this hypothesis, those cells that maintain the i(Xq) chromosome inactivate 1 of the centromeres, conferring stability.

Wolff DJ, et al. *Am J Hum Genet* 1996;58:154-160.

**Editor's comment:** This is a breakthrough in our understanding of the mechanisms of isochromosome formation and supports some previous studies. More studies are needed to find out whether other regions of breakpoints on the X chromosome and other mechanisms for isochromosome formation occur. Investigation defining whether the breakage follows



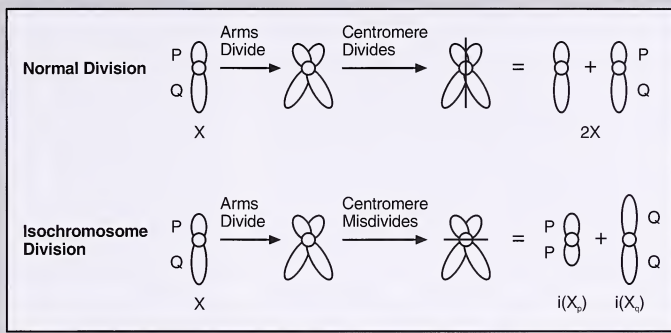
a particular nucleotide sequence, or is sequence dependent, also will help clarify X chromosomes that are predisposed to isochromosome formation. It also may help to determine whether certain X chromosomes are more predisposed to producing germ cells or zygotes with sex chromosome loss,

addition, or changes. The hypothesis cited above regarding inactivation of 1 centromere in a dicentric  $i(Xq)$  requires further study.

Judith G. Hall, MD

Figure 1  
Previous Concept

This concept may be outdated if report by Wolff et al is confirmed for all instances of  $i(X_c)$ .



## A Regression Method Including Chronological and Bone Age for Predicting Final Height in Turner's Syndrome (PTS), With a Comparison of Existing Methods

Van Teunenbroek et al present a new method for predicting final height (FH) in girls with Turner syndrome using either the Greulich and Pyle (GP) or Tanner and Whitehouse (TW) bone age determinations. The predicted final height in these Turner girls was either PTS by Greulich Pyle ( $PTS_{GP}$ ) or by TW using radius, ulna, and short bones ( $PTS_{RUS}$ ). To develop their regression equations, they utilized data from 57 Dutch women (235 measurements points). These women were born between 1934 and 1973 and, with the exception of estrogen, received no other growth-promoting agents. Criteria for the achievement of final height included: (1) a follow-up to at least age 20 years; or (2) a height velocity of  $<0.5$  cm over the previous year; or (3) a height velocity of  $<1$  cm over the previous 2 years and a bone age (TW) of at least 15 years of age. The PTS, which they developed, can be calculated as follows: FH (final height in centimeters) =  $a \times H$  (actual height) +  $b \times CA$  (chronologic age) +  $c \times BA$  (bone age) plus a constant. Smoothed regression coefficients and constants were created for chronologic ages 6 through 19 years for both the TW and GP systems. A prediction error was calculated to compare other prediction methods with this new equation. The mean prediction errors of both the  $PTS_{RUS}$  and the  $PTS_{GP}$  were small and similar except for the chronologic ages of 15 through 18 years. There was an overall tendency to over predict final height; however, the mean error of all final height predictions was less than for the Bailey-Pinneau (BP) methods.

**Editor's comment:** The authors point out the importance of having a single variable prediction method for FH in girls with Turner syndrome. In addition, they restate that BP and TW methods were developed from data on healthy children and included predictions of a pubertal growth spurt. Thus, these methods are not particularly useful in the prediction of FH in girls with Turner syndrome. Accurate FH predictions could be useful in deciding whether to initiate growth hormone therapy and in evaluating the effects of growth hormone and other anabolic agents on FH.

I agree with the authors' conclusions: "Of the single-variable FH prediction methods, the smallest mean prediction errors at most ages were observed using the modified PAH [projected height], with a good accuracy from the age of 9 years onwards. Averaging mPAH [modified PAH] with methods allowing for BA increased the accuracy of the more inaccurate method substantially. Thus, if population-specific Turner reference data are available, a number of calculations (with possible errors) can result in a smaller mean prediction error and a higher accuracy. On the other hand, the simplest methods—the mPAH and PAH—were remarkably good at most ages." This article should be read by all groups evaluating the effects of therapeutic agents on the ultimate heights of children.

Van Teunenbroek A, et al. *Acta Paediatr* 1996;85:413-420.

William L. Clarke, MD



## The Role of Proteoglycans in Overgrowth Syndrome

**Editor's comment:** The comment is presented before the abstract to alert the reader to the importance of the topic.

Beckwith-Wiedeman syndrome (BWS) is characterized by intrauterine overgrowth but normal adult stature, hemihyperplasia, and an increased incidence of a variety of embryonal tumors; Simpson-Golabi-Behmel syndrome (SGBS) is associated with prenatal and postnatal overgrowth, resulting in tall adult stature (often males reach >195 cm); cleft lip/palate; polydactyly; vertebral, rib, and sternal malformations; congenital heart disease; cryptorchidism; and hypospadias. The 2 syndromes overlap as both display macroglossia, omphalocele, and an increased incidence of Wilms' tumor. BWS is associated with overexpression of paternally imprinted IGF-2 (paternal heterodisomy or isodisomy, maternal deletion of 11p15.5).

Glypicans are proteoglycans containing complex sugar molecules, such as dermatan, chondroitin, and heparin sulfate, that are anchored to the exterior of the cell membrane through glycosylphosphatidylinositol links. Four molecules currently comprise the human glypican-related integral membrane proteoglycans (GRIPs) family. Glypican-3 may modulate the growth-promoting effects of IGF-2 by acting as a coreceptor with the IGF-2 (mannose-6-phosphate) receptor (Figure 1). In the absence of glypican-3, the growth-promoting effects of IGF-2 may be unregulated. It will be interesting to learn if abnormalities in GPC3 are found in other overgrowth syndromes such as cerebral gigantism.

Allen W. Root, MD

The investigators have identified deletions in the gene *GPC3* for a cell-surface proteoglycan termed glypican-3 in patients with SGBS. SGBS is an X-linked (Xq26) recessive overgrowth syndrome related to, but distinct from, BWS (see above). Glypican-3 is a 580 amino acid protein whose gene contains 8 or more exons. Studying female patients with SGBS and translocations between the long arm of the X chromosome and autosome 1 and 16, the authors identified the *GPC3* gene and its deletions at the translocation breakpoint. Gene sequence was 94% homologous with a previously identified rat cell-surface proteoglycan, thus permitting characterization of the *GPC3* product as a glypican. Utilizing *GPC3* probes, the investigators detected deletions of 1 to 3 exons in 3 families with male-limited SGBS. They did not detect gross exon deletions in 3 other families, suggesting that in their affected members more subtle mutations (point mutations) in *GPC3* may be present (or that another gene defect exists with a similar phenotype to that of SGBS). *GPC3* is expressed primarily during embryologic development in mesenchymal tissues (lung, kidney, liver) and not in brain or white blood cells. With an antiserum against glypican-3, the authors demonstrated that this proteoglycan associated with IGF-2 and its binding protein(s). They suggest that SGBS may be due to defective binding of IGF-2 by glypican, thus permitting IGF-2 to exert unrestrained growth-promoting effects during embryologic development and tumor formation in later life.

Pilia G, et al. *Nature Genet* 1996;12:241-247.

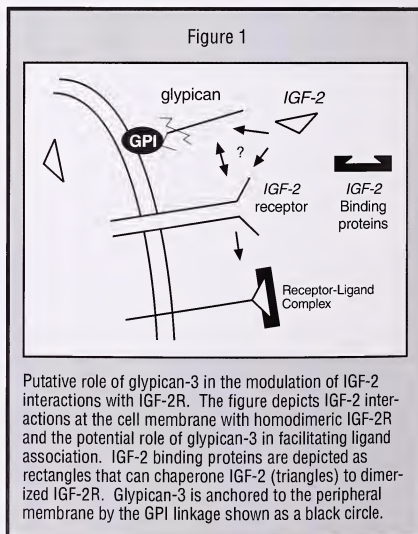
Weksberg R, Squire JA, Templeton DM. *Nature Genet* 1996;12:225-227.

**Second Editor's comment:** Several points are made in this article. First, diagnoses of rare syndromes are not always what they seem, or are said to be, even when registered in the NIGMS repository. Investigators who use cell lines from this repository should keep this in mind when studying cells from patients whose diagnoses are difficult to make and for which specific criteria evolve over time, as commonly occurs for rare dysmorphic syndromes.

Secondly, we are reminded to keep an open mind about molecules and their biologic functions. For example, proteoglycans were originally considered boring molecules that primarily occupied space in connective tissues. Now it appears that some proteoglycans may play important roles in ligand-receptor interactions of growth factors. Another example of this phenomenon involves another proteoglycan, heparin sulfate, which appears to be required for FGF ligands to bind their receptors.

Finally, the report demonstrates how several different disciplines can interact to advance the understanding of a process that may be an important regulator of growth. Indeed, this work could not have been completed without collaborations among dysmorphologists, endocrinologists, gene mappers, and molecular biologists.

William A. Horton, MD



## Mutations in the $\text{Ca}^{2+}$ -Sensing Receptor Gene Cause Autosomal Dominant and Sporadic Hypoparathyroidism

The 7-transmembrane, G-protein-associated,  $\text{Ca}^{2+}$ -sensing receptor gene is present on chromosome 3q. Abnormalities in this gene have been associated with familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia of varying severity. Baron et al have identified 3 mutations in this receptor: Gln681His (first extracellular loop) and Ala116Thr (amino terminal, extracellular domain) in 2 different families with autosomal dominant hypocalcemia and Phe806Ser (sixth transmembrane domain) in a third patient but with sporadic hypocalcemia. Symptoms varied from muscle cramping to neonatal seizures. All had hypercalciuria despite hypocalcemia, reflecting the role of the  $\text{Ca}^{2+}$ -sensing receptor in the modulation of renal calcium excretion. The authors point out that conventional treatment of this disorder with calcitriol with or without supplemental calcium may increase urinary calcium excretion. Thus, for optimal treatment of this disorder it may also be neces-

sary to administer an agent that lowers urine calcium excretion (a thiazide).

Baron J, et al. *Hum Mol Genet* 1996;5:601-606.

**Editor's comment:** The reader is referred to Dr. Shenker's article, Activating Mutations in G Protein-Coupled Signaling Pathways As a Cause of Endocrine Disease (GGH 1996;12[3]:33-38). These subjects are closely related. The reader also may wish to read a comprehensive review by Pearce and Brown concerning defects of the  $\text{Ca}^{2+}$ -sensing receptor (J Clin Endocrinol Metab 1996;81[6]:2030-2035).

The current report is of interest because of the severity of the hypocalcemic symptoms in some of these patients. In previous subjects, hypocalcemia has been modest and the patients often mildly symptomatic or asymptomatic.

Allen W. Root, MD

## Protein Turnover During Puberty in Normal Children

Arslanian and Kalhan performed leucine turnover studies in 20 prepubertal Tanner I and 21 pubertal Tanner II through IV nondiabetic children and adolescents. The aim of their study was to determine whether the insulin resistance of puberty involves protein metabolism. Leucine flux, oxidation, and nonoxidative disposal were measured during a primed constant infusion of [ $1\text{-}^{13}\text{C}$ ] leucine at baseline and during a stepwise hyperinsulinemic (10 and 40  $\text{mU/m}^2/\text{min}$ ) euglycemic clamp. Indirect calorimetry was performed as well. Breath samples were collected every 5 minutes for the analysis of  $\text{C}_{13}$  enrichment in the expired  $\text{CO}_2$ , and continuous indirect calorimetry by ventilated hood system was used to measure  $\text{CO}_2$  production and energy expenditure. During the hyperinsulinemic-euglycemic periods, the glucose was clamped at approximately 100  $\text{mg/dL}$ , and arterial blood was sampled every 10 to 15 minutes for determination of isotopic enrichment of plasma ketoisocaproate, amino acids, and insulin.

Fasting plasma glucose and insulin concentrations were similar in both groups, as were leucine and other branched-chain amino acids. Whole body leucine flux, an indicator of proteolysis, was lower in the pubertal versus prepubertal subjects. Similarly, leucine oxidation was lower in pubertal than prepubertal subjects, while nonoxidative leucine disposal (an indicator of protein synthesis) did not differ between the 2 groups. There were no gender-related differences in leucine kinetics. Resting energy expenditure correlated positively with leucine turnover, oxidation, and nonoxidative disposal.

IGF-1 correlated negatively with whole body leucine flux and nonoxidative disposal. Fasting insulin correlated negatively with leucine oxidation but not with leucine flux and nonoxidative leucine disposal.

During the hyperinsulinemic-euglycemic clamp, leucine flux was suppressed from baseline and the suppression was significantly lower in pubertal than in nonpubertal subjects.

The authors conclude that whole body proteolysis is approximately 12% lower in pubertal adolescents compared with prepubertal children, and protein oxidation is 24% lower; however, protein synthesis is similar. They state that this is the first study to demonstrate changes in protein turnover during puberty compared with prepuberty. Protein turnover explained 24% of the variability in resting metabolic rate in these children. They note that the positive correlations between resting energy expenditure and leucine kinetics support the notion that protein turnover is a significant regulator of resting metabolic rate. They also note the inverse relationship between IGF-1 levels and leucine turnover, ie, the higher the IGF-1 level the lower the rate of proteolysis. In addition, studies with the hyperinsulinemic clamp show that pubertal adolescents demonstrate lower levels of proteolysis suppression.

Arslanian SA, Kalhan SC. *Am J Physiol* 1996;270:E79-E84.

**Editor's comment:** This is an important and carefully conducted study that significantly advances the understanding of some factors associated with growth during adolescence. The data suggest that (1) puberty is characterized by reduced protein breakdown; (2) pubertal elevations in IGF-1 may play a role in suppressing postabsorptive proteolysis; (3) approximately 20% of resting energy expenditure can be attributed to protein turnover; and (4) during puberty, whole body proteolysis is resistant to suppression by insulin. They carefully point out how their data differ from those collected by others.

Importantly, the authors point out that this study was done in the postabsorptive state and, therefore, conclusions with regard to postprandial metabolism cannot be extrapolated from their data. It is hoped that such data will be forthcoming, although such studies are significantly more complex to perform and their data are significantly more complex to analyze.

Arsanian and Kalhan have substantially increased our knowledge with regard to the events that contribute to growth during adolescence.

William L. Clarke, MD

## Morphogenesis and Tumors "Patched" Together in Gorlin Syndrome

Discoveries related to rare genetic syndromes also may provide insight into common diseases. A case in point is the recent delineation of the molecular defect in Gorlin syndrome, or nevoid basal cell carcinoma syndrome (NBCCS). A predisposition to basal cell carcinoma, medulloblastoma, and ovarian fibroma occurs in this autosomal dominant condition, as do diverse malformations involving the ribs, craniofacial structures, digits, and spine. Many of these manifestations reflect localized overgrowth. The underlying defect turns out to be in a gene called *patched* (*PTC*), which was studied first in fruit flies as an important developmental control gene. A similar defect may be involved in the most common human cancer, basal cell carcinoma of the skin.

Two teams connected NBCCS to *PTC*. Johnson et al<sup>1</sup> started with their work on the fly gene. When they cloned and mapped human *PTC*, they discovered that it resided very close to or where NBCCS had been mapped. Subsequent analysis in 2 families with NBCCS revealed *PTC* mutations. One was a 9-bp insertion; the other was an 11-bp deletion. They also found a point mutation in a basal cell carcinoma not associated with NBCCS.

Hahn and colleagues<sup>2</sup> used positional cloning to identify *PTC* as the NBCCS gene. Mutations predicted to inactivate *PTC* were found in 6 unrelated NBCCS patients and in tumors from 2 non-NBCCS patients.

Both papers,<sup>1,2</sup> as well as related editorials,<sup>3,4</sup> discussed the *PTC* gene product's normal function and its possible role in the pathogenesis of NBCCS and sporadic basal cell carcinoma. In flies, and presumably in humans, *PTC* encodes a transmembrane glycoprotein that acts as an antagonist in the Hedgehog signaling pathway; it influences the effects of a number of growth factors and morphogens, such as members of the transforming growth factor- $\beta$  and BMP families,

on early embryologic development. Given the inactivating nature of the mutations and the occurrence of tumors in NBCCS, *PTC* must also function as a tumor suppressor gene.

Hahn et al<sup>2</sup> and Shilo<sup>3</sup> speculated that 3 sets of features in NBCCS can be explained by a 2-step mechanism. The first step is the inherited mutation that causes constitutional loss of function at one *PTC* allele, haploinsufficiency; the second step is a sporadic mutation that leads to loss of function at the second allele. They postulated that symmetrical defects, such as craniofacial and overgrowth defects, result from disruption of dosage-sensitive pathways involving *PTC* during early development. Manifestations that are found in random clusters, ie, rib and spine malformations, may reflect sporadic mutations at the second allele in progenitor cells that contribute populations of cells to relevant tissues. Such tissues would be mosaic with regard to *PTC* alleles. Finally, loss of function at the second allele in adulthood leads to basal cell carcinoma and other tumors.

1. Johnson RL, et al. *Science* 1996; 272:1668-1671.
2. Hahn H, et al. *Cell* 1996; 85:841-851.
3. Shilo B-Z. *Nature* 1996; 382:115-116.
4. Pennisi E. *Science* 1996; 272:1583-1584.

**Editor's comment:** The authors of all of these reports acknowledge that precisely how *PTC* acts to influence the Hedgehog signaling pathway and how this pathway works in humans is poorly understood. Nevertheless, it seems clear that *PTC* influences the proliferation and perhaps survival of cells during development, growth, and carcinogenesis given the clinical manifestations of NBCCS.

William A. Horton, MD

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Richard M. Pauli, MD, PhD

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Cognitive Abilities Associated With the Silver-Russell Syndrome

Molecular Basis of Mammalian Sexual Determination: Activation of Müllerian Inhibiting Substance Gene Expression by SRY

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Catch-up Growth, Persisting Short Stature, and Adult Height of Children Born Small for Gestational Age

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# GROWTH

## Genetics & Hormones

Vol. 13 No. 1

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## The Neuroendocrinology of Stress: Its Relation to the Hormonal Milieu, Growth, and Development

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*Development*

*National Institutes of Health*

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Life exists by maintaining a complex dynamic equilibrium, termed "homeostasis," that is constantly challenged by intrinsic and extrinsic adverse forces, the stressors.<sup>1</sup> "Stress," a term borrowed from physics first by W. Cannon and subsequently used

by H. Selye, refers to factors threatening homeostasis. The human mind and body react to stress by activating a complex repertoire of adaptive central nervous system (CNS) and peripheral responses, the familiar "fight-or-flight" response.<sup>2</sup> Successful adaptive responses are generally specific to a stressor, but can become relatively non-specific when a stressor of any kind exceeds a threshold magnitude. Alterations in the ability of the organism to respond to stressors, with the responses being either excessive or inadequate in magnitude and duration, may lead to disease.

The adaptive response of an individual to stress is determined by a multiplicity of genetic and environmental factors, with development being an important consideration.<sup>3</sup> Indeed, prenatal life, infancy, childhood, and adolescence are critical periods characterized by increased vulnerability to stressors.<sup>4</sup> If stresses are excessive and/or these adaptive responses are prolonged, personality development and, hence, behavior may be disturbed. Adverse consequences on physiologic functions, including growth, metabolism, reproductive function, and the inflammatory/immune response, may result. Thus, stressors in early life may lead to developmental, psychiatric, growth, metabolic, reproductive, and immunologic disorders.

In this brief article, the reader will be introduced to the neuroendocrinology of the stress response; the stress system itself; the regulation of affect; and the effects of stress on endocrine functions, with emphasis on the hormonal milieu, growth, and the effect of stress on the immune system. The interdigitation of these are emphasized.

### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

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## NEUROENDOCRINOLOGY OF THE STRESS RESPONSE

The adaptive response to stress is characterized by both behavioral and physical changes.<sup>2,4</sup> These include increased arousal and alertness, heightened attention, and suppression of "vegetative" functions, which include sexual activity, feeding behavior, and growth. There is redirection of energy, ie, oxygen and nutrients, to the body site that is stressed and to the CNS, where it is most needed. The adaptive response is coordinated by the central and peripheral components of the stress system. The central components constantly receive information from higher and lower centers of the CNS, from the periphery of the organism, and from the environment. These then are integrated to help coordinate the dynamic equilibrium of the organism.

As diagramed in Figure 1, the central coordinators of the stress system include the parvocellular ("composed of small cells") corticotropin-releasing hormone (CRH) neurons; the arginine-vasopressin

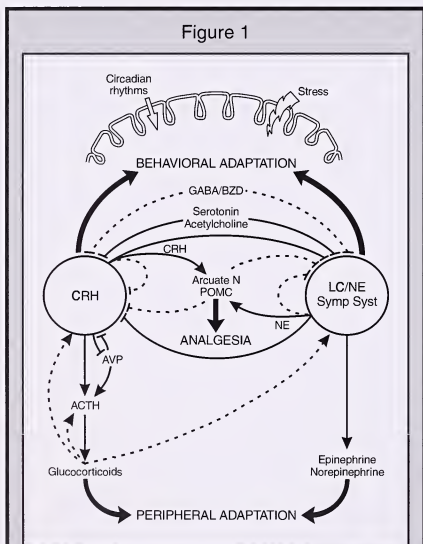
(AVP) neurons of the paraventricular nuclei (PVN) in the hypothalamus; the CRH neurons of the paraventricular nuclei and other nuclei in the medulla; and the catecholaminergic neurons of the locus ceruleus (LC) and other cell groups in the medulla and the pons. The hypothalamic-pituitary-adrenal (HPA) axis and the efferent sympathetic/adrenomedullary system represent the peripheral limbs of the central coordinating body. Reciprocal neural connections exist between the CRH and catecholaminergic neurons of the CNS, and there are autoregulatory ultra-short negative feedback loops exerted by CRH on the CRH neurons and on the catecholaminergic neurons exerted by norepinephrine via collateral fibers and presynaptic receptors. Both CRH and noradrenergic neurons are stimulated by serotonin and acetylcholine, and inhibited by glucocorticoids,  $\alpha$ -aminobutyric acid (GABA),  $\alpha$ -melanocyte-stimulating hormone (MSH), and opioid peptides.

Parvocellular neurons of the PVN produce CRH and AVP (Figure 1), and reciprocally innervate and are innervated by opioid peptide (pro-opiomelanocortin [POMC])-producing neurons of the arcuate nucleus of the hypothalamus. Thus, activation of the stress system stimulates hypothalamic POMC-peptide secretion, which reciprocally inhibits the activity of the stress system and, in addition, produces analgesia through projections to the hindbrain and spinal cord. CRH, of course, stimulates and also is permissive for pituitary ACTH secretion. On the other hand, AVP is a potent synergistic factor of CRH, but it has very little ACTH secretagogue activity by itself. During stress, AVP and CRH secretion into the hypophyseal portal system results in increased ACTH and, hence, cortisol release into the systemic circulation. Additional CRH, AVP, ACTH, and cortisol secretagogues are recruited during the various types of stress, further potentiating the activity of the HPA axis. These include the inflammatory cytokines, angiotensin II, and other mediators.

Glucocorticoids are the final effectors of the HPA axis and participate in the control of homeostasis in a multifaceted way. They play a key regulatory role in the basal activity of the HPA axis and in the termination of the stress response by exerting negative feedback at the CNS components of the stress system. A major function of glucocorticoids is the protection of the organism from the consequences of excessive adaptive responses. An example of such a role is the profound anti-inflammatory/immunosuppressive activity of glucocorticoids.

The sympathetic division of the autonomic nervous system provides a rapidly responding mechanism that controls mostly the acute response of the organism to a stressor. Peripherally, it widely innervates vascular smooth muscle cells, as well as

Figure 1



A simplified representation of the central and peripheral components of the stress system, their functional interrelations, and their relations to other CNS systems involved in the stress response. LC = locus ceruleus, NE = norepinephrine. Activation is represented by solid lines, and direct or indirect inhibition by dashed lines. Adapted with permission from Chrousos and Gold.<sup>2</sup>



the kidneys, gut, and many other organs, and the adrenal medulla. In addition to acetylcholine, norepinephrine, and epinephrine, the sympathetic and the parasympathetic divisions of the autonomic nervous system secrete a variety of neuropeptides, such as CRH, neuropeptide Y (NPY), somatostatin (STS), galanin, enkephalin and neurotensin, adenosine triphosphate (ATP), prostanoids, and nitric oxide (NO).

## STRESS SYSTEM AND THE REGULATION OF AFFECT

Activation of the stress system occurs in diametrically opposed situations, such as pleasure and dysphoria.<sup>2,4</sup> Indeed, novelty seeking and self-driven activation of the stress system — an important component of human development — is associated with pleasure if the response is adaptive and the individual has a sense of control. In contrast, stress may lead to dysphoria if the response is maladaptive and if the individual has the perception of no control. The teleology of these phenomena is obvious, for this is how an individual respectively seeks favorable changes and avoids, or learns to avoid, situations that may be detrimental to existence. The crucial nature of the stress system in human survival is underscored by the fact that it is activated not only by novelty but also by both feeding and sexual activity, *sine qua non* functions for self-preservation and species preservation.

The mechanisms regulating the stress-activated mood response are complex and poorly understood. It appears that the stress system has reciprocal interactions with at least 3 other elements of the CNS that participate in the regulation of emotions: (1) the mesocortical and mesolimbic dopamine systems, which include the prefrontal cortex and nucleus accumbens and which are involved in anticipatory and motivational/reinforcement and reward phenomena, respectively; (2) the amygdala/hippocampus complex, which is involved in emotional stressors such as conditioned fear; and (3) the arcuate nucleus opioid peptide-secreting neurons, which alter sensitivity to pain and perhaps influence the emotional tone of an individual.

Several emotional disorders may represent dysregulation of the generalized stress response. Thus, excessive and prolonged activity of the stress system characterizes melancholic depression, whose cardinal symptoms are hyperarousal (anxiety), suppression of feeding (anorexia) and sexual behaviors (loss of libido), and excessive and prolonged redirection of energy (tachycardia, hypertension, carbohydrate intolerance, dyslipidemia). All of these are extremes of the classic manifestations of

the “generalized” stress response.<sup>5</sup> The dysphoria that accompanies this condition may represent a response to a perceived uncontrollable stressor and could be due to tachyphylaxis of the mesocorticolimbic system in response to chronic activation by the stress system, while the obsessiveness that characterizes depressive individuals appears to represent a maladaptive increase in the attention span.<sup>5</sup>

Both the HPA axis and the sympathetic system are chronically activated in melancholic depression, in which hyperarousal of the stress system occurs, producing increased CRH. Increased CRH causes or is associated with insomnia, depressed mood, inability to concentrate, decreased appetite, decreased libido, and weight loss. Melancholic depression rarely afflicts children and adolescents. However, chronic activation of the HPA axis and/or the sympathetic system has been shown in a host of other conditions that afflict children, adolescents, and young adults in a major fashion, including malnutrition, anorexia nervosa, panic disorder, obsessive-compulsive neurosis, chronic active alcoholism, alcohol and narcotic abuse, excessive exercising, and post sexual abuse traumatic disorder.<sup>2,6</sup> Animal studies are confirmatory of the association between increased CRH secretion, chronic activation of the HPA axis, and affective disorders. For example, traumatic separation of infant rhesus monkeys and laboratory rats from their mothers causes behavioral agitation and increased CRH, ACTH, and cortisol responses to stressors throughout their lives.<sup>7,8</sup> This is in accordance with human studies showing that melancholic depression has a strong association with precipitating environmental stressors.<sup>9</sup>

Interestingly, the HPA axis and/or the sympathetic system appear hyperactive in several pathologic states, including seasonal affective disorder, in the postpartum period, and the period following the cessation of smoking, as well as in the chronic fatigue and fibromyalgia syndromes — all dysphoric, hypoarousal states.<sup>2,10,11</sup> These conditions are usually characterized by an increase in appetite, weight gain, somnolence, and fatigue — manifestations compatible with low CRH secretion and hypoactivation of the mesocorticolimbic system.

There is general agreement that adolescence is a challenging period of life during which significant physical, psychological, and social changes take place.<sup>12</sup> Adolescents are in a chronic state of “threatened homeostasis,” and their adaptive responses are crucial for a successful and happy adulthood. Dysregulation of the stress system in adolescence by the mechanisms indicated above could be the reason behind the emergence of a number of disorders during this period, including



depression, eating disorders, and substance abuse. Recently, it became apparent that it is the "atypical" form of depression that primarily afflicts adolescents and that this form appears to be genetically distinct from the melancholic form usually seen in adults.<sup>13,14</sup>

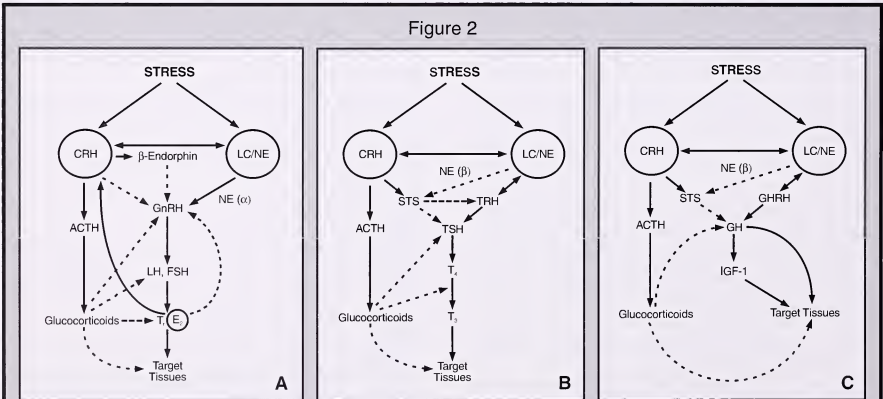
### STRESS AND ENDOCRINE FUNCTIONS: EFFECTS ON THE HORMONAL MILIEU

Reproduction and growth are profoundly influenced by stress.<sup>2,4</sup> The reproductive axis is inhibited at all levels by various components of the stress system (Figure 2A). Thus, CRH suppresses the secretion of gonadotropin hormone-releasing hormone (GnRH) by arcuate neurons of the hypothalamus, either directly or via the stimulation of arcuate POMC peptide-secreting neurons. Moreover, glucocorticoids exert inhibitory effects at the level of the GnRH neuron, the pituitary gonadotrope, and the gonads themselves, and render target tissues of sex steroids resistant to these hormones. Suppression of gonadal function caused by chronic HPA axis activation has been demonstrated in highly trained runners of both sexes and ballet dancers. These subjects have increased evening plasma cortisol and ACTH, increased 24-hour urinary free cortisol excretion, and blunted ACTH responses to exogenous CRH. Males have low levels of luteinizing hormone (LH) and testosterone, and

females have amenorrhea. Characteristically, obligate athletes go through withdrawal symptoms and signs if for any reason they have to discontinue their exercise routine. This syndrome is possibly the result of withdrawal from the daily exercise-induced activation of the dopaminergic system and/or elevation of opioid peptides.

The interaction between CRH and the gonadal axis appears to be bidirectional.<sup>15</sup> We recently demonstrated the presence of estrogen-responsive elements in the promoter area of the CRH gene and direct stimulatory estrogen effects on CRH gene expression. This finding implicates CRH and, therefore, the HPA axis as a potentially important target of ovarian steroids and a potential mediator of gender-related differences in the stress response. Indeed, Kirschbaum et al<sup>16</sup> recently showed that estrogen administration to normal male volunteers resulted in excessive stress system responses to mental stress.

In parallel to the gonadal axis, the stress system suppresses function of the thyroid axis (Figure 2B).<sup>2,4</sup> During stress, there is suppressed secretion of thyrotropin and decreased conversion of the relatively inactive thyroxine ( $T_4$ ) to the potent triiodothyronine ( $T_3$ ) in peripheral tissues. This situation is similar to what is observed in the euthyroid sick syndrome, a phenomenon that serves to conserve energy during stress. The mediators of these changes in thyroid function include glucocorticoids,



A schematic representation of the interactions between the stress system and other neuroendocrine axes: (A) the reproductive axis; (B) the thyroid axis; and (C) the growth axis. Note that the LC/NE system provides positive stimulation to all 3 axes; this effect is overcome by the inhibitory effects of the HPA axis during stress. Hypoactivity of the LC/NE in several human states, such as atypical depression and the chronic fatigue/fibromyalgia syndromes, may be responsible for the mild central hypogonadism, hypothyroidism and hyposomatotropism observed in such states. Adapted with permission from Chrousos and Gold.<sup>2</sup>

which suppress thyrotropin secretion; the activity of the peripheral enzyme 5'-deiodinase, which converts L-T<sub>4</sub> to L-T<sub>3</sub>; somatostatin, which suppresses both TRH and thyrotropin; and, in the case of inflammatory stress, the cytokines, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin 1 (IL-1), and interleukin 6 (IL-6), all of which activate CRH secretion and also directly inhibit the 5'-deiodinase. Accordingly, patients with melancholic depression, anorexics, and chronically ill patients have significantly lower thyrotropin and T<sub>3</sub> hormone concentrations than controls.

## STRESS AND ENDOCRINE FUNCTIONS: EFFECTS ON GROWTH AND DEVELOPMENT

The growth axis also is inhibited at many levels during stress (Figure 2C).<sup>2,4</sup> Thus, prolonged activation of the HPA axis leads to suppression of growth hormone (GH) release and inhibition of insulin-like growth factor 1 (IGF-1) effects on its target tissues. CRH-induced increases of somatostatinergic tone have been implicated as a potential mechanism of stress-induced chronic suppression of GH secretion. It is noteworthy that acute elevations of GH concentrations in plasma occur at the onset of the stress response and after acute administration of glucocorticoids, presumably through stimulation of the GH gene by its glucocorticoid-responsive elements (GREs).

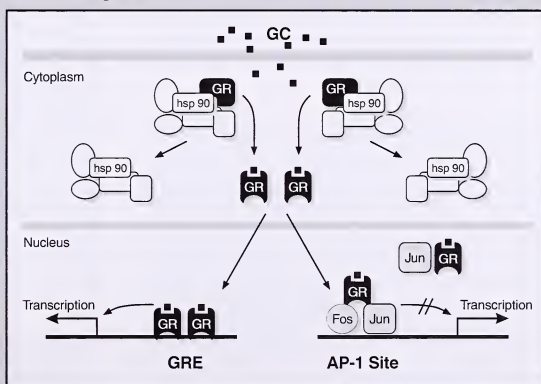
One of the major ways by which the HPA axis inhibits growth is by glucocorticoid-induced resistance of target tissues to IGF-1. Indeed, children

with Cushing syndrome have delayed or arrested growth and lose an average of 7.5 to 8.0 cm of their final height as adults.<sup>17-19</sup> The molecular mechanism by which glucocorticoids render tissues resistant to IGF-1 and other growth factors is complex.<sup>20</sup> A major mechanism is the inhibition of growth factor third messengers, such as the cJun-Fos heterodimer or AP-1 transcription factor, by protein-protein interactions between this factor and the ligand-bound, "activated" glucocorticoid receptor (Figure 3).

Psychosocial short stature (PSS) is a term describing severe childhood or adolescent short stature and/or delayed puberty due to emotional deprivation or psychological harassment.<sup>21</sup> This topic is discussed in an abstract and letters to and from the editor on page 8 of this issue. Decreased GH secretion that is reversible after separation of the child from the responsible environment is a very frequent finding in this condition.<sup>22</sup> PSS is also associated with a variety of behavioral abnormalities, such as depression and bizarre eating behaviors. This condition was first studied in infants in foundling homes or orphanages who failed to thrive or had decreased growth. It was hypothesized that failure to thrive and/or grow resulted from lack of attention and stimulation and/or deficient nutrition. Later it was shown that weight gain was independent of food intake, whereas with a caring and attentive environment, growth advanced and the psychological profile improved. In addition to low GH secretion, these patients often had decreased cortisol secretion and/or a dysfunctional thyroid axis—all manifestations compatible with a hyperfunctioning stress system.<sup>23</sup>

Figure 3

A simplified model of glucocorticoid-mediated transcriptional modulation. Hormone binding causes dissociation of the glucocorticoid receptor/hsp complex and nuclear translocation of the ligand-bound receptor. Within the nucleus, the "activated" receptor can act in 2 ways: As indicated on the left, it can bind to glucocorticoid responsive elements (GREs) in the regulatory region of target genes. This interaction causes either stimulation or, less frequently, inhibition of transcription. As indicated on the right, the activated glucocorticoid receptor can also interact with, and inhibit the transactivational activity of, other transcription factors important for growth or immune function, such as, respectively, the cJun-Fos heterodimer and the NF- $\kappa$ B transcription factor. From Bamberger CM, et al. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews* 1996;17(3):247; © The Endocrine Society.



We have reported a nonhuman primate model in which the quality of parental care correlated well with infant and child growth and development.<sup>24</sup> Improvement of care resulted in catch-up growth in this model. Ovine (o)CRH testing results were compatible with a hyperactive HPA axis, as we had seen in patients with adult melancholic depression and sexually abused preadolescent girls.<sup>2,6</sup> Pine et al<sup>25</sup> recently reported a small loss of final height in young girls with childhood anxiety disorder, but not in those with childhood and adolescent depression.<sup>25</sup> These data are in agreement with the slightly hyperactive HPA axis in the former and the normal or hypoactive HPA axis in the latter.<sup>13</sup> No compromise in the final stature of boys with anxiety disorder was observed in the study by Pine et al. This may be explained by the higher estrogen levels in girls than in boys, enhancing CRH secretion.<sup>15</sup>

Premature infants are at risk for delayed growth and/or development, especially after prolonged hospitalization in the intensive care nursery. The condition is similar to PSS, but is known as "reactive attachment disorder of infancy" and can be prevented and/or treated with loving attention.<sup>26</sup> Interestingly, activation of the human fetal HPA axis is also associated with growth retardation. Elevated levels of CRH, ACTH, and cortisol have been reported in growth-retarded fetuses.<sup>27</sup>

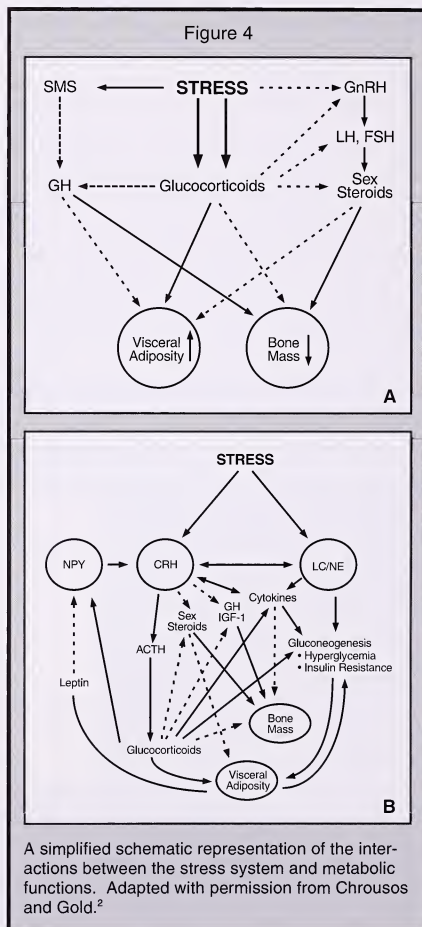
Infantile and childhood malnutrition is characterized by hypercortisolism, decreased responsiveness to CRH and incomplete dexamethasone suppression, and the euthyroid sick syndrome pattern of thyroid hormone abnormalities, all of which are restored after nutritional rehabilitation. It is noteworthy that in this condition, increases rather than decreases of GH secretion are present, possibly resulting from starvation, which induces hyposecretion of IGF-1 and resultant decreased negative feedback upon GH secretion.

## STRESS AND METABOLISM

Long-term administration of glucocorticoids or endogenous Cushing syndrome is associated with visceral obesity, insulin resistance, hypertension, and elevated cholesterol and triglyceride levels (Figure 4A). Thus, hypercortisolism resembles "metabolic syndrome X" (MS-X) in both its somatic and biochemical phenotypes. Interestingly, MS-X was recently associated with increased 24-hour urinary free cortisol excretion, suggesting that glucocorticoids may represent a common denominator of both states.<sup>28</sup> Moreover, both hypercortisolism and MS-X are associated with increased atherosclerosis and resultant cardiovascular morbidity and mortality.

The association between chronic, experimentally induced psychosocial stress and a hypercortisolism/MS-X-like state, with resultant marked coronary atherosclerosis, was recently reported in cynomolgus monkeys.<sup>29</sup> In these animals, chronic, stress-induced activation of the HPA axis and, therefore, hypercortisolism and suppressed GH secretion apparently led to visceral obesity, insulin resistance, hypertension, and dyslipidemia (Figure 4B), all converging to the development of varying degrees of the physical and biochemical phenotype of MS-X.

"Low turnover" osteoporosis is almost invariably seen in association with hypercortisolism and GH





deficiency, reflecting the detrimental effect of the combination of high cortisol and low GH and/or IGF-1 concentrations on the osteoblasts. Osteoporosis may be further potentiated by the stress-related hypogonadism. We recently reported increased prevalence of "low turnover" osteoporosis associated with decreased plasma osteocalcin levels in relatively young women with depression or a history of depression.<sup>30</sup>

## STRESS AND IMMUNE FUNCTION

Activation of the HPA axis takes place during the stress of an infectious disease, autoimmune inflammatory process, and accidental or operative trauma.<sup>31</sup> The mechanisms of this association have been unraveled recently. The 3 "inflammatory cytokines"—TNF- $\alpha$ , IL-1, and IL-6—cause stimulation of the HPA axis *in vivo*, alone or in synergy with each other (Figure 4B). This is mediated by hypothalamic CRH and AVP secretion and by direct effects at the pituitary and adrenocortical levels. IL-6, the main endocrine cytokine, causes major elevations of ACTH and cortisol, elevations well above those observed with maximal stimulatory doses of CRH, suggesting that AVP and potentially other ACTH secretagogues are also stimulated by this cytokine. Glucocorticoids and prostanoid synthesis inhibitors suppress the stimulatory effects of cytokines on the HPA axis.

Glucocorticoids, the end-hormones of the HPA axis, play a major role in the stress-induced suppression of the immune/inflammatory reaction.<sup>31</sup> On the other hand, the autonomic system also participates in the effects of stress on the immune/inflammatory reaction, both by being reciprocally connected with the CRH system and by transmitting neural signals from the CNS to the immune system. The latter is mediated by a dense innervation of both primary and secondary lymphoid organs, and by reaching sites of inflammation via postganglionic sympathetic neurons. The sympathetic system, when activated, causes systemic secretion of IL-6, which by directly inhibiting the other 2 inflammatory cytokines, TNF- $\alpha$  and IL-1, and by activating the HPA axis participates in the stress-induced suppression of the immune/inflammatory reaction. Also, catecholamines via  $\beta$ -adrenergic receptors inhibit IL-12 and stimulate IL-10 and IL-4 secretion and, hence, cause suppression of cellular immunity and stimulation of humoral immunity.<sup>32</sup>

The HPA axis and the immune system function in balance in the physiologic state.<sup>31</sup> An excessive response of the HPA axis to inflammatory stimuli mimics the hypercortisolemic state and leads to increased susceptibility of an individual to certain viral and bacterial infections or neoplasia. On the

other hand, a defective HPA axis response to inflammatory stimuli reproduces the glucocorticoid-deficient state and leads to increased susceptibility to allergic/autoimmune/inflammatory diseases.

## SUMMARY

In response to a stressor that exceeds a threshold magnitude, or multiple stressors applied simultaneously, the organism alters its behavior and physiology with the aim of maintaining homeostasis. The adaptive changes that occur are coordinated and mediated by the stress system in the CNS — which includes CRH-peptidergic and noradrenergic neurons in the hypothalamus and the brain stem, respectively — and its peripheral limbs, the HPA axis and the autonomic (sympathetic) system. Controlled or self-driven challenges to homeostasis and a normally functioning stress system are crucial for normal development and the preservation of self and species. In childhood and adolescence, during which psychosexual maturation and growth take place, appropriately functioning neuroendocrine responses to stressors are necessary to allow these processes to progress normally. Maladaptive neuroendocrine responses, ie, dysregulation of the stress system, may lead to disturbances in growth and development, and cause developmental/psychiatric, endocrine/metabolic, and/or autoimmune disorders or vulnerability to such disorders not only during childhood and adolescence but also in adulthood.

**Editor's comment:** Pertinent to the content of this excellent article is the abstract elsewhere in this issue, entitled "A New Stress-Related Syndrome of Growth Failure and Hyperphagia in Children Associated With Reversibility of GH Insufficiency," and the letter to the editor from the authors.

Robert M. Blizzard, MD

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## Erratum

In the last issue of *GGH*, the starting date that Dr. Lifshitz became Chief of Staff at Miami Children's Hospital was incorrectly stated as February 1, 1977. The correct date is February 1, 1997.

## Letter to the Editors

Dear Dr. Blizzard:

This letter concerns a paper by Tillotson et al (*GGH* 1996;12[2]:30). The paper contains a methodologic error and the results are invalid. However, the authors' conclusion that the mental and neurologic features of phenylketonuria (PKU) are not caused prenatally can rest on other evidence and is perfectly sound. Other workers who by luck or judgment avoid this error and get different results may tend to discard Tillotson et al's conclusion with their results. This is the reason for submitting this letter.

Since PKU is not panethnic and ethnicity affects birth weight, it is not valid to compare birth weights of affected infants with statistical norms for an entire multiethnic population such as that of the United Kingdom (*Eur J Pediatr* 1995;154(10):847-849). In investigations (*Int J Neuroscience* 1990;54:259-266) in Ireland and west Scotland, where there is a very high incidence of PKU in an ethnically homogeneous population, there was no significant difference in birth weight between PKU infants and their unaffected siblings ( $P>0.5$ ), as others had previously found. However, closely matched control infants drawn from the same populations had mean birth weights 107 g > than the PKU infants and their unaffected siblings ( $P<0.02$ ). A similar finding was later made in the

Netherlands, although the authors did not investigate the birth weights of unaffected siblings of PKU infants (*Arch Dis Child* 1994;71[2]:114-118).

In the Irish and west Scottish investigation, the birth weights of the PKU infants and their unaffected siblings lay on a single normal distribution curve with no evidence of bimodal or trimodal distribution. Since the unaffected sibs are mentally and neurologically normal, but show a reduction in birth weight equal to that of PKU infants, the lower birth weight cannot be related to the pathogenesis of PKU or to the fetal genotype. The reduction in birth weight can be a reflection only of maternal heterozygosity and constitutes a previously unknown effect of the PKU gene in a single dose.

Respectfully submitted,

**L.I. Woolf, PhD**  
Professor Emeritus  
University of British Columbia  
Vancouver, British Columbia

**Editor's comment:** Dr. Tillotson was contacted twice regarding this letter from Dr. Woolf, but no response was forthcoming.

Robert M. Blizzard, MD



## ***GROWTH, Genetics, & Hormones***

**Volume 13, Number 1**

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## A New Stress-Related Syndrome of Growth Failure and Hyperphagia in Children, Associated With Reversibility of Growth-Hormone Insufficiency

Growth failure without organic cause but associated with behavioral disturbances and psychosocial stress has been termed psychosocial short stature (PSS), reversible hypsomatotropism, the garbage can syndrome, and maternal deprivation. The authors state that PSS does not describe a valid diagnostic entity, but encompasses failure to thrive, stunting secondary to chronic malnutrition, and idiopathic hypopituitarism. Some of these children show spontaneous catch-up growth when removed from the source of stress, but until now precise definition of this subgroup for the purpose of clinical identification has not been possible.

The authors compared 31 normal children with short stature identified from an epidemiologic survey versus 51 children with growth failure unrelated to organic pathology who were referred to the hospital between 1986 and 1994. An additional subject who was community-identified was added to the hospital-referred group, raising the total number of hospital referred patients to 52 and the total sample to 83 patients. Growth hormone (GH) dynamics were studied in the hospital group of 52 patients by a combination of diurnal GH profiles and provocative tests for GH release. The tests were repeated after a hospital stay of 3 weeks away from familial stress. The mean age of referral of the stressed children was 7.9 years (range, 3.8 to 13.7 years). All but 2 were prepubertal. Nine sibling-pairs, including 2 sets of dizygotic twins, were included. Height for age was below the 3rd percentile. Only 8 of the 52 had not been emotionally, sexually, or physically abused.

In a distinctive subgroup (29 of the 52) growth hormone insufficiency (GHI) was associated with hyperphagia, poly-

dipsia, and normal body mass index. When the children were removed from their stressful homes, GHI spontaneously resolved *only* in formerly hyperphagic subjects. A distinctive correlation was reported between GHI, hyperphagia, and growth recovery when the children were removed from the home. Seventy-four percent of the hospital-referred group ( $n=23$ ) were identified as being nonhyperphagic with anorexia, low body mass index, and normal GH response to provocation tests.

The authors identified 9 key symptoms that distinguished the hyperphagia/polydipsia group ( $n=29$ ) from the nonhyperphagic ( $n=23$ ) and community comparison ( $n=31$ ) groups (Table 1). The hyperphagic children were distinguishable from the nonhyperphagic children usually by using standard provocative tests of GH secretion. Five of the hyperphagic children were admitted for at least 3 weeks for testing of spontaneous changes in growth hormone secretion. The remaining 16 hyperphagic children had initial provocative tests of growth-hormone secretions as inpatients on the day of admission. Fourteen of these 16 hyperphagic children (88%) were found to be growth hormone insufficient initially. After about 3 weeks of restricted parental contact, 10 of the 16 had a distinctly increased GH release. Sixteen of the nonhyperphagic children were investigated by at least 1 GH provocative test. Six nonhyperphagic children (38%) had evidence of GHI on initial testing. The mean initial response was greater than in the hyperphagic children initially, 22.6 mU/L versus 8.9 mU/L. The mean of the peak GH in these nonhyperphagic children was 26.7 mU/L, when testing was repeated at 3 and 6 weeks.

Table 1  
Characteristics of Appetite Disturbance

|                               | Hyperphagic Patients<br>(n=29) | Nonhyperphagic Hospital-Referred<br>(n=23) | Community Comparisons<br>(n=31) |
|-------------------------------|--------------------------------|--|---------------------------------|
| Eats too much                 | 25 (86%)                       | 2 (9%)*                                    | 0*                              |
| Gorges and vomits             | 23 (79%)                       | 1 (4%)*                                    | 0*                              |
| Steals food at home           | 28 (97%)                       | 3 (13%)*                                   | 3 (10%)*                        |
| Steals food at school         | 16 (55%)                       | 0*   | 0*                              |
| Hoards food                   | 19 (66%)                       | 2 (9%)*                                    | 1 (3%)*                         |
| Drinks excessively            | 17 (59%)                       | 2 (9%)*                                    | 12 (39%)*                       |
| Pica                          | 18 (62%)                       | 0*   | 0*                              |
| Eats from bins/discarded food | 16 (55%)                       | 0*   | 0*                              |
| Searches for food at night    | 18 (62%)                       | 1 (4%)*                                    | 0*                              |

Analyses were planned comparisons between hyperphagic subjects and those in the other 2 groups separately. Symptoms were recorded as present if they were happening now or if they had occurred within the previous 6 months. \* $P<0.001$ .

Skuse D, et al. A new stress-related syndrome of growth failure and hyperphagia in children associated with reversibility of growth-hormone insufficiency. *Lancet* 1996;348:355.



In the discussion, the authors suggested that the 29 patients with hyperphagia and polydipsia constitute a previously undefined syndrome of growth failure. They propose that the condition of hyperphagic short stature has predictive and discriminant validity on the basis of its symptom profile: GHI, associated intellectual impairment, and familial aggregation. The authors state that failure to thrive during infancy occurred in 95% of the hyperphagic subjects, but also in 40% of the nonhyperphagic hospital cases. The authors suggest that nosologic confusion could be avoided if the term hyperphagic short stature is used to identify patients with hyperphagia/polydipsia, and these patients should strongly be suspected of having GHI. They suggest that in the nonhyperphagic patients (75% with anorexia), chronic nutritional deficiency is very frequently present and causes stunting.

The authors further interpret the data by stating that the explicit behavioral and developmental criteria by which the novel syndrome of hyperphagic short stature may be clinically recognized was described. Such children were stated to have a capacity for spontaneous recovery of GH production when removed from the adverse environment. Discriminant and predictive validity of the core symptoms was demonstrated and preliminary familial studies indicated a possible genetic predisposition, as there were 9 sets of sibs, including 2 pairs of dizygotic twins.

Skuse D, et al. *Lancet* 1996; 348:353-358.

**Editor's comment:** The readers are referred for summary of current knowledge about PSS to Chapter 6 in the Third Edition of *Pediatric Endocrinology*, edited by Fima Lifshitz. (Marcel Dekker Publishers, 1996). The table below is a classification published in that text.

Skuse et al are to be commended on furthering our knowledge about the variability within the group of patients described as having PSS type II. Skuse et al have added clarification to the variability of the syndrome if one uses the umbrella term of PSS for children who are short and who experience a high degree of parental psychological neglect or trauma. Undoubtedly, the data dividing hyperphagic and nonhyperphagic patients is a service and clarification. The demonstration that in type II PSS, as defined by Blizzard and Bulatovic, there may be subdivisions of hyperphagic and nonhyperphagic patients is a contribution. The fact that hyper-

phagic patients are those who most frequently have associated GHI is a significant contribution also.

I disagree that this can be called a "new" stress-related syndrome of growth failure and hyperphagia in children, associated with reversibility of GHI. Instead, I interpret these findings to further clarify the variable types of PSS. The need to have a valid diagnostic entity broken out of PSS may be tenable for medical administrative reasons, and that is acceptable, if necessary. However, as pointed out by Blizzard and Bulatovic in Lifshitz' text, there are different types of PSS, including failure to thrive in some cases brought on by psychological stress, and other types as described by Boulton et al (GGH 1992;8[4]:13). The latter was labeled as type III in the overall classification of PSS by Blizzard and Bulatovic. Perhaps the designation of type IIA for the PSS of the hyperphagic type and type IIB PSS for the anorexic type would be more preferable for classification purposes, until we have additional data to break out the subtypes of growth retardation that should be considered under the umbrella of PSS.

An additional approach to the hyperphagic versus the anorexic patients looked at by Skuse et al would be to examine these patients in respect to depression. It has been pointed out previously that depression is a common finding in all PSS types. Another route of investigation for the authors would be to examine their 2 groups of affected patients in respect to their growth response to GH treatment. Type II patients as recorded by Blizzard and Bulatovic are resistant to GH treatment.

I urge that pediatric endocrinologists incorporate in their thinking the type of hyperphagic PSS described by Skuse et al as being only one type, therefore type IIA. For purposes of further study, it is better to call their hyperphagic patients a subtype of PSS at this point in time. Further study comparing type III as described by Boulton et al with the nonhyperphagic/anorexic group described by Skuse et al is desirable, as the anorexic patients described by Skuse et al may fall in type IIB, as proposed above, or in type III, which would eliminate the need to have 2 subtypes of category II. The authors of this fine paper and all others are urged to further clarify the variability of recognized PSS syndromes rather than describe new syndromes.

Robert M. Blizzard, MD

Characteristics of Various PSS Syndromes

| Type | Age of Onset     | Failure to Thrive        | Bizarre Behavior | Depression | GH Secretion              | Parental Rejection     | GH Responsiveness        |
|------|------------------|--------------------------|------------------|------------|---------------------------|------------------------|--------------------------|
| I    | Infancy          | Usually                  | No               | Often      | Normal                    | No                     | ?                        |
| II   | ≥3 years         | Some and some overweight | Usual            | Very often | Decreased or absent often | Usual                  | Minimal at doses used    |
| III  | Infancy or later | Not usual                | Not usual        | Yes        | Normal                    | Concern, not rejection | Significant at dose used |

Lifshitz F. *Pediatric Endocrinology*. New York, NY: Marcel Dekker Inc; 1996:3rd Edition, Chapter 6.

**Response by Authors:** We agree with Professor Blizzard's comments. We wish to identify patients with potentially reversible GH secretion who could achieve catch-up growth without GH therapy. Eighty percent of our patients with hyperphagic short stature experienced occult physical, emotional, or sexual abuse. Treating such children with GH daily for many years, without recognizing child abuse, would add insult to injury.

There was no evidence of depression in our patients. GH reversibility was demonstrated in only 1 of 6 nonhyperphagic children in the hospital-referred comparison group who were not anorexic. Two patients had no reversibility of GH secretion.

We understand the point of view that a new syndrome has not been described, but rather a more specific subtype within type II classification of PSS. Nevertheless, considerable evidence of the syndrome's distinctive nature has been gathered. This includes evaluation of a new series of patients. The syndrome is not apparently on a qualitatively similar continuum with other types of PSS, as our data strongly indicate only some children are predisposed to develop it, even within a sibling group. We agree that if classified within Blizzard and Bulatovic's scheme, hyperphagic short stature will fall into category type II, or possibly type IIA. In this condition, GH deficiency reverses spontaneously with environmental manipulation, and such patients often are resistant to GH treatment. Our nonhyperphagic subjects without an eating disorder, which in our experience is rarely associated with GH deficiency, could possibly be classified as type IIB. Patients in this group only occasionally respond to GH treatment.

As induced injury can be considered as a spectrum of disorders, ranging from factitious injury to the Munchausen by Proxy syndrome, PSS also is a spectrum of morbidity ranging from environmentally induced growth failure due to chronic undernutrition to growth failure caused by the endo-

crinopathy originally described by Powell and colleagues in 1967. Our work has not only confirmed these findings in that hyperphagia and polydipsia [were part of the clinical picture of their cases, but we also have shown that hyperphagia and polydipsia] are very sensitive markers that suggest the patient probably has reversibility of GH deficiency. The syndrome is a remarkable example of the potential responsiveness of a neuroendocrinologic system to stress.

Our clinical impression is that many children with hyperphagic short stature are labeled as having true GH deficiency following a day-case evaluation of GH secretion. The true diagnosis is missed. We emphasize the importance of asking the appropriate questions in order to make the correct diagnosis and to reveal that occult child abuse is probable in children with the disorder. The consistency of the symptom constellation in affected children is remarkable. The physician should routinely ask whether a child with growth failure and GH deficiency eats excessively; gorges and vomits if given unlimited access to food; steals food at home and school; hoards food; has polydipsia or pica; scavenges from trash cans; or searches for food at night. We wish to emphasize that among children with GH deficiency, the syndrome of hyperphagic short stature is not as rare as previously believed.

#### **Richard Stanhope, MD, FRCP**

Consultant Pediatric Endocrinologist  
Great Ormond Street Hospital for Children and Institute of Child Health  
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#### **Professor David Skuse, MD, FRCP**

Consultant Pediatric Psychiatrist  
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London, England

## **Holoprosencephaly and Sonic Hedgehog**

The "hedgehog" story is emerging rapidly. The hedgehogs are a family of developmentally important signaling proteins first discovered in the fruit fly, *Drosophila*. There are 3 human hedgehog genes known to date: *Sonic Hedgehog (SHH)*, *Indian Hedgehog (IHH)*, and *Desert Hedgehog (DHH)*. They encode secreted proteins that undergo autocatalytic cleavage to produce a carboxy-terminal fragment and a biologically active amino-terminal fragment that tends to remain near the cell of origin.

The hedgehog proteins have been shown to have effects on the developing embryo in many species, including patterning effects on the midline central nervous system (CNS) structures and on developing limbs. Indeed, genetic inactivation of *SHH* in mice produced cyclopia and other CNS abnormalities, raising the possibility that mutations of *SHH* could be responsible for some human birth defects involving these structures.

These latter observations prompted groups headed by Muenke and Tsui to consider *SHH* as a candidate for alobar holoprosencephaly type 3 (HPE3), which behaves as a dominant trait with wide clinical variability in some families. It had been previously mapped to chromosome 7q36. This form of holoprosencephaly involves failure of the forebrain to divide into right and left hemispheres. At the severe end of the spectrum, it is typically associated with midline facial abnormalities, including cyclopia, a primitive nasal structure (proboscis), and clefting. Manifestations at the mild end of the spectrum may be limited to microcephaly, mild hypotelorism, midline facial clefts, and a single maxillary central incisor.

In the first paper, Belloni et al defined a critical region of about 500 kb for HPE3. This was done from physical mapping of breakpoints for chromosomal rearrangements in several HPE3 patients. Next, they mapped *SHH* to this interval. Interestingly, none of the breakpoints disrupted *SHH*.

From analysis of *SHH* in 30 families, Roessler et al subsequently detected heterozygous mutations, which segregated with *HEP3* phenotype, in 5 families. Two mutations were nonsense mutations predicted to cause premature termination of the *SHH* protein. Another predicted disruption of the autocatalytic cleavage site. All 3 would be expected to produce loss of function of one of the *SHH* alleles, haploinsufficiency. The paper, as well as invited comments, speculated about how such mutations could cause such profound effects on craniofacial development.

Belloni E, et al. Identification of *Sonic hedgehog* as a candidate gene responsible for holoprosencephaly. *Nature Genet* 1996;13:353-356. Letter.

Dean M. Polarity, proliferation and the *hedgehog* pathway. *Nature Genet* 1996;14:245-247. News and Views.

Roessler E, et al. Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly. *Nature Genet* 1996;13:357-360. Letter.

**Editor's comment:** Signaling through the *hedgehog* family of proteins is becoming very interesting. In the past year, IHH has been implicated in a negative feedback loop controlling the rate of endochondral bone growth. Mutations of the *hedgehog* receptor, patched, have been found in the Gorlin syndrome and in sporadic basal cell carcinoma; and now mutations of *SHH* appear to cause some forms of holoprosencephaly. Given the apparent importance of *hedgehog* signaling in so many regulatory circuits, one wonders how many other sporadic disorders, especially those involved in craniofacial and limb development, might also be due to defects in *hedgehog* signaling.

William A. Horton, MD

## Constitutively Activated Receptors for Parathyroid Hormone and Parathyroid Hormone-Related Peptide in Jansen's Metaphyseal Chondrodysplasia

Jansen's metaphyseal chondrodysplasia is a rare form of short-limbed dwarfism associated with hypercalcemia and normal or low serum concentrations of parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP). It is an autosomal dominant genetic disorder. Most cases are due to new mutations. Jansen's metaphyseal chondrodysplasia is recognized in the newborn period by rhizomelic short stature, severe bowing of the legs, fronto-orbital asymmetry, hypertelorism, and hypoplasia of the mandible. X-ray films show cupping and irregularity of the growth plates. All metaphyses are severely involved and appear markedly enlarged, wide, irregular, and cystic. Laboratory findings include increased serum calcium and alkaline phosphatase, with normal or low PTH and PTHrP.

The actions of both PTH and PTH-related hormones are mediated through PTH-PTHrP receptors, and their intracellular signaling is mediated by both cyclic AMP (cAMP) and calcium. PTH-PTHrP receptors belong to the family of G protein-coupled receptors, which have dual signaling properties. They are expressed in many fetal and adult tissues and found in abundance in kidney, bone, and growth-plate cartilage.

Schipani et al have confirmed the presence of a mutation in the gene for PTH-PTHrP receptors in 4 of 6 additional individuals with Jansen's metaphyseal chondrodysplasia. A similar mutation has previously been identified by Schipani et al in an individual with Jansen's metaphyseal chondrodysplasia.

Three of the mutations found had the histidine changed to arginine at position 223 (H223R). One had a novel missense mutation that changed a threonine in the receptor's sixth membrane-spanning region to proline (T410P). None of these mutations were found in the healthy relatives. In one family, the H223R mutation was found in the affected mother and her affected daughter but not in the healthy father.

Mutations cause activation of PTH-PTHrP receptors, resulting in hypercalcemia and hypophosphatemia resembling that of humoral hypercalcemia of malignancy seen in some breast cancer tissues and some hematologic cancers such as adult T-cell leukemia. The mutant receptor seems to be constitutively active in Jansen's metaphyseal chondrodysplasia and its actions appear to be independent of PTH and PTHrP. When the authors compared the PTH and PTHrP receptors containing the H223R mutation with those containing the T410P mutation, they found that receptors containing the T410P mutation had significantly higher ligand-stimulated accumulation of AMP and inositol phosphate and that receptor activation (receptor function) was independent of PTH and PTHrP. Although there were differences in receptor functions between the 2 types of mutations, the manifestations of the disease were similar with both types of mutations in affected individuals. The 2 individuals without identifiable mutations had somewhat milder disease, with less severe hypocalcemia, normal serum phosphorus and alkaline phosphatase activity, normal serum PTH concentrations, and normal urinary cAMP excretion.

Schipani E, et al. *N Engl J Med* 1996;335:708-714.

**Editor's comment:** The discovery of this constitutively activating mutation has brought to light yet another physiologically important role of PTHrP in fetal bone growth and cellular differentiation. With recent advances in molecular biology as well as in tissue engineering, perhaps it may be possible to correct the abnormality during embryonic and fetal development by in utero gene manipulation and thus eliminate the disorder. This also may be true for some other activating receptors in disorders of growth and other metaphyseal dysplasias.

Judith G. Hall, MD



## A Placebo-Controlled, Double-Blind Trial of Growth Hormone (GH) Treatment in Prepubertal Children After Renal Transplant

Although it has been shown that GH therapy can increase growth velocity (GV) in children with chronic renal failure, similar data have not been shown in prepubertal children following renal transplantation. The initial increase in growth rate following transplantation declines such that up to 70% of prepubertal children do not show significant catch-up growth in the first 2 years after transplantation. The most likely cause of this is prolonged immunosuppressive therapy.

Hokken-Koelega et al, used a double-blinded, placebo-controlled, 6-month cross-over trial of biosynthetic human GH (hGH) in 11 prepubertal patients (9 boys and 2 girls) post renal transplantation. Inclusion criteria included (1) at least 12 months post transplantation; (2) no rejection episodes within the last 6 months; (3) height standard deviation score (SDS) for chronologic age below -1.88; (4) height velocity (HV) for chronologic age below the 50th percentile or a height SDS for chronologic age above -1.88 with a HV below the 25th percentile; (5) prepubertal; (6) bone age (BA) < 10 years for girls and < 12 years for boys; (7) prednisone dose not exceeding 0.25 mg/kg/d; (8) normal thyroid function studies; (9) normal acid-base balance; (10) no previous treatment with sex steroids; and (11) no evidence of specific reasons for growth retardation other than renal transplantation.

Subjects had a mean age of  $12.1 \pm 2.9$  years, with a range of 8 to 18 years. Immunosuppressive therapy consisted of prednisone with either azathioprine or cyclosporine, or a combination of both. Children were randomly and blindly assigned to receive 1 subcutaneous injection a day of either

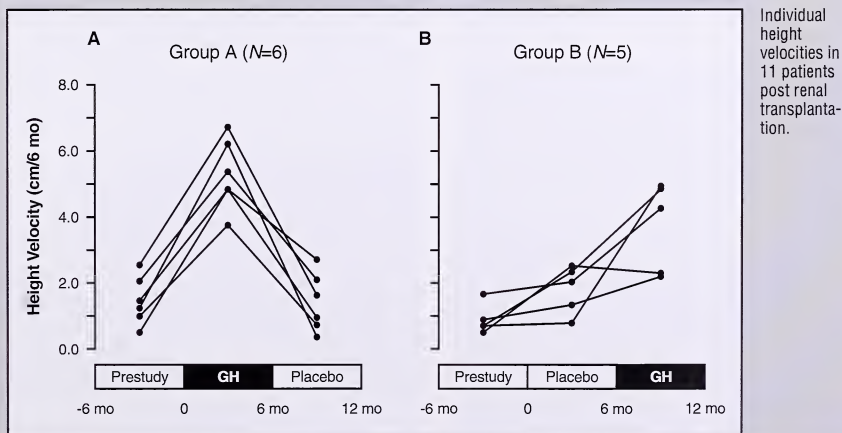
biosynthetic hGH (4 IU/m<sup>2</sup>, roughly equal to 0.05 mg/kg) or an equal volume of reconstituted placebo. Patients were measured and weighed every 3 months. Mean height was expressed as SDS for CA, as was HV. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), glucose, and oral glucose tolerance were determined at 0, 6, and 12 months, and insulin-like growth factor 1 (IGF-1), IGF-2, IGF-binding protein 1 (IGFBP-1), and IGFBP-3 were measured.

Children on biosynthetic hGH therapy increased their HV significantly more than those receiving the placebo:  $5.3 \pm 1.0$  cm per 6 months versus  $1.9 \pm 0.7$  cm per 6 months;  $p < 0.0001$  (see Figure 1). The mean increase in HV during biosynthetic hGH treatment was significantly higher for those who started the trial with biosynthetic hGH than for those who started with placebo. No significant increase in bone maturation occurred during the study. Mean IGF-1 increased significantly while the concentration of IGF-2 and IGFBP-3 did not. No patient had an acute rejection episode, and there were no differences in GFR or ERPF between the 2 groups.

The authors state that their data show that synthetic hGH therapy results in an improvement in HV in prepubertal children with growth retardation after renal transplantation. Long-term studies are necessary in order to determine whether the effect will be sustained and whether there will be significant improvement in final height.

Hokken-Koelega A, et al. *Kidney Int* 1996;49(suppl 53):S128-S134.

Figure 1





**Editor's comment:** This is an important study but the data should be viewed as preliminary. The lack of significant changes in plasma IGF-2 and IGFBP-3 may have been due to the relatively small number of individuals studied since trends were evident. In addition, the short length of the study may have contributed to the lack of failure to demonstrate any significant changes in skeletal maturation secondary to biosynthetic hGH therapy. Finally, it may not be appropriate to report HV for CA when the subject population includes individuals as old as 18 years who are prepubertal. Despite these

shortcomings, the authors should be commended for utilizing a placebo-controlled trial of biosynthetic hGH in prepubertal children. Their data are encouraging, and we look forward to further data as they continue to use biosynthetic hGH therapy in these children.

The reader is referred to the lead article by Fine in GGH (1996;12[4]:49-53) regarding the use of biosynthetic hGH over several years in patients with chronic renal failure.

William L. Clarke, MD

## Serum Leptin in Children With Obesity: Relationship to Gender and Development

The aim of this study was to investigate whether leptin can be detected in the serum of obese children; to determine whether it directly correlates with body fat, as it does in adults; and to identify any variations of leptin concentration in relation to gender, race, and growth and development. All values were expressed as mean  $\pm$  SD.

Seventy-seven children (44 girls and 33 boys; mean age  $11.3 \pm 4$  years) with obesity (body mass index [BMI])  $> 95$ th percentile; mean BMI  $34.4 \pm 7.6$  kg/m<sup>2</sup>, and 30 normal weight children (20 girls and 10 boys; mean age  $13.3 \pm 3.5$  years and mean BMI  $18.9 \pm 3.1$  kg/m<sup>2</sup>) were studied.

Serum leptin was measured by radioimmunoassay in all study subjects in a blood sample obtained after an overnight fast. Children in the control group were slightly older than the obese children but differences in Tanner staging were not significant. Serum leptin in the obese group was significantly higher than that in the control group ( $38.6 \pm 21$  ng/mL vs  $7.8 \pm 6.5$  ng/mL). All children had detectable leptin concentrations. The range of leptin concentrations in the obese group was 4.9 to 84.6 ng/mL. Serum leptin directly correlated with BMI and upper-arm fat area analysis in the combined (obese

and control) group ( $r=0.88$ ,  $P<0.001$  and  $r=0.88$ ,  $P<0.001$ , respectively). This is similar to the correlation described in adults. A mild direct correlation ( $r=0.51$ ,  $P<0.001$ ) was found between serum leptin and fasting insulin levels. Girls demonstrated higher serum leptin levels than boys. The effects of sexual development on leptin concentration were also positive. Subjects with advanced Tanner stages displayed lower leptin levels than earlier Tanner stages, independently of adiposity.

There were no effects detected among different races independent of the estimate of body fat. The authors conclude that, as in adults, obese children have high concentrations of leptin, which directly correlates with arm fat and BMI. They hypothesize that a relative central "leptin resistance" is part of the normal process of growth and development during childhood.

Hassink SG, et al. *Pediatrics* 1996;98:201-203.

**Editor's comment:** A new world order for pediatric endocrinologists is now upon us. The fat tissue is the source of leptin. This paper is only the beginning of what will be forthcoming in this area. The authors' interpretation of the inverse relation between leptin levels and the degree of sexual maturation independent of the adiposity leads to the hypothesis that children display a relative central "leptin resistance,"

### In Future Issues

#### Tyrosine Kinases and Cancer

Brian Druker, MD

#### GH Secretagogues

Allen Root, MD

#### Insulin, the IGF System, and Insulin-Dependent Diabetes Mellitus

Cheri Deal, MD

#### The Pathophysiology of Growth Failure in Renal Disease: An Update

David Powell, MD

#### Genetic Basis of Human Chondrodysplasias: A Review

William Horton, MD

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which, if true, would favor an increased energy intake necessary for growth and development. A switch to an increased leptin sensitivity would occur at the end of puberty, signaling the end of growth. Even though the finding of lower leptin levels at more advanced pubertal stages found in this study is appealing to formulate that hypothesis, more direct evidence of such a relation is necessary. The perpetuation of this "physiologic" leptin resistance beyond the time of full pubertal development as a result of a disturbance in the switch to normal leptin sensitivity could be a theoretical cause for the development of obesity in early adulthood. However, this would not explain the cases of childhood obesity.

The greater levels of leptin in girls than boys, independent of adiposity, led to the hypothesis that central leptin resistance in girls may be necessary for the accumulation of the adipose tissue stores necessary for reproduction. This is an attractive thought, but the evidence provided to support this hypothesis is very indirect.

The study of leptin levels in children will provide clues on the interactions between adipose tissue and the endocrine system in the developing individual. Note that 2 articles concerning leptin recently were abstracted in GGH (1996;12[2]: 31-32). Readers may wish to refer to these abstracts.

Fima Lifshitz, MD

## Growth Hormone (GH) Replacement in Healthy Older Men Improves Body Composition But Not Functional Ability

The objective of the study was to determine whether GH replacement in older men improves functional ability. The design was to perform a randomized, controlled, double-blind study utilizing 52 healthy men >69 years old with well-preserved functional ability but low baseline insulin-like growth factor 1 (IGF-1) levels. Recombinant human GH (rhGH; 0.03 mg/kg of body weight) or placebo was given 3 times a week for 6 months. Body composition, knee and hand grip muscle strength, systemic endurance, and cognitive function were measured.

At 6 months, lean mass increased on average by 4.3% and fat mass decreased by an average of 13.1% in the rhGH-treated group, in contrast to slightly decreased percentages in the placebo-treated group. No statistically or clinically significant differences occurred in knee or hand grip muscle strength, physical performance, systemic endurance, or cognitive function or mood as measured by the Geriatric Depression Scale, the Mini-Mental Status Examination, the Digit Symbol Substitution Test, and the Trails B Test, which is a neuropsychological test battery assessing visual and motor tracking and attention.

The dose of rhGH was considered physiologic replacement for young adults, and IGF-1 levels were maintained between 190 and 350 ng/mL by adjusting the doses. A dose decrease was necessary in 26% of the rhGH-treated group versus 0% of the control group because of side effects, which usually developed in the first month of treatment. The most common side effects were pitting lower extremity edema and diffuse arthralgias.

The authors conclude that the data do not support the hypothesis that an age-related decline in GH secretion is responsible for the functional decline of aging. Because of the lack of demonstrable efficacy in the study sample, coupled with the frequent side effects and substantial expense, rhGH should not be used to preserve or improve functional ability in healthy, functionally intact older men.

**Editor's comment:** Some readers may argue that the use of rhGH in the elderly does not deal with growth or genetics, and I would agree they are correct. However, the use of rhGH in the elderly deals with the possible use of hormones to prevent or reverse aging, and is of enough significant interest to endocrinologists, particularly older ones, to prompt publication of this abstract.

Rudman et al (N Engl J Med 1990;323:1-6) also reported an increase in lean body mass and a decrease in fat mass when rhGH was given to aging but otherwise normal men. Appearances before the media by aging study subjects and participating investigators conveyed the impression that rhGH was a positive force on endurance, energy, and sense of well-being. The studies reported in 1996 by Papadakis et al do not support the impressions conveyed by the media regarding Rudman et al's studies. As a proband in a similar study in (1982 through 1985), along with 4 other aging men, I can add from my own experience and from conducting body composition studies on the other 4 men that the changes in both physique and psyche were equivocal at best. Therefore, I concur with the conclusions of Papadakis et al: "GH should not be used to preserve or improve functional ability of aging men." Further studies may prove otherwise but the burden of contradicting this conclusion remains a heavy one.

Robert M. Blizzard, MD

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Papadakis MA, et al. *Ann Intern Med* 1996;124:708-716.

**Post-Program Self-Assessment/CME Verification**  
**Post Self-Assessment Test Questions**

**Instructions:** The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The CNS element that alters sensitivity to pain and may influence the emotional tone of an individual is:
  - a. the amygdala/hippocampus complex.
  - b. the arcuate nucleus opioid peptide-secreting neurons.
  - c. the mesocortical dopamine system.
  - d. the mesolimbic dopamine system.
2. Stress-induced melancholic depression is characterized by all but which one of the following symptoms:
  - a. anorexia
  - b. hypotension
  - c. loss of libido
  - d. anxiety
  - e. tachycardia
3. Chronic activation of the HPA axis and the sympathetic system:
  - a. causes melancholic depression in children and adolescents.
  - b. may cause seasonal affective disorder and postpartum depression.
  - c. is implicated as a cause of anorexia nervosa, malnutrition, and panic disorder in young adults.
  - d. is evident in patients with chronic fatigue and fibromyalgia syndromes.
  - e. all of the above
4. Choose the correct answer:
  - a. Chronic HPA axis activation has little effect on gonadal function in highly trained athletes.
  - b. CRH and the HPA axis are potential mediators of gender-related responses to stress.
  - c. Lower concentrations of thyrotropin and  $T_3$  hormones result from excessive and prolonged activity of the stress system.
  - d. b and c above
  - e. All of the above
5. In infantile and childhood malnutrition, GH secretion is:
  - a. increased, inducing hyposecretion of IGF-1.
  - b. decreased, possibly resulting from starvation.
  - c. about the same as in well-nourished patients.

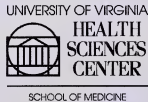
1. B 2. B 3. C 4. D 5. A  
Answer Key

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Dr. Chrousos reports no conflicts; Dr. Lifshitz reports no conflicts; Dr. Clarke reports no conflicts; Dr. Horton reports no conflicts; Dr. Root serves on Genentech's National Cooperative Growth Study (NCGS) Advisory Committee; Dr. Hall reports no conflicts; Dr. Blizzard is the President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

Vol. 13 No. 2

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## Limb Lengthening in the Skeletal Dysplasias and Short Stature Conditions: State of the Art in 1997

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*Detroit, Michigan*

### INTRODUCTION

Limb lengthening was first described in the Western literature in 1905 by Codivilla.<sup>1</sup> Until relatively recently, the indications for limb lengthening were largely confined to leg length discrepancies due to congenital or acquired conditions.

### HISTORICAL ASPECTS

Limb lengthening as a technique has evolved over the last 8 decades and has involved a variety of external distraction methods. Until the introduction of the Ilizarov technique, limb lengthening was achieved by osteotomy and relatively rapid distraction of the bone ends (1 to 2 mm daily).<sup>2</sup> A gap resulted, usually requiring bone grafting and plate application. The most popular of these techniques, the Wagner method, was widely used in North America.<sup>3</sup> Numerous publications outlined complications such as hypertension, joint displacement or stiffness, compartment syndrome, and nerve palsy. Bone problems, including delayed union or non-union and osteomyelitis, were common. Furthermore, patients required a third operation for plate and screw removal and the prolonged hospitalization of these patients often had significant psychosocial ramifications.

As Wagner popularized his techniques, a Siberian surgeon, Gavriil Ilizarov, was developing a new

### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

### In This Issue

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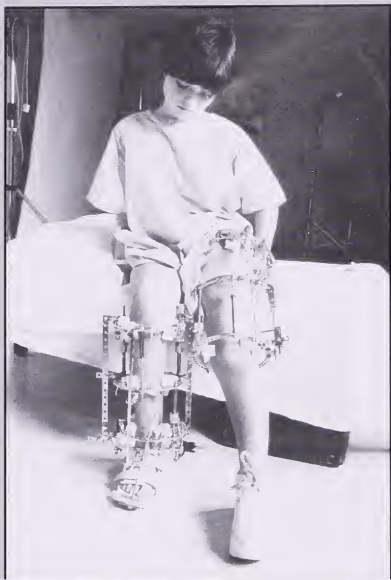
**Abstracts** ..... page 23

**GGH Glossary** ..... insert

**CME Information** ..... page 32



Figure 1



Clinical appearance during initial left femoral and right tibial Ilizarov lengthening.

biology of limb lengthening.<sup>4</sup> As with prior methods, the technique involved the use of an external fixator. This fixator of the bones (Figure 1) differed from previous devices. It was composed of metal rings, and stability was achieved via tensioned transosseous wires. The bone was cut through a small (~1 cm) incision, with care being taken to avoid disruption of the periosteal blood supply. Gradual incremental distraction of no more than 1.0 mm daily in 3 to 4 increments resulted in new bone formation in the developing distraction gap. Once the desired bone length was achieved, the fixator was left in place until the bone consolidated. The total treatment time was roughly 1 month per centimeter of length gained. Generally, no immobilization was used following fixator removal. As opposed to the other methods, this technique encouraged weight-bearing and patient activity during treatment, thus promoting more rapid bone healing and patient rehabilitation.

The advent of this new incremental method of bone lengthening preserved bone biology, thus eliminating the need for bone grafting and plate

application, and bone complications were significantly reduced. Other surgeons in Europe, North America, and Great Britain have devised other types of external fixators.<sup>5,6</sup> Regardless of the device, however, the current state of the art of limb lengthening involves gradual incremental distraction of the bone ends to allow new bone formation in the evolving gap.

## COMPLICATIONS OF LIMB LENGTHENING

Early enthusiasm for the Ilizarov technique after its introduction to North America in 1986 saw many surgeons avidly applying the technique to an expanded list of pathologies, including short stature conditions. As with many new techniques, this early enthusiasm has been tempered by the realization that a multitude of potential complications remain.

Despite the improvement in bone healing, the Achilles heel of limb lengthening continues to be the effects on the soft tissue envelope surrounding the bone and the adjacent joints. Injuries to muscle and adjacent joints due to distraction have been described in both clinical and experimental settings.<sup>7</sup> Joint stiffness due to intra-articular cartilage injury and joint dislocation or subluxations are still problematic despite controlled gradual bone distraction.

## LIMB LENGTH INEQUALITY

From an orthopedic perspective, the most common indication for limb lengthening is the patient with an actual or predicted leg length inequality of  $\geq 5$  cm. Smaller discrepancies and patients without limb deformity and normal stature are still most safely treated by appropriately timed epiphysiodesis or by femoral shortening.

Limb length inequality and limb deformity are common in the skeletal dysplasias.<sup>8,9</sup> The most common dysplasias causing inequality of limb length are fibrous dysplasia and Ollier disease. Bone formation occurs quite readily in these conditions, and bone in the lengthened limb ultimately mimics the quality of the bone in the original area of osteotomy. Conradi-Hünemann chondrodysplasia punctata also can be associated with limb length inequality. Successful limb lengthening has been reported in this condition and in Silience types I and IV osteogenesis imperfecta, although this should be undertaken cautiously in the latter due to the abnormal fragility of the bone and slow bone formation. Neurofibromatosis is commonly associated with limb length inequality. Surgery should be approached cautiously in these patients as the short limb may have very abnormal bone, such as in congenital pseudarthrosis of the tibia, which will not heal predictably or remain healed.

Patients with congenital limb hypoplasia syndromes can be appropriate candidates for limb lengthening, provided that the adjacent joint stability and function can be maintained and that the foot is a suitable plantigrade weight-bearing surface. Femoral hypoplasia includes the spectrum from mild shortening to subtotal absence of the femur. Femoral hypoplasia and less severe forms of proximal femoral focal deficiency, in which the hip is stable or can be made stable surgically, are amenable to one lengthening or a series of lengthenings as indicated by the extent of the predicted limb length inequality. All of these disorders have intrinsic knee instability that can be managed if lengthening is done carefully.

Distal deficiency, particularly fibular hemimelia, also can be managed by limb lengthening. Foot abnormality often accompanies this condition. However, if there are 3 or more rays in the foot, a reasonable result can be anticipated. The milder forms of tibial hypoplasia may also be manageable by limb lengthening. Due to the rarity of this condition, there are currently no reported series of limb lengthenings for tibial hemimelia. Severe fibular hemimelia and tibial aplasia are still best managed by amputation.

## LIMB LENGTHENING IN SHORT STATURE

The first reference to limb lengthening for short stature was by Bier in 1923.<sup>10</sup> Since that time it has gained significant popularity in Europe, particularly Italy and Spain. The effects of short stature on psychological development are well documented.<sup>11-13</sup> A number of issues must be considered prior to undertaking limb lengthening. These include: the diagnosis; which limb segments are involved; and how much should the leg be lengthened and when. One of the most important factors to consider from an outcome perspective is the natural history of each disorder.<sup>14</sup> The epiphyseal dysplasias such as pseudoachondroplasia and spondyloepiphyseal and multiple epiphyseal dysplasia all characteristically have a natural history of early degenerative joint disease. Thus, a procedure such as limb lengthening, which may negatively impact on joint function, must be undertaken cautiously to avoid precipitating early osteoarthritis.<sup>15</sup>

Patients with metaphyseal dysplasias such as the Schmid and McKusick types of chondrometaphyseal dysplasia may be appropriate candidates for lengthening. However, there are no reported series involving lengthening specifically in patients

Figure 2



Radiographs at the conclusion of stage 1 (7 months postoperative), with 10 cm of right tibial and 8 cm of left femoral elongation.

Figure 3



Radiographs taken 6 weeks following initiation of stage 2, right femoral and left tibial lengthening.

with these syndromes or those with epiphyseal dysplasia. Patients with Turner syndrome who have normal intelligence also represent good candidates for lengthening. Again, there are no published and very little accumulated data in this group of patients.

Patients with achondroplasia and hypochondroplasia are probably the ideal candidates for limb lengthening (Figures 1 through 5). Their joints are intrinsically normal and they have significant trunk-limb disproportion. One of the first English language publications concerning lengthening for short stature was by Saleh et al in Sheffield, England.<sup>14</sup> They reported surgery on 28 patients, 17 of whom had achondroplasia. Three had hypochondroplasia and the other 8 had other disorders. Ninety-four lower limb segments were lengthened an average of 9.6 cm. A variety of techniques were used, including the Ilizarov technique. The overall complication rate per limb segment was 71%. Of these complications, 49% were considered to be of moderate severity and the other 51% of less severe degree. Therefore, 1 out of 3 patients had moderate but significant complications.

Cattaneo et al<sup>16</sup> reported humeral lengthening in 29 patients, aged 10 to 36 years. Of these humeral lengthenings, 14 were in patients with achondroplasia.

Total arm lengthening averaged 9 cm. All final results were excellent or good, with few complications. This currently remains the only English reference known to this author discussing elongation of the upper extremities in short stature conditions. This author's personal experience has been extremely favorable in lengthening the humeri of such patients, with complications limited to superficial pin site infection and, in one instance, pin loosening.

The results of Cattaneo et al's series of 37 lower extremity lengthenings in achondroplasia were also quite favorable.<sup>6</sup> Increases of 14 to 18 cm were achieved in patients who underwent femoral and tibial lengthening. The paper reported few serious complications, although critical review of ultimate functional outcome was lacking. The most significant reported complications were deformation of the bone after fixator removal; bowing and valgus deformity of the tibia; and equinus contractures at the ankle.

Both the Vilarubias technique of limb lengthening for achondroplasia and the DeBastiani technique have gained wide popularity in Europe.<sup>5,17,18</sup> Both techniques utilize uniplanar external fixators. The former, however, stresses non-weight-bearing wheelchair existence during treatment. Although

Figure 4



A left knee flexion contracture obscured mild valgus deformity of the left tibia during lengthening that was cosmetically unacceptable to the patient.

Figure 5



Clinical and radiographic appearance following left tibial osteotomy at the final height of 5 ft 1 in.



results have reportedly been excellent, this encourages loss of function and independence. In North America, Price<sup>19</sup> has reported stature lengthening in achondroplasia using the DeBastiani technique in carefully selected patients, achieving good results and a mean height increase of 15.4 cm.<sup>19</sup>

A recent report<sup>20</sup> in 1995 discussed staged lengthening in the prevention of dwarfism in children. These authors utilized 2 separate operations on the tibiae at the ages of 5 and 10 years and 2 on the femora at 6 and 12 years. Most surgeons previously have operated primarily on older children and adults when doing bilateral lengthening for short stature. Six children reportedly had completed the first 3 stages with a total increase in length of 18 to 23 cm. This was a preliminary report and a follow-up report in a few years will be very helpful.

Issues that have not yet been adequately addressed with respect to lengthening in short stature are the ultimate functional outcomes and the effect of lengthening on limb growth. Large lengthenings put significant stress on both adjacent joints and, when open, the adjacent physes because of the increased soft tissue tension developing during distraction. The potential negative effects on joints have been discussed. Whether this stress can in fact inhibit physeal growth remains controversial in the human situation. In experimental animals, various effects, including growth stimulation and inhibition, have been reported. Maintenance or improvement in function is the goal of most surgical interventions. Limb lengthening should be no exception. However, to date, there are no adequate functional outcome studies related to limb lengthening in patients with short stature conditions.

The specific strategy adopted for limb lengthening in patients with short stature must be directed toward the goals for each individual. Ultimate height and limb proportion must be considered before developing a scheme. Patients with mesomelic dysplasia often elect only bilateral tibial lengthening, as 8 to 10 cm of length can be achieved with normalization of lower limb proportions. This strategy also may be used for patients with hypochondroplasia or Turner syndrome who require only 3 to 4 inches of height gain.

In those patients who elect more substantial lengthenings involving femora, tibiae, and, perhaps, humeri, there are several possible strategies. Bilateral tibial lengthening using either circular or cantilever fixation is generally well tolerated. This can be followed by 1 femoral lengthening and then by lengthening of the contralateral femur. In general, bilateral femoral lengthening is impossible if circular fixation is used. Occasionally, modest

**Editor's comment:** *The policy of GROWTH, Genetics, & Hormones is to not call attention to texts or similar publications, which might be interpreted as advertising. However, because there is very limited information published in English regarding limb lengthening, your attention is called to one publication that is important reading for those who are interested. This is Limb Lengthening: For Whom, When & How? edited by Z. Laron, S. Mastragostino, and C. Romano and published by Freund Publishing House Ltd, London-Tel Aviv. The content consists of presentations by experts on limb lengthening. GROWTH, Genetics, & Hormones will make this book available for a 1-week period. If the book is not mail-stamped for return within 10 days of receipt, there will be a \$50.00 charge for each week the book is overdue. Requests should be made with Ms. Juanita Bishop or staff at the Genentech Foundation for Growth and Development (telephone 804-977-8192; fax 804-977-9450).*

Robert M. Blizzard, MD  
Editor-in-Chief

bilateral femoral lengthenings can be achieved using cantilever fixation. It is possible to do "crossed" lengthenings with either type of fixation. Adapting this scheme (see the case history below), 1 femur and the contralateral tibia are lengthened with either type of fixation. Eventually, bilateral humeral lengthening, which is very well tolerated, is performed. However, humeral lengthening should not be done during lower extremity lengthenings, since crutch or walker use is impossible.

### Case History

The patient depicted in Figures 1 through 5 is a skeletally mature 14-year-old girl with hypochondroplasia. Her preoperative height at 14 years was 4 ft 5 in. Figure 1 shows her clinical appearance during initial crossed lengthenings of the left femur and right tibia using the Ilizarov technique with circular fixation. The first stage of lengthening, including distraction and consolidation, took approximately 7 months to complete. Figure 2 consists of radiographs taken at the conclusion of her first lengthening, with 10 cm of tibial and 8 cm of femoral elongation. Six months later, she embarked on her second crossed lengthenings of the right femur and left tibia. Figure 3 consists of a radiograph taken 6 weeks after initiation of these lengthenings.



A left knee flexion contracture developed during lengthening, obscuring a mild valgus deformation of the left tibia that developed during lengthening. This led to the deformity noted in Figure 4, which was cosmetically unacceptable to the patient. She subsequently underwent a left tibial osteotomy with elimination of the deformity. Figure 5 shows the final clinical and radiographic appearance. It is now 8 years since the completion of treatment.

## DISCUSSION

The indications for limb lengthening in limb length inequality are clear. The indications for limb lengthening in short stature conditions remain controversial. The decision must be based on both psychological and physical factors. As discussed by Peretti,<sup>20</sup> Saleh,<sup>14</sup> and others, a lengthening "plan" must be developed. This includes the decision concerning which segments to lengthen and how the lengthening will be staged.<sup>20</sup> Epiphyseal dysplasias should be approached with extreme caution. However, individuals with short stature should have the choice of what are now well-established techniques in order to enhance appearance and improve function. The decision of when to perform lengthening procedures should involve the patient, not just the physician, surgeon, or parent. The author's approach to those patients seeking limb lengthening for short stature has been a team effort, involving the services of social workers, psychologists, physical therapists and consultations with other patients and families who have undergone the procedure.

Despite the technical advances in limb lengthening in North America over the last decade, there appears to be only moderate enthusiasm for limb lengthening in conditions with short stature. This is due to potential surgical problems and societal factors. In North America, as opposed to some European countries, it appears that society is more accepting of physically "different" individuals. Nevertheless, our environment is built for individuals of normal stature. Limb elongation for significant short stature cannot be considered merely cosmetic. Use of public facilities, including bus stops and washrooms, is extremely difficult for patients under 4 ft 6 in. Many patients cannot get onto toilets easily or use public sinks, drinking fountains, or cafeteria counters. These factors, however, must be weighed against the potential difficulties and complications of limb lengthening. It is difficult to lengthen both tibiae and femora without any complications and to be able to have them of equal length and appropriately aligned.

Finally, patient and family motivation and involvement must be considered. A strong commitment to this long and arduous process is necessary in order to achieve excellent functional and cosmetic results.

In 1997, the ideal short-statured candidate for limb lengthening is one for whom normal or nearly normal height can be achieved or for whom substantial lengthening will greatly enhance activities of daily living. The patient should have normal or nearly normal joints and adjacent musculature and must be psychologically prepared for and committed to a long, arduous process. Despite potential complications, the results to date have been encouraging, and continued judicious application of these techniques is warranted.

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## Letter From the Editor

In *GROWTH, Genetics, & Hormones* (12 [3]:44), an article by Thalange et al, entitled "IGFBP-3 Generation: An Index of Growth Hormone Insensitivity," was published. Dr. Lifshitz and I each commented, and invited the authors to respond to our comments. They have done so, and their letter follows. You, the reader, may wish to refer to the original abstract and comments to better understand Dr. Clayton's reply.

Robert M. Blizzard, MD

## Letter to the Editor:

There has been much recent interest in the clinical characterization, biochemical definition, and molecular analysis of the GH receptor (GHR) in congenital GH insensitivity (Laron syndrome). The classic biochemical criteria are not necessarily fulfilled by all patients who have the condition, and marked heterogeneity exists within the clinical phenotype and the degree of height retardation. This has instigated studies screening for GH receptor mutations in children classified as having idiopathic short stature (ISS). The finding of various heterozygous GH mutations within this population suggests that minor dysfunction within the GH receptor or its signaling pathways may be relevant to their short stature.

Our study, performed prior to the first reports of heterozygous GHR mutations in ISS, was intended to assess GH sensitivity (by IGF-1 and IGFBP-3 generation) in patients who were short (height SDS < -2) but had an arbitrarily defined high basal and/or peak GH level. Their bone ages were similar to chronologic age or slightly delayed, and 2 patients were in early puberty. A control group with GH deficiency was used for comparison.

Our data demonstrated the marked variability in response to acute administration of GH in both groups. This provides evidence for a wide

spectrum of individual responsiveness to GH, regardless of the etiology of the short stature. The finding of GH receptor mutations in children with ISS, whose auxology is similar to those included in our proposed GH-insensitive group, adds further support to the concept of a range of GH sensitivity. We were not surprised, therefore, that we have not been able to define a precise biochemical tool to detect partial GH insensitivity. We suspect the definition of the latter from a biochemical perspective will prove as difficult as the definition of "partial GH insufficiency." Molecular analysis not only of the GH receptor, but also of other molecules involved in GH signaling is more likely to help us precisely locate the site(s) of GH insensitivity. Some children with unexplained short stature, in fact, may have a dysfunctional GH transduction pathway. An IGF-1/IGFBP-3 "generation test" can only provide useful supportive but not definitive evidence for such a diagnosis.

We appreciate the opportunity to respond to the editorial comments in the review of our article published in *GROWTH, Genetics & Hormones*.

Yours sincerely,  
Dr. P. Clayton  
Senior Lecturer in Child Health  
Royal Manchester Children's Hospital  
N.K.S. Thalange, et al

## Abstracts From the Literature

### A Receptor in Pituitary and Hypothalamus That Functions in Growth Hormone Release

The investigators cloned the endogenous receptor for the synthetic growth hormone-releasing peptides (GHRPs) and nonpeptidyl mimetics of GHRP (termed GH secretagogues [GHSs] by the authors). This receptor is present in the human, chimpanzee, swine, cattle, mouse, and rat pituitary, as well as in the arcuate, ventromedial, and infundibular regions of the primate (rhesus monkey) hypothalamus. There are 2 species of GHS receptors. The major GHS receptor (type Ia) is a 366 amino acid peptide with 7 transmembrane spanning domains coupled to G<sub>11</sub> with apparently very short extracellular (42 amino acids) and intracellular (30 amino acids) domains. GHS receptor type Ib is a 289 amino acid protein with 5 transmembrane domains followed by a 24 amino acid intracellular domain. The GHS receptors have 30% identity with receptors for neurotensin and thyrotropin-releasing hormone (TRH). Radiolabeled MK-0677, a nonpeptidyl GHS, binds avidly to the GHS receptor and is displaced by unlabeled MK-0677, GHRP-2, and GHRP-6 with decreasing potency; GHRH, GnRH, TRH, ACTH, and galanin do not displace the radioligand.

Howard AD, et al. *Science* 1996;273:974-977.

**Editor's comment:** Characterization of the endogenous receptor for the synthetic GHS validates further the presence of a third system involved in the regulation of pituitary GH secretion in addition to GHRH and SRIH. Utilizing the GHS receptor, it is now likely that its endogenous ligand will be identified. It is of interest that the GHS receptor is expressed not only in the pituitary but also in the hypothalamus. This finding confirms previous observations that the GHRPs act at both the pituitary and hypothalamic levels. The endogenous GHSs may be involved in the integration of GHRH and SRIH regulation of pituitary GH synthesis and/or secretion. (Besides the noteworthy scientific accomplishments reported in this paper, it was of interest to count the number of coauthors; 32 were listed.) This indeed was a team effort.

Allen W. Root, MD

## Intrauterine Growth Retardation and Postnatal Growth Failure Associated With Deletion of IGF-1

The authors report the case of a 15.8-year old boy referred for evaluation of short stature and growth delay in whom a diagnosis of GH insensitivity was suggested because of elevated basal and poststimulation GH levels, absent response to rhGH treatment, and low serum IGF-1 concentration. The patient was born at 37 weeks gestation with symmetric IUGR. His birth weight, length, and head circumference were 3.9, 5.4, and 4.9 SD below the mean, respectively. The placental weight was 1.3 SD below the mean. The patient had severe growth failure throughout infancy and childhood. At age 8 years, he underwent evaluation, which showed normal thyroid function tests, normal male karyotype, and elevated serum basal and peak GH levels (18 ng/mL and 94 ng/mL, respectively) after the administration of the clonidine. He received rhGH treatment for 1.7 years, starting at age 11 years, with no effect on his growth rate. IGF-1 levels performed at age 14 years were markedly below normal range (0.05 U/mL, normal for age = 0.48 to 2.8 U/mL). The patient was diagnosed with profound bilateral sensorineural deafness, moderately delayed motor development, hyperactivity, and short attention span. Additionally, some dysmorphic features were recognized, including micrognathia, bilateral ptosis, low hairline, and bilateral clinodactyly. The patient's parents were first cousins once removed. His father, mother, and 10-year-old sister had less severe growth impairment, with heights 1.8, 1.4 and 1.0 SD below the mean, respectively. Subsequent endocrine tests performed on the patient showed normal fasting blood glucose, thyrotropin, prolactin, and cortisol, and pubertal levels of DHEAS although pubic hair was at Tanner Stage 1. The gonadotropin response to GnRH was pubertal (patient was at Tanner Stage 2 for genitalia). The testosterone response to hCG was significant, and the GH peaks on the overnight GH secretion test were supernormal. A normal basal level of 2.2 ng/mL, but high poststimulation of

61 ng/mL levels of GH, were present. There was an absent response of IGF-1 in the generation test and normal IGF-2, IGFBP-3 and GHBP levels. Brain MRI studies were essentially normal, and electrophysiology studies of the CNS were also normal. Detailed DNA studies using PCR and reverse transcriptase PCR were able to identify homozygosity for the D12S346 polymorphism, consisting in the partial deletion of the IGF-1 gene at the level of the exons 4 and 5, and heterozygosity for such polymorphism in both parents and his sister. Both parents and his sister had low-normal levels of IGF-1 and normal IGF-2 and IGFBP-3.

Woods KA, et al. *N Engl J Med* 1996;335(18):1363-1367.

**Editor's comment:** *It has been known that GH has no direct impact on intrauterine growth. Indirect evidence suggests that insulin, IGF-1, and IGF-2 are the possible mediators of intrauterine growth. This report yields evidence for the pivotal role of IGF-1 in the process of intrauterine and postnatal growth. Unfortunately, this patient was not treated with IGF-1 to ascertain the growth response to this hormone. The coexistence of postnatal growth failure with a history of IUGR should prompt us to think of problems in the expression or action of IGF-1. IUGR patients who do not exhibit catch-up growth need to be assessed, as was this patient. Another interesting finding of this case is the description of less severe growth retardation in the parents and the sister of the proband, all of whom had normal levels of IGF-1, IGF-2, and IGFBP-3. We must ask: "How many individuals do we see with moderate growth failure and without definitive GH alterations who might be cases of heterozygous deletions of portions of the IGF-1 gene?"*

Fima Lifshitz, MD

## Newborn Screening Fact Sheets

The Committee on Genetics of the American Academy of Pediatrics has developed and published fact sheets regarding newborn screening that are very useful resources for physicians dealing with children who have metabolic disorders. These guidelines were designed to help physicians understand and interpret the various tests employed for newborn screening. They take into consideration the variations in screening procedures in different states, and give information concerning early detection, treatment, and follow-up of infants with metabolic disorders. For the purpose of counseling and referral, the information also covers the identification of asymptomatic "carrier couples."

The availability of newborn screening is discussed, and professional and public educational materials are suggested. References for additional reading, notes on early hospital discharge, and costs in each state also are provided.

There is detailed information about biotinidase deficiency; maple syrup urine disease; congenital adrenal hyperplasia; congenital hypothyroidism; cystic fibrosis; galactosemia; homocystinuria; phenylketonuria; sickle cell disease; toxoplasmosis; and tyrosinemia. For each condition, the following are reviewed: State Newborn Screening Availability; Brief Clinical Description; Genetics (including chromosomal map location, incidence, inheritance, racial and ethnic variability,



SCHOOL OF MEDICINE

## ***GROWTH, Genetics, & Hormones***

### **Volume 13, Number 2**

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# GROWTH

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## Genetics & Hormones

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Dear Colleagues:

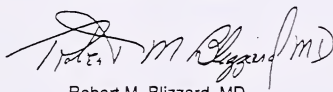
The field of genetics is where the action is today. The members of the Editorial Board have been aware for some time that the fields of genetics, pediatric endocrinology, nutrition, and growth are intimately intertwined. This knowledge prompted establishment of *GROWTH, Genetics, & Hormones* to stimulate and facilitate intellectual exchange of important knowledge among these disciplines. Drs. William Horton and Judith Hall have been key in representing members of the genetic subspecialty on our Editorial Board. The glossary that you are holding in your hands, which is an updated revision of the one first published in *GGH* Vol 9, No. 1, 1993, results from their efforts to simplify and interpret terms that recently have appeared in the vocabulary of geneticists. This they have done to permit us to more readily understand that which we read. We thank them for their effort and contribution.

We also thank Genentech, Inc. for the additional funds placed in our educational grant so this glossary can be brought to you.

Please note that this glossary is physically separate from the remainder of the publication. This is by design to permit you to readily access the information in the glossary when you need it in interpreting the articles that you will read in the future, both in *GROWTH, Genetics, & Hormones* and elsewhere.

We hope this endeavor constructively assists you in quickly understanding more fully the important and pertinent articles that will be appearing in future issues of *GROWTH, Genetics, & Hormones*.

Respectfully,  
For the Editorial Board



Robert M. Blizzard, MD  
Editor

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# GROWTH

## Genetics & Hormones

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### GENETICS GLOSSARY

**acceptor splice site** The boundary between the 3' end of an intron and the 5' end of the adjacent exon.

**alogeneic** The allelic variation seen among members of the same species.

**anticipation** Phenomenon in which the severity of a genetic condition appears to become more severe and/or arise at an earlier age with subsequent generations (seen in many trinucleotide repeat permutations).

**ascertainment** The selection of individuals for inclusion in a genetic study (severity, age of onset, certain features of the trait).

**apoptosis** Programmed cell death; a physiologic process conserved to remove unwanted cells.

**association** In a specific population, the occurrence together of 2 or more different phenotypes more often than expected by chance.

**ATP** Abbreviation for adenosine triphosphate. The energy-yielding molecule in cells that is used to drive chemical reactions.

**autophagy** Digestion of the cell's own organelles.

**autosome** Any chromosome other than the X or Y. Humans have 22 pairs of autosomal chromosomes.

**autosomal disease** A disease encoded by a gene on one of the 22 pairs of autosomes.

**autosomal dominant** A trait that is expressed in an individual who is heterozygous for a particular gene when the mutant allele is on one of the autosomes.

**autosomal modifier gene** A gene that modifies the action of the autosomes.

**autosomal recessive** A trait that is expressed in an individual who is homozygous for a particular gene.

**BAC** See bacterial artificial chromosome.

**backcross** A genetic crossing of a heterozygous organism and one of its homozygous parents.

**bacterial artificial chromosome (BAC)** Artificial chromosome vector derived from bacteria used for cloning relatively large DNA fragments.

**balanced translocation** A rearrangement translocation with no apparent loss or gain of chromosomal material, resulting in a clinically normal but genetically "abnormal" person.

**banding** The differential staining of a chromosome by a variety of techniques that results in a specific pattern of positively and negatively stained bands for each chromosomal pair.

**base analogue** A substance that can mimic the chemical behavior of 1 of the 4 DNA bases.

**base pair substitution** The replacement of 1 base pair by another.

**Barr body** The sex chromatin mass located adjacent to the nuclear membrane in interphase nuclei, which corresponds to an inactivated X chromosome. One Barr body is seen in the cells of 46,XX and 47,XXY individuals, and none in the cells of 45,X and 46,XY individuals.

**Bayesian analysis** Mathematical method for calculating probability of the carrier state in mendelian disorders by combining several independent likelihoods.

**bioinformatics** The discipline of using computers to address information problems in the life sciences; it involves the creation of electronic data bases on genomes, protein sequences, etc.

**bivalent** A pair of homologous chromosomes in association as seen at metaphase of the first meiotic division.

**CAG/CTG repeats** Abbreviation for cytosine-adenine-guanine triplet nucleotide and cytosine-thymine-guanine triplet nucleotide repeats; they are associated with unstable mutations.

**candidate gene** A gene known to be located in the region of interest whose product has biochemical or other properties suggesting that it may prove to be the disease gene being sought.

**candidate gene approach** Strategy to identify disease-associated genes based on finding candidate genes in a chromosome region in which a disorder is mapped.

**cap** A modified nucleotide added to the 5' end of a growing mRNA chain, apparently required for normal processing, stability, and the translation of mRNA.

**cap site** The site of initiation of transcription.

**CAT assay** Reporter gene assay used to measure activity of a promoter under different conditions, such as to define elements of a promoter or to study signals that activate an intact enhancer/promoter. CAT is the abbreviation for the enzyme, chloramphenicol acetyl transferase, the activity of which is measured in the assay.

**cell line** A cultured cell type that can be reproduced indefinitely, ie, immortalized.

**CG island** Unmethylated cytosine-guanine sequences that are often found near the 5' ends of some genes.

**chain termination mutation** A mutation that generates a stop codon, thus preventing further synthesis of the polypeptide chain.

**chromosome aberration** An abnormality of chromosome number or structure.

**clinical genetics** That part of medical genetics concerned with health and illness in individuals and families.

**clinical heterogeneity** Refers here to the production of clinically different phenotypes from mutation in the same gene.

**codominance** The expression of both alleles in a heterozygous individual, eg, presence of both hemoglobin A and S on electrophoresis in an individual heterozygote for sickle-cell disease.

**complementary DNA (cDNA)** DNA synthesized from an mRNA template, using reverse transcriptase.

**complementation analysis** A genetic test for determining whether 2 mutations producing a similar phenotype are allelic.

**concordance** Presence of the same trait in both members of a pair of twins (or set of individuals).

**confined placental mosaicism** Mosaicism that is seen only in the placenta but not in the fetus.

**congenic mouse strain** A strain that differs from another in the region containing 1 genetic locus.

**consultand** Individual seeking, or referred for, genetic counseling.

**contig** A series of contiguous, overlapping, cloned DNA fragments.

**copy number** The number of copies of a transgene integrated into a host genome; used to describe transgenic animals.

**crossing over** Reciprocal breaking and rejoining of homologous chromosomes in meiotic prophase I that results in exchange of chromosomal segments.

**deletion** Loss of part or a whole chromosome or loss of DNA nucleotide bases.

**dicentric** Refers to an aberrant chromosome that contains 2 centromeres.

**diploid** The number of chromosomes in most somatic cells, which is double the number found in the gametes (the haploid number). In humans, the diploid chromosome number is 46.

**discordant** A twin pair (or set of individuals) in which one member exhibits a certain trait and the other does not.

**dizygotic** The product of fertilization of 2 separate eggs by 2 separate sperm; nonidentical twin pair.

**DNA construct** A DNA sequence that has been modified to yield a recombinant DNA molecule.

**DNA ligase** Enzyme that catalyzes religation (reconnection) of 2 fragments of DNA.

**DNA rearrangements** Recombination of DNA segments, eg, in cells of the immune system, the variable (V), diversity (D), and joining (J) regions somatically rearrange to generate functional antibody genes.

**dominant (trait)** Those conditions that are expressed in heterozygotes, ie, individuals with 1 copy of the mutant gene and 1 copy of the normal allele; refers to phenotype.

**double heterozygote** An individual with 1 mutant allele at each of 2 different loci.

**donor site** Guanine-thymidine sequence that defines the splice site at the 5' end of an intron.

**duplication** The presence of an extra copy of chromosome material. At the gene level, this refers to the presence of more than 1 copy of a structured gene, usually having arisen through unequal crossing over. At the chromosomal level, this refers to an unbalanced state in which there may be a triple dose of a portion of an autosome, usually occurring as the result of unequal segregation of a translocation in meiosis (trisomy).

**ecogenetic disorder** A disorder resulting from the interaction of a common environmental factor with a specific genetic predisposition, eg, cigarette smoking causing emphysema in alpha<sub>1</sub>-antitrypsin deficiency.

**electroporation** Application of a short, high-voltage electric pulse to cells in the presence of DNA to permit DNA to enter the cells.

**embryo biopsy** Potential method for preimplantation diagnosis of genetic disorders used in conjunction with in vitro fertilization in which cells are removed and analyzed at a very early stage in embryonic development.

**embryonic stem cells** Cells derived from early embryos that can replicate indefinitely and differentiate into many cell types. Stem cells serve as a continuous source of new cells; they may become incorporated into many tissues to produce chimeric animals when introduced into early embryos, ie, blastocysts.

**empiric risk** Risk of recurrence for multifactorial or polygenic disorders based on family studies (observed risk).

**endonuclease** Enzyme that cleaves bonds between nucleotides of single- or double-stranded DNA or of RNA at specific sequences of nucleotides.

**env gene** Encodes capsule or envelope protein of a retrovirus.

**epigenetic** A factor that changes the phenotype without changing the genotype.

**episome** A plasmid that can exist either independently in the cytoplasm or as an integrated part of the genome of its bacterial host.

**ES cells** See embryonic stem cells.

**EST** See expressed sequence tag.

**exonuclease** An enzyme that cleaves nucleotide chains at their terminal bonds only.

**expressivity** The degree to which a heritable trait is expressed in an individual. "Variable expressivity" refers to the variation in phenotype and in severity produced by the same gene in different individuals.

**expressed sequence tag** A short fragment of an expressed sequence, cDNA, which serves as a landmark for gene mapping.



**expression** The observable effects of an active gene.

**F<sub>1</sub> hybrids** The first generation of animals generated from 2 different inbred strains. These animals are genetically identical to one another but different from either inbred parent.

**F<sub>2</sub> hybrids** The progeny produced from matings between F<sub>1</sub> animals. These animals are different from one another and will contain different mixtures of the genetic variations that were present in the original inbred progenitors.

**flanking sequence** A region of a gene preceding or following the transcribed region.

**footprinting (DNA footprinting)** Assay used to study DNA-binding proteins.

**founder** Refers to animals generated from genetically altered eggs or embryos, ie, eggs microinjected with a transgene.

**founder effect** The high frequency of a mutant gene in a rapidly expanding population founded by a small ancestral group when 1 or more of the founders were, by chance, carriers of the mutant gene.

**gel-shift assay** An assay used to detect specific protein binding to DNA. Such binding creates complexes that migrate more slowly during gel electrophoresis than free DNA. Also known as mobility-shift assay.

**gene family** A group of genes having similar DNA sequence evolved from a single ancestral gene. These genes may or may not be located in the same region of a chromosome.

**genetic code** The base triplets that specify the 20 different amino acids.

**gene flow** Gradual diffusion of genes from one population to another, as a result of migration and intermarriages.

**gene therapy** A strategy in which therapeutic genes are introduced into a person's cells to correct a disease or genetic flaw.

**genetic drift** Random fluctuations in gene frequencies, most evident in small populations.

**genetic heterogeneity** Different mutations causing a similar phenotype; allelic heterogeneity refers to different mutations at the same locus, whereas locus heterogeneity refers to mutations at different loci.

**genetic lethal** A genetic disease that prevents fertility.

**genetic mapping** Determination of the relative positions of genes on a DNA molecule (chromosome or plasmid); distances are measured in linkage units, ie, centimorgans (cM), between them.

**genetic marker** A polymorphic genetic property that can be used to distinguish the parental origin of alleles.

**haplotype** A set of closely linked genes that tends to be inherited together as a unit, as occurs with the A, B, and C loci of the human leukocyte antigen (HLA) gene complex.

**HA** Abbreviation for heteroduplex analysis.

**heteroduplex** Refers to a region of a double-stranded DNA molecule with noncomplementary strands that originated from different duplex DNA molecules.

**heteromorphism** A normal morphologic or staining variant of a chromosome.

**heteroplasmy** The existence of more than 1 mitochondrial type in the cells of an individual, ie, the presence of both normal and mutant mt DNA in a single individual.

**heteroploid** An individual with an abnormal number of chromosomes (as compared to euploid, which is the normal number of chromosomes).

**heterotetramer** A molecule consisting of 4 subunits, at least 1 of which differs from the others.

**homoplasmy** The presence of a single population of mt DNA in the cells of a single individual. This is normal.

**homotetramer** A molecule consisting of 4 identical subunits.

**hybrid cell** A cell formed by fusion of 2 cells of different origin in which the 2 nuclei have merged into 1. Can be cloned to produce hybrid cell lines.

**inbred mouse strain** A strain of mice that has been maintained by successive brother to sister matings over many generations, eg, BALB/c and C57BL/6 mice strains.

**inducer** A molecule that induces the expression of a gene.

**initiation factor** A protein that associates with the small subunit of a ribosome when protein synthesis begins.

**insertional mutagenesis** The production of a mutation by insertion of 1 or more copies of a transgene into a host genome.

**in situ** Refers to carrying out experiments or tests with intact tissues.

**intergenic DNA** The untranscribed DNA of unknown function that makes up a large proportion of the total DNA.

**inversion** A structural rearrangement of a chromosome in which 2 breaks occur, followed by the reinsertion of the chromosome segment but in reversed order. It may be either paracentric, ie, it does not include the centromere, or pericentric, ie, it does include the centromere.

**in vitro** Refers to a biologic or biochemical phenomenon that occurs outside of a living organism.

**in vivo** Refers to a biologic or biochemical phenomenon that occurs within a living organism.

**isochromosome** A structural chromosome rearrangement caused by the division of a chromosome along an axis perpendicular to the usual axis of division; results in chromosomes with either 2 short arms or 2 long arms.

**isodisomy** The presence of 2 identical homologues of a transmitted chromosome from only 1 of the parents.

**junk DNA** DNA with no apparent function.

**kinetochore** A structure at the centromere to which the spindle fibers are attached.

**lagging strand of DNA** The new strand of a DNA replicating in the 3' to 5' direction. It is synthesized in short fragments in the 5' to 3' direction that are subsequently joined together.

**lethal factor** An abnormality of the genome that leads to death in utero, eg, numerous chromosomal anomalies.

**ligand** A molecule that can bind to a receptor and thereby induce a signal in the cell, eg, a hormone.

**linker DNA** A synthetic DNA that carries the recognition site for a restriction enzyme and that can bind 2 DNA fragments. Also, the stretch of DNA between 2 nucleosomes.

**linkage map** A chromosome map showing the relative positions of genetic markers of a given species, as determined by linkage analysis; not the same as a physical, or gene, map, which uses linkage analysis, cytogenetic examination, and physical techniques to generate the map.

**linkage phase** The arrangement of alleles of linked loci on chromosomes.

**loss of heterozygosity** Describes a locus (or loci) at which a deletion or other process has converted the locus from heterozygosity to homozygosity or hemizygosity. Phenomenon can lead to cancers by loss of tumor suppressor genes.

**lyonization** A term used for the phenomenon of X inactivation, which was first proposed by the geneticist Mary Lyon.

**Maxam-Gilbert method** A method for determining the exact nucleotide sequence via a chemical degradation process.

**mendelian inheritance** A trait obeying Mendel's first law of independent segregation of the alleles at the same locus conveyed by each parent.

**minimal promoter** The minimal elements of a promoter, including the TATA box and transcription initiation site, which is inactive unless regulatory elements that enhance promoter activity are placed upstream; used to test candidate sequences for enhancer activity.

**mismatch** The presence in 1 chain of double-stranded DNA of a base that is not complementary to the corresponding base in the other chain. Also known as mispairing.

**mitochondria** A small, intracellular, spherical to rod-shaped cytoplasmic organelle, enclosed by 2 membranous spaces; the inner membrane is folded, forming a series of projections called cristae. Mitochondria are the principal sites of ATP synthesis; they contain enzymes of the tricarboxylic acid cycle and enzymes for fatty acid oxidation, oxidative phosphorylation, and many other biochemical pathways. They contain their own nucleic acids and ribosomes, replicate independently, and code for the synthesis of some of their own proteins.

**molecular hybridization** The ability of a single-stranded DNA or RNA to anneal to its complementary single strand by Watson-Crick base pairing.

**mobile elements** DNA sequences that are capable of inserting themselves into other locations in the genome.

**mobility-shift assay** An assay used to detect specific protein binding to DNA. Such binding creates complexes that migrate more slowly during gel electrophoresis than free DNA. Also known as gel-shift assay.

**modifier gene** A gene that alters the expression of a gene at another locus.

**molecular genetics** The study of the structure and function of genes at the molecular level.

**monoclonal** A group of cells that consist of a single clone, ie, all cells are derived from the same single ancestral cell.

**monogenic** Describing a single gene or mendelian trait.

**morphogen** A protein present in embryonic tissues in a concentration gradient that induces a developmental process.

**mosaic** An individual or tissue with at least 2 cell lines differing in genotype or karyotype, derived from a single zygote.

**monosomy** An aneuploid condition in which a specific chromosome is present in only single copy, giving the individual a total of 45 chromosomes.

**monozygotic** Refers to twins derived from a single fertilized egg.

**multipoint mapping** A type of genetic mapping in which the recombination frequencies among 3 or more loci are estimated simultaneously.

**murine** Relating to mice or rats.

**mutagen** A substance that causes a mutation.

**neurofibromin** The protein product of the neurofibromatosis type 1 gene.

**new mutation** An alteration in DNA sequence that appears for the first time in a family as the result of a mutation in 1 of the parent's germ cell.

**nondisjunction** The failure of homologous chromosomes (in mitosis or meiosis I) or sister chromatids (in meiosis II) to separate properly into different progeny cells.

**nonpenetrance** Lack of clinical expression of the mutant phenotype in an individual with the appropriate genotype.

**nuclear family** A pair of biologic parents and their children.

**nude mice** Immunologically deficient mice used to permit growth of tumor cells from mouse and other species, such as human.

**null mutation** An allele that results in either the absence of the gene product or the absence of any function at the phenotypic level.

**obligate heterozygote** An individual who is clinically unaffected but, on the basis of pedigree analysis, must carry a particular mutant allele.

**oligogenic diseases** Diseases or traits that result from the effects of relatively few genes, some of which have rather large effects.

**oligoprobe** A short DNA probe whose hybridization is sensitive to a single base mismatch.

**oncogenes** Normal genes of vertebrates that are involved in control of cell growth and have been preserved throughout evolution. When mutated, overexpressed, or amplified in somatic cells, oncogenes may cause neoplastic transformation.

**organelles** Membrane-bound intracellular, cytoplasmic structures having specialized functions, eg, mitochondria, plastids, Golgi apparatus, lysosomes.

**origin of replication (ORI)** The site where DNA replication starts.

**outbred mouse strains** Strains of mice propagated by nonstandardized matings. These mice retain substantial genetic variability.

**PAC** The artificial chromosome vector derived from the temperate bacteriophage, P1, used for cloning 100- to 200-kb DNA fragments.

**palindrome** In molecular biology, a nucleotide sequence in which the 5' to 3' sequence of 1 strand of a segment of DNA is the same as that of its complementary strand. The sites of many restriction enzymes are palindromes.

**PEP** Abbreviation for primer extension preamplification.

**peptide fingerprint** The chromatographic pattern of peptides obtained after partial hydrolysis of a protein or peptide. The technique also may be applied to DNA and RNA.



**peroxisomal enzymes** Enzymes localized to the peroxisomes. These enzymes are initially synthesized by the free polyribosomes and then enter the cytoplasm and eventually are localized to the peroxisomes. There are at least 40 enzymes. Some are involved in the production and decomposition of hydrogen peroxide and some are concerned with lipid and amino acid metabolism.

**peroxisome** A subcellular organelle surrounded by a single membrane containing at least 40 enzymes involved in energy production.

**physical mapping** The determination of the linear positions of genes on a DNA molecule; distances are measured in physical units, ie, base pairs, kilobases, and megabases.

**phytohemagglutinin** Lectin isolated from the red bean used to agglutinate red blood cells and stimulate lymphocytes to divide; used in preparation of peripheral blood karyotypes.

**platelet-derived growth factor (PDGF)** A protein, produced by platelets and other cells, that strongly stimulates cell growth and division and is involved in normal wound healing. The gene for PDGF is identical to the proto-oncogene *sis*.

**pulsed field electrophoresis** An electrophoretic technique that allows the separation of relatively long (>5,000 kb) sequences of DNA.

**point mutation** A mutation in a single nucleotide.

**polyadenylation** The addition of approximately 200 adenine residues at the 3' end of messenger RNAs, apparently involved in their transport of the nucleus and stability.

**polymerases** Enzymes that catalyze the combining of nucleotides to form RNA or DNA (genetic transcription and DNA replication).

**polysomes (polyribosomes)** Structures composed of multiple ribosomes attached to mRNA in the process of translation.

**pronucleus** Either of the 2 haploid gamete nuclei just prior to their fusion in the fertilized ovum. Transgenic lines are often generated by microinjection of the transgene into the pronuclear region of these haploid gametes.

**proofreading** The correction of errors in the nucleotide sequence that can occur during replication, transcription, or translation.

**protein suicide mechanism** In dominant disorders, 1 mutant subunit leads to the loss of function of an entire multimeric protein, eg, collagen.

**proto-oncogenes** Normal genes that are found in normal eukaryotic cells concerned with various aspects of cell division. If amplified, mutated, rearranged, or picked up by a retrovirus, they may give rise to oncogenes that can cause cancer.

**pseudoautosomal region** The distal tip of the Y chromosome short arm, which undergoes crossover with the distal tip of the X chromosome short arm during meiosis in the male.

**quasidominance** The pattern of inheritance produced by the mating of an affected homozygote with an individual heterozygous for the same recessive trait so that homozygous affected members appear in 2 or more successive generations.

**Q-banding** The pattern of bright and dim fluorescent cross-bands seen on chromosomes under ultraviolet light after quinacrine mustard staining.

**R-banding** A chromosome banding technique in which chromosomes are heated in a phosphate buffer; produces dark and light bands in patterns that are the reverse of those produced by G-banding.

**receptor** A transmembrane or intracellular protein involved in transmission of a cell signal.

**recombinant chromosome** A chromosome in an offspring that has a genotype not found in either parent, due to crossing over in meiosis.

**recombination fraction** In linkage analysis, the fraction of meiotic events that show a recombination between 2 loci.

**regulatory gene** A gene coding for a protein that regulates other genes.

**replication** The identical duplication of DNA.

**replication fork** The unwound region of the DNA double helix in which replication takes place.

**replication segregation** Refers to changes in the proportions of mitochondrial DNA alleles as the mitochondria reproduce.

**reporter gene** A gene used to analyze another gene.

**restriction digest** The process in which DNA is exposed to restriction enzymes (restriction endonuclease), causing it to be cleaved into fragments of DNA called restriction fragments.

**restriction map** A map of a DNA sequence with restriction enzyme recognition sites serving as landmarks.

**restriction site** A short sequence in DNA that can be recognized and cut by a specific restriction endonuclease.

**reverse genetics** The application of human gene mapping to clone the gene responsible for a particular disease when no information about the biochemical basis of the disease is available.

**ribosomes** Cytoplasmic organelles composed of ribosomal RNA and protein, on which polypeptide synthesis from messenger RNA occurs.

**ring chromosome** A structurally abnormal chromosome in which the end of each chromosome arm has been deleted and the broken arms have reunited to form a ring.

**RT-PCR** Abbreviation for reverse transcriptase polymerase chain reaction.

**Sanger method** The enzymatic method for determining the exact nucleotide sequence of a cloned fragment of DNA.

**satellite DNA** A portion of the DNA that differs enough in base composition so that it forms a distinct band on cesium chloride gradient centrifugation; usually contains highly repetitive DNA sequences.

**scaffold** The nuclear structure observed when histones are experimentally removed from chromosomes. Thought to represent a structural component of the nucleus and of chromosome.

**segregation** The separation of allelic genes at meiosis. Because allelic genes occupy the same locus on homologous chromosomes, they pass to different gametes.

**sequence-tagged site (STS)** A short fragment of DNA whose exact sequence is found nowhere else in the genome; typically about 200 to 300 bp. Polymerase chain reaction can be used to amplify the known sequences, which can serve as physical landmarks for mapping.

**sibship** The group comprising all the siblings (brothers and sisters) in a family.

**silent gene** A mutant gene that has no detectable phenotypic effect.

**silencer** The *cis* regulatory element that reduces transcription of a gene.

**site-directed mutagenesis** The process of creating mutations at specific locations, in contrast to naturally occurring random mutations.

**skewed X-inactivation** A nonrandom pattern of inactivation of 1 of the X chromosomes in a female that can arise through a variety of mechanisms. When this occurs, the active X chromosome may bear the mutant allele and the female will show signs and symptoms of the disease. The female is called a manifesting heterozygote or a carrier.

**somatic cell gene therapy** The insertion of new DNA material into a particular tissue of an affected individual in such a way that the inserted DNA does not enter the germline.

**SSCP** Abbreviation for single-strand conformation polymorphism.

**SSP** Abbreviation for sequence-specific primer. These are used in PCR reactions.

**STR** Abbreviation for short tandem repeat. These often serve as polymorphic markers.

**STS** See sequence-tagged site.

**STRP** Abbreviation for short tandem repeat polymorphism.

**syngeneic** Refers to genetically identical members of the same species.

**transcript map** A genetic map in which expressed sequences, eg, mRNAs, mRNA transcripts, corresponding to genes are mapped; a functional blueprint of the genome.

**transforming retrovirus** A retrovirus carrying an additional DNA sequence (often an oncogene) that confers the ability to transform infected cells to malignant phenotype.

**transgene** A foreign gene; typically, a gene produced by recombinant DNA techniques.

**transposable element** A DNA sequence that can move from one chromosomal location to another.

**transversion** A mutation in which purine is substituted for pyrimidine or vice versa.

**triplet** A sequence of 3 nucleotides comprising a codon of a nucleic acid and representing the code for an amino acid (triplet code, codon).

**tumor-suppressor gene** A gene thought to suppress formation of tumors; loss of suppression leads to malignant transformation. P53 is an example of a tumor-suppressor gene.

**unequal crossing over** Crossing over between similar DNA sequences that are misaligned, resulting in sequences with deletion or duplication of DNA segments. A cause of a number of genetic variants, eg,  $\alpha$ -thalassemia and Lepore hemoglobin.

**variable expressivity** Refers to the variable severity of a genetic trait. Individuals with the same mutant gene with pleiotropic effects frequently show variable expressivity due to either environmental effects or effects of other genes modifying the expression of the mutant gene.

**wild type** The term used to indicate the normal allele (often symbolized as +) or the normal phenotype.

**X-autosome translocation** The reciprocal translocation between the X chromosome and 1 of the autosomes.

**X-linked dominant** A trait that is manifested in the heterozygous female as well as in the male who has the mutant allele on 1 of the X chromosomes.

**X-linked recessive** A disorder manifested exclusively in a male who is a heterozygote or a homozygous female when the abnormal gene is carried on the X chromosome. A female is usually a carrier if she is heterozygous and transmits the disease to the son.

**zinc finger proteins** Transcription-activator proteins containing finger-like structures containing zinc atoms.

**zoo blot** A Southern blot containing conserved DNA sequences from related genes of different species. It is taken as evidence that the sequences are coding sequences from a specific gene.

## New References

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molecular pathology, potential for symptomatic diagnosis, genotype-phenotype correlation, and genetic counseling); Severity and Variability Without Screening (including mortality, developmental disabilities, and physical findings); Clinical Outcome With Screening and Treatment (including mortality, clinical disability, variability, and possible interventions); Screening Test Characteristics and Confirmation (including type of test, timing, stability of specimen, confirmation, accuracy of screening, and ongoing studies); Special Concerns and Issues; and Professional and Public Education.

Information on additional newborn screening tests that are available in some university-based laboratories and commercial laboratories also is provided. These include adenosine deaminase deficiency; arginase deficiency; urea cycle defects; Duchenne muscular dystrophy; glucose-6-phosphate dehydrogenase deficiency; pyroglutamic aciduria; medium-chain acetyl-CoA dehydrogenase deficiency; and other organic acidemias.

American Academy of Pediatrics, Committee on Genetics. *Pediatr* 1996;98(3):473-500.

**Editor's comment:** This is a useful resource for pediatricians who suddenly find they need information about newborn screening. Information regarding many different metabolic disorders is contained in this one article. Each section provides current information on screening variability among different states, appropriate therapies, as well as the recent advances in genetics regarding metabolic disorders. The morbidity and mortality of metabolic diseases can be improved significantly with early detection and treatment. The field of metabolic diseases is changing rapidly because of molecular genetic techniques and new types of therapy. These guidelines will help physicians help their patients. Obtain your copy soon.

Judith G. Hall, MD

## A Month-Long Effect From a Single Injection of Microencapsulated Human Growth Hormone

The investigators have prepared a sustained-release form of rhGH using zinc and incorporating it into biodegradable polymers of DL-lactic co-glycolic acid (PLGA), producing 50- $\mu$ m diameter microspheres. The monomeric form of rhGH is

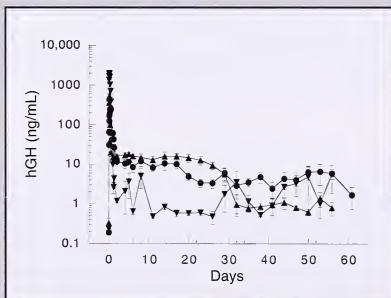
released from these microspheres. One subcutaneous (sc) injection did not cause an inflammatory reaction or fibrosis at the site of injection. When this microencapsulated form of rhGH (24 mg) was injected sc into juvenile rhesus monkeys, the peak serum rhGH concentration (260 ng/mL) was achieved within 12 hours after injection. Levels of rhGH declined and were maintained at 10 ng/mL through day 20 and thereafter at 4 to 5 ng/mL through day 60 after administration (this is a calculated rate of release of rhGH from the microspheres 0.4 mg/d) (see Figure 1). Insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) values increased 2- to 3-fold within 3 days and were maintained for 30 days. These data were similar to those recorded in another group of animals receiving microsphere-equivalent amounts of rhGH by osmotic pump. Daily injections of rhGH (0.86 mg/d) resulted in lower levels of IGF-1 and IGFBP-3. One of 4 animals developed a low titer of anti-rhGH antibodies.

Johnson OFL, et al. *Nat Med* 1996;2(7):795-799.

**Editor's comment:** The availability of a clinically useful preparation of hGH that can be administered once a month or less will be of great benefit in the management of patients with GH deficiency and analogous to the utility of depot forms of GnRH agonist in central precocious puberty. If further studies demonstrate the safety and effectiveness of this preparation of rhGH, it may have more utility than oral forms of GH secretagogues, which need to be taken at least once daily and which will be ineffective in patients with primary pituitary dysfunction.

Allen W. Root, MD

Figure 1



Recombinant hGH serum concentration levels in rhesus monkeys. Values are means  $\pm$  SEM. Treatment groups were 160 mg microspheres (24 mg rhGH) (●), 24 mg rhGH in solution (▼), or 3.4 mg rhGH in solution followed by surgical implantation of an osmotic pump containing 20.8 mg rhGH in solution (for a total dose of 24 mg) (▲).

From Johnson OL, Cleland JL, Lee HJ, et al. A month-long effect from a single injection of microencapsulated human growth hormone. *Nat Med* 1996;2(7):797.

## Over Expression of an Osteogenic Morphogen in Fibrodysplasia Ossificans Progressiva

Fibrodysplasia ossificans progressiva was first described in 1692. It is a rare autosomal dominant inherited disorder of the connective tissue with a high rate of new spontaneous mutations. Most cases represent new mutations and are sporadic. The disorder is characterized by short great toes, broad femoral necks, short metacarpals, and, with age, progressive formation of ectopic bone in the soft tissue and muscles. Ectopic bone formation is usually provoked by trauma and can occur at any age but is most often first seen between birth and 10 years. A significant number of patients with fibrodysplasia ossificans progressiva have heterotopic ossification at injection sites following DPT vaccinations.

The disorder is usually progressive, with a characteristic pattern of involvement. There is a predilection for developing ossification in the paraspinal and scalp muscles, jaw muscles, and muscles of the arms and legs; however, the facial muscles, muscles of the tongue, diaphragm, visceral smooth muscles, and abdominal muscles are usually spared. The disorder eventually leads to ankylosis (fixation of joints); severe scoliosis (from ossification of paravertebral muscles); starvation (from involvement of the jaw muscles); and premature death due to complications of ankylosis.

The authors speculate that overexpression of bone morphogenetic protein 4 in lymphocytes of patients with fibrodysplasia ossificans progressiva is the causative factor for heterotopic ossification in these individuals. They also suggest a mechanism to explain the pathophysiology of heterotopic bone formation in these individuals.

Bone morphogenetic proteins are potent osteogenic agents that belong to the transforming growth factor  $\beta$  (TGF- $\beta$ ) family, a superfamily of peptides that is responsible for endochondral osteogenesis and fracture healing. The gene for bone morphogenetic protein 4 has been mapped to chromosome 14q22-23.

The authors examined the expressions of bone morphogenetic proteins 1 to 7 as well as their mRNAs in the lymphoblastic cells of individuals with fibrodysplasia ossificans progressiva and in normal healthy individuals. They found overexpression of bone morphogenetic protein 4 and its mRNA in lymphoblastic cell lines from 26 of 32 individuals with fibrodysplasia ossificans progressiva and increased expression in 1 of 12 normal individuals. The authors speculate that following an injury, those lymphocytes having increased bone morphogenetic protein 4 migrate to the site of trauma to aggregate in large numbers. They postulate that these lymphocytes then bind to the type IV collagen present in the basement membrane of endothelial cells and muscle cells. This binding leads to a high local concentration of the bone morphogenetic protein 4, triggering preosseous fibroproliferative lesions.

Bone morphogenetic protein 4 is markedly increased during embryonic life as well as later on in development when healing of fractures occurs. This suggests a common molecular

basis for prenatal and postnatal osteogenesis. Fibrodysplasia ossificans progressiva is the only known genetic disorder of osteogenesis that is associated with overexpression of a bone morphogenetic protein. An error could be in the regulatory regions of the *BMP-4* itself or in some other gene whose product controls *BMP-4* production. The commentary by J.M. Connor in the same issue of the journal is helpful but speculative in predicting care of patients with fibrodysplasia ossificans progressiva. Readers who wish more information should seek out and read this commentary.

Connor JM. *N Engl J Med* 1996;335(8):591-593.

Roush W. *Science* 1996; 273(30):1170.

Shafritz AB, et al. *N Engl J Med* 1996;335(8):555-561.

**Editor's comment:** *Fibrodysplasia ossificans progressiva provides a unique opportunity to study the role of morphogenetic proteins in endochondral bone formation. Whether the overexpression is due to a defect in the gene itself or in the receptors needs to be determined. Once this is known perhaps these proteins can be modified therapeutically to turn off the expression of bone morphogenetic protein and thereby decrease ectopic bone formation.*

*Understanding the biology of these proteins will have major clinical and therapeutic implications. It will help define the etiology and mechanism of ectopic bone formation in other diseases, such as soft-tissue ossification (myositis ossificans), which may occasionally occur as a complication of major or repeated minor muscle trauma, hip replacement, major burns, immobilization after paraplegia, or prolonged comas in otherwise healthy subjects.*

*It also seems possible that once their biology is known, these proteins might be useful in tissue engineering and may even possibly replace bone grafts in the future.*

Judith G. Hall, MD

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## Growth Hormone Therapy in Silver-Russell Syndrome: 5 Years Experience of the Australian and New Zealand Growth Database (OZGROW)

This report details the experience of 33 patients (22 males, 11 females) who were diagnosed with the Silver-Russell syndrome through the OZGROW data base and subsequently treated with exogenous growth hormone (GH) for 3 to 5 years. The inclusion criteria for Silver-Russell syndrome were: (1) short stature 2 standard deviations (SD) or more below the mean; (2) birth weight 2 SD or more below the mean for gestational age; and (3) lack of other recognized syndrome or etiology causing short stature. Of the 33 patients, 23 had at least one GH stimulation test, and 2 of the 23 had GH deficiency defined as a peak of  $<20$  mIU/L in 2 different tests. Subjects were eligible for GH therapy if their height was less than the 1st percentile and their growth velocity was less than the 25th percentile for bone age and sex (the guidelines for GH therapy in Australia and New Zealand). The children received subcutaneous GH 6 or 7 days per week at a starting dose of 14 IU/m<sup>2</sup>/wk (approximately 4.6 mg/wk). Growth was assessed every 3 months and bone age was assessed yearly. The dose was subsequently increased at 6-month intervals if the growth response was considered inadequate.

The median age at commencement of treatment was 6.7 years, and the median height SD score (SDS) for chronologic age was -3.2. The median birth weight and the median birth length for gestational age were -3.2 and -4.0 SD, respectively. Bone age was delayed in girls by 0.8 years and in boys by 2.2 years. Height SDS for chronologic age, growth velocity (cm/y), and SDS growth velocity for chronologic age increased during therapy. The median change in height SDS for chronologic age after 3 years of therapy (N=21) was 1.0 SD; after 4 years (N=14), 1.5 SD; and at 5 years (N=9),

1.8 SD. No significant increase in height SDS for bone age was observed. The gain in height SDS over the first year accounted for 30% of the total in over 3 years. The increase in growth velocity was maintained throughout the 3 years, and the height gain over 3 years as compared to the median base line was 5.7 cm. Using multiple regression analysis for the 21 subjects who had completed 3 years of therapy, the authors demonstrated that age combined with birth weight SDS, height SDS, or catch-up SDS significantly predicted 39% of the variance in height SDS over the 3 years. The authors conclude that younger, shorter children have the greatest increase in height SDS, a finding similar to that in children with GH deficiency treated similarly. Further studies are necessary to follow these children to final height in order to demonstrate the effectiveness of GH therapy.

Rakover Y, et al. *Acta Paediatr* 1996;155:851-857.

**Editor's comment:** This is a very interesting and important study. Children with Silver-Russell syndrome are often severely handicapped due to both their short stature and low weight to the point that participation in age-appropriate activities is not only difficult but dangerous. Thus, even if final height were not significantly increased by GH therapy, acceleration of growth velocity to achieve a height SDS closer to the normal range at an earlier age would be clearly beneficial to these children. It is interesting to recall that guidelines for GH therapy in New Zealand and Australia include individuals whose height is less than the 1st percentile and growth velocity is less than 25th percentile for bone age and sex. Demonstration of biochemical GH deficiency is not a requirement for therapy in these countries. Thus, these investigators have the opportunity to gather therapeutic information on the effects of GH in a variety of different clinical syndromes and situations that may not be possible in other parts of the world. They should be encouraged to exercise extreme care in the collection of their anthropometric data and in their presentation of such data. It would have been preferable in this particular study to have seen mean and standard deviations rather than median data.

Previous data on children with Silver-Russell syndrome treated with GH have shown variable results, such as no gain in height SDS for chronologic age after 1 year of therapy or no significant change in height SDS for bone age. Thus, it would be interesting to determine how the individuals identified in the OZGROW data base may be different from those reported in other studies.

William L. Clarke, MD

### In Future Issues

#### Molecular Genetics of Human Chondrodysplasias

William A. Horton, MD

#### Insulin, the IGF System, and IDDM

Cheri Deal, MD

#### GH Secretagogues

Allen W. Root, MD

#### Safety and Effectiveness of Human Growth Hormone Using Pharmacological Dosing

Arnold Slyper, MD



## Growth Hormone Increases Breast Milk Volumes in Mothers of Preterm Infants

Recombinant human growth hormone (rhGH) was given for a period of 7 days at 0.2 IU/kg/d (0.53 mg/kg/wk) to a maximum of 16 IU/d to 9 mothers of infants born between 26 to 34 weeks of gestation whose milk production was insufficient to supply their infants' needs. They were compared to 9 mothers of similarly premature infants, who received placebo instead of rhGH (Table 1). The infants of the rhGH-treated mothers were slightly heavier at birth and older at enrollment than those of mothers receiving the placebo. Maternal milk production, measured as the volume of milk obtained by 5 to 6 breast expressions a day and by the gained weight of the infants after breast feedings, increased significantly from 139  $\pm$  49 mL/d at baseline to 175  $\pm$  46 mL/d at 7 days (31% increase) in the rhGH-treated mothers. No significant increase was observed in the placebo-treated mothers, whose baseline production was 93  $\pm$  50 mL/d and rose to 102  $\pm$  69 mL/d at day 7 (7.6% increase). All 9 rhGH-treated mothers had an increase in milk production, whereas 4 of the placebo-treated mothers had a decrease (Figure 1). Insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 increased only in the rhGH-treated group. No adverse effects were seen in the rhGH-treated mothers or their infants. The authors conclude that rhGH treatment in mothers with lactational insufficiency can modestly improve breast milk volumes, although infants' milk needs could be met only with supplementary feeding even when rhGH was used.

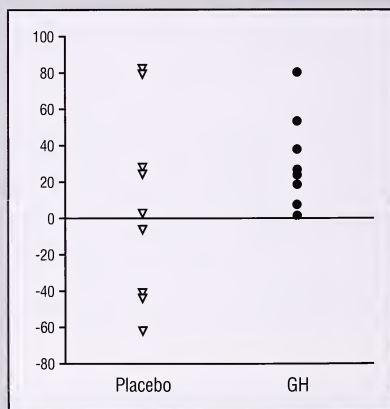
Gunn AJ, et al. *Pediatrics* 1996;98:279-282.

**Editor's comment:** This paper elicits an interesting concept and expands the currently growing list of uses of rhGH in humans. All pediatricians agree that human milk is best for babies; however, lactation failure continues to be the main cause for its early termination. Nevertheless, clinicians should not immediately prescribe rhGH for the treatment of mothers who do not have sufficient milk production. Sucking and appropriate breast-feeding technique are the single most efficient methods for stimulation of breast milk production. In premature babies who are unable to perform effective suction and, hence, are at increased risk for early termination of

maternal milk feedings, mechanical devices to express milk from the mother have been very helpful. When this resource is not successful, a short period of rhGH treatment for the mother may be preferable to a switch to cow's milk-based formulas. The less than optimal matching between babies of rhGH- and placebo-treated mothers, ie, larger and older babies in the rhGH-treated group, could have introduced a bias in the generation of results. An increased production of breast milk could be theoretically expected from mothers of bigger babies independently from the use of rhGH. Further studies with better infant matching need to be performed to prove this efficacy of rhGH.

Fima Lifshitz, MD

Figure 1  
Percent Increase in Milk Volume



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Table 1  
A Comparison of Study Groups Receiving Either hGH or Placebo for 1 Week

|         | Birth Weight<br>(g) | Gestational Age<br>(weeks) | Age<br>(days)   | Weight of Infant<br>(g) | Mothers Age<br>(y) |
|---------|---------------------|----------------------------|-----------------|-------------------------|--------------------|
| hGH     | 1398 $\pm$ 397      | 30.6 $\pm$ 3.2             | 39.7 $\pm$ 32   | 2206 $\pm$ 455          | 32.4 $\pm$ 3.6     |
| Placebo | 1239 $\pm$ 552      | 30.1 $\pm$ 3.2             | 31.3 $\pm$ 18.9 | 1576 $\pm$ 661 *        | 35.7 $\pm$ 4.6     |

\*  $P < .05$

Reproduced by Permission of *Pediatrics*, Vol. 98, Pages 279-282, Table 1, Copyright 1996.

## The New Genomics: Global Views of Biology

The Human Genome Project is well along—by some accounts, ahead of schedule—making it highly likely that the entire human genome will be sequenced by the year 2005. This has led many in the genetics community to ask: "What will be done after this goal is attained?" In the recent *Genome* issue of *Science*, one of the leaders in this field, Eric Lander, has addressed the question by putting forth several specific goals for what he calls "the new genomics."

First and importantly, Lander views the genome project as the biologist's equivalent of the periodic table. Just as the periodic table gave chemists and physicists building blocks to understand their 19th-century world, the genome project will provide scientists of the next century the building blocks to understand biology. Accordingly, he proposes 10 goals for the next phase of genomics, which he sees as a transition from structural to functional genomics.

1. Routine resequencing of large regions of the human and mouse genome. The rationale is that this will be needed to fully define the extent of variation, eg, polymorphism, in the human genome. Such information will be necessary to understand how genetic variation contributes to the causation of common diseases.
2. Systematic identification of all common variants in human genes. This represents an extension and ordering of the previous goal.
3. Rapid sequencing of other organisms. Lander argues that comparative DNA sequencing will unlock evolutionary relationships not previously appreciated. He notes that sequence conservation will provide a powerful tool to determine functional constraints of genes and their products and a means to identify regulatory regions and important structural features of proteins.
4. Simultaneous monitoring of the expression of all genes. This is needed to generate a complete picture of the state of a cell and a basis for distinguishing among many different cell types. This goal would be achieved through description and cataloging of cell- and tissue-specific gene expression.
5. Develop generic tools for manipulating cell circuitry. Lander reasons that monitoring gene expression is insufficient to understand biologic functions. Rather, he points out that they must be disrupted and manipulated to fully define them; and he urges improvements of tools to accomplish this in model organisms in which functions can be monitored.
6. Monitor the level and modification state of all proteins. This goal focuses on gene products—proteins—rather than genes themselves. Lander argues that many functions of proteins reflect posttranslational modifications, which cannot be determined by analysis of the gene alone.

7. Systematic catalogs of protein interactions. Proteins do not function in a vacuum. Rather, their functions reflect interactions with other molecules; and diseases are due to disturbances in these interactions. This goal would lead to a comprehensive "interaction map" of the genome.
8. Identification and cataloging of basic protein shapes. This represents a more complex level of defining proteins.
9. Increased attention to ethical, legal, and social issues. This goal addresses the need to use the new knowledge in a responsible way.
10. Public education. This is related to goal 9. Lander emphasizes that the new information will provide people with choices regarding how the information will be used. He holds that education is the best safeguard to prevent its misuse.

Lander ES. *Science* 1996;274:536-539.

**Editor's comment:** This is a thoughtful and insightful commentary that is useful for both geneticists and nongeneticists alike. It underscores that the Human Genome Project is not an end in itself, but a stepping stone to a more complete understanding of biology and disease.

William A. Horton, MD

**2nd Editor's comment:** Dr. Lander's proposals to study gene aspects are in the broadest perspectives of development, physiology, and microbiology—and are mind-boggling. The analogy that comes to my mind is: "We now have the land, what are we going to build?" The important question to be asked is: "What will all this constructively mean to the human race?" The answers are unknown, but very exciting prospects exist.

Robert M. Blizzard, MD

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Allen W. Root, MD  
St. Petersburg, Florida

## Insulin-Like Growth Factor-1 and IGF Binding Protein-3 Remain High After GnRH Analogue Therapy in Girls With Central Precocious Puberty

Kanety et al measured estradiol, insulin-like growth factor 1 (IGF-1), and IGF-binding protein 3 (IGFBP-3) prior to and after 1, 2, and 3 months of GnRHa (D-Trp<sup>6</sup>-GnRHa, Decapeptyl 3.75 mg, Ferring, Malmo, Sweden) in 10 girls, aged 7 to 8 years, with central precocious puberty (CPP). Results were compared to those from 7 prepubertal girls aged 8 to 10 years, with no known endocrine abnormalities. The results were analyzed using the nonparametric Wilcoxon signed rank tests and the Pearson  $\chi^2$ -test. The values were expressed as mean  $\pm$  SEM.

Pretreatment estradiol levels were in the pubertal range in all 10 patients, fell to levels below the detection limits 1 month after therapy, and remained depressed. Serum IGF-1 was significantly higher in CPP patients as compared to controls ( $48.8 \pm 6.5$  nmol/L vs  $23.1 \pm 4.9$  nmol/L;  $P < 0.01$ ). Although serum IGF-1 levels decreased after 1 injection of GnRHa, the decrease was not significant and no further decrease was noted after 2 to 3 months. The changes in IGF-1 levels, when analyzed individually, were heterogeneous and did not follow a specific pattern. Serum IGFBP-3 levels were also significantly higher in CPP patients than in controls ( $4.70 \pm 0.37$  mg/L vs  $3.71 \pm 0.42$  mg/L,  $P < 0.01$ ) at baseline and did not change significantly after 1, 2, or 3 months of GnRHa therapy. IGFBP-3 levels were also heterogeneous and did not fall into any specific category. Interestingly, IGF-1 and IGFBP-3 failed to correlate before or during the 3 months of therapy.

The authors review previous studies of the effects GnRHa in CPP on growth hormone (GH), IGF-1 and IGFBP-3. Variable results have been reported, including a decrease in basal and GRF-stimulated GH levels after 3 months of GnRHa

therapy without a decrease in serum IGF-1, and a decrease in IGF-1 only after a year of therapy. The authors conclude their data suggest that while estradiol has a role normally in increasing GH, IGF-1, and IGFBP-3, it may not be important in maintaining the levels of these hormones once the increases over the prepubertal state have been established. They speculate that the lack of change in IGF-1 and IGFBP-3 "with GnRHa therapy which has been proven to reduce growth velocity underscores the known effect of sex steroids in the growth process."

Kanety H, et al. *Clin Endocrinol* 1996;45:7-12.

**Editor's comment:** This is an interesting article. When one looks at individual data, it would appear that 7 out of the 10 girls had reductions in IGF-1 by 3 months of therapy while 6 out of 10 had reductions in IGFBP-3 during the same period. The lack of consistent patterns and the fact that some girls' levels rose probably accounts for the inability of the data to be significant. It may be that statistical significance could be obtained by studying a larger number of girls. However, the speculation of the authors remains provocative. Should these findings be verified in studies of larger numbers of girls? This may account for the observation that bone age advanced in girls with CPP treated with GnRHa despite adequate suppression of gonadotropins. In addition, this may account for some of the lack of success in achieving significantly greater final heights in children treated with exogenous GH simultaneously with GnRHa therapy.

William L. Clarke, MD

## Increased Energy Expenditure in Growing Adolescents With Crohn's Disease

Growth failure is common in children and adolescents with Crohn's disease and may be the result of reduced energy intake, impaired absorption, or protein-losing enteropathy. In addition, patients with Crohn's disease may have increased energy requirements related to the increase in metabolic activity of their inflamed tissue. Zoli et al measured resting energy expenditure via indirect calorimetry in adolescents with inactive Crohn's disease, both those who were growing and those who had completed their growth. In addition, a control group of healthy growing adolescents was studied. Ten growing adolescents with inactive Crohn's disease (aged  $17.8 \pm 1.4$  years) and 9 who had ceased growing matched for disease, site, and duration (aged  $19.0 \pm 1.3$  years) participated. Subjects had to have histologically proven Crohn's

disease with onset prior to age 16, and to have been diagnosed a minimum of 2 years. Height was assessed every 3 months, and those whose height had increased by 2 cm or more during the previous 12 months were considered growing. Nutritional status was assessed by anthropometric measurements from which body mass index, percent body fat, and free fat mass were calculated. Food intake was assessed by 7-day food diary. No subjects were currently receiving corticosteroids and both interleukin 6 and C-reactive protein levels were in the normal range for all subjects.

Resting energy expenditure per kilogram of body weight was significantly higher in growing patients compared to disease controls ( $32.1 \pm 1.6$  kcal vs  $27.6 \pm 0.9$  kcal;  $P < 0.05$ ) or healthy controls ( $24.5 \pm 1.0$  kcal;  $P < 0.001$ ).



Similar relationships held when resting energy expenditure was expressed per kilogram of fat-free mass. Dietary intake in 5 of the growing patients averaged 97% of the amount recommended for age, sex, weight, and physical activity. Thus, the growing adolescents with inactive Crohn's disease had increased energy expenditure as compared to healthy growing adolescents and nongrowing subjects with inactive disease. The cause of this increase in resting energy expenditure is unknown but could be the result of subclinical disease activity. The authors conclude that nutritional therapy should be directed towards increasing energy intake in these subjects to maximize growth potential.

Zoli G, et al. *Dig Dis Sci* 1996;41(9):1754-1759.

**Editor's comment:** *The abnormal resting energy expenditure demonstrated in these individuals with inactive Crohn's*

*disease suggests that the metabolic implications of this disease are not necessarily quiescent when the disease becomes clinically inactive. As pointed out by the authors, growth retardation may be the sole manifestation of Crohn's disease in approximately 5% of patients. Until we understand more about the pathophysiology of Crohn's disease, especially in its quiescent state, it may not be possible to suggest alternatives to increased nutrient intake as a means of improving growth in these individuals. Whether antibiotic agents could be of benefit remains to be shown. For further reading regarding management of growth failure in Crohn's disease, see J.A. Walker-Smith's article, "Management of growth failure in Crohn's disease," in Archives of Disease in Childhood 1996; 75(4):351-354.*

William L. Clarke, MD

## Shortened and Diminished Pubertal Growth in Boys and Girls Treated for Acute Lymphoblastic Leukemia

The longitudinal patterns of growth and sexual maturation in 11 Dutch boys and 17 Dutch girls with acute lymphoblastic leukemia (ALL) treated before age 7 years with chemotherapy (including vincristine, prednisone, asparaginase, mercaptopurine, intrathecal methotrexate, and prednisone) and cranial irradiation (24 Gy) were studied. The mean age of onset of the pubertal growth spurt in girls ( $8.9 \pm 0.2$  years) was significantly less than that of the (Swiss) reference group ( $9.7 \pm 1.0$  years), as were the ages at peak height velocity ( $10.6 \pm 0.7$  years vs  $12.2 \pm 0.8$  years); the age at the end of the pubertal growth spurt ( $12.1 \pm 0.8$  years vs  $13.8 \pm 0.8$  years); and the duration of the pubertal growth spurt ( $3.2 \pm 0.9$  years vs  $4.1 \pm 0.5$  years). This pattern led to a decrease in the pubertal height gain of the ALL girls ( $20.9 \pm 5.3$  cm vs  $24.7 \pm 2.6$  cm) and a lower final height ( $160.8 \pm 5.8$  cm vs  $165.3 \pm 5.8$  cm). In comparison to normal Dutch females, girls with ALL were younger ( $10.6 \pm 0.71$  years vs  $11.9 \pm 0.84$  years) and shorter ( $146.1 \pm 7.5$  cm vs  $152.8 \pm 5.6$  cm) at peak height velocity and younger ( $12.0 \pm 0.4$  years vs  $13.2 \pm 0.4$  years) and shorter ( $152.5 \pm 3.0$  cm vs  $162.5 \pm 4.1$  cm) at menarche, but achieved similar postmenarchal growth ( $7.1$  cm). In comparison to normal Dutch girls with early pubertal onset, girls with ALL were younger ( $10.3 \pm 0.6$  years vs  $10.7 \pm 0.3$  years), shorter ( $144.7 \pm 5.7$  cm vs  $151.8 \pm 3.5$  cm), and had decreased growth ( $8.0 \pm 0.6$  cm/y vs  $9.2 \pm 1.4$  cm/y) at peak height velocity. For males with ALL there were no differences relative to (Swiss) control subjects for age at onset of puberty ( $11.2 \pm 0.7$  years), peak height velocity ( $13.7 \pm 0.6$  years), or end of the pubertal growth spurt ( $15.0 \pm 0.7$  years), but the duration of the pubertal growth spurt was shorter ( $3.8 \pm 0.3$

years vs  $4.5 \pm 0.6$  years). Pubertal height gain was less in ALL males ( $25.9 \pm 3.1$  cm vs  $28.8 \pm 4.0$  cm) as was final height ( $170.8 \pm 7.6$  cm for ALL males vs  $177.5 \pm 6.7$  cm for Swiss males vs  $182.0 \pm 6.7$  cm for Dutch males). In both boys and girls, skeletal maturation progressed more rapidly during puberty than in control subjects. The authors concluded that in children successfully treated for ALL, not only was there a deceleration in growth during treatment, but the pubertal growth spurt was attenuated as well.

Groot-Loonen JJ, et al. *Acta Paediatr* 1996;85:1091-1095.

**Editor's comment:** *These data should prove a valuable resource with which to determine the effect of intervention (eg, administration of growth hormone, delay of pubertal onset with gonadotropin hormone releasing hormone agonists or antagonists, or dual therapy) on the growth of children who have survived therapy for ALL.*

Allan W. Root, MD

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**GROWTH, Genetics, & Hormones Volume 13, Number 2**  
**Post Program Self-Assessment/CME Verification**

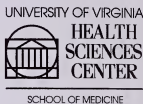
**Instructions:** The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The most common indication for limb lengthening is leg length inequality of:
    - a.  $\geq 3$  cm
    - b.  $\geq 4$  cm
    - c.  $\geq 5$  cm
    - d.  $\geq 7$  cm
    - e.  $\geq 10$  cm
  2. The Ilizarov method of limb lengthening was an improvement over prior osteotomy and rapid bone distraction methods because of all of the following *except*:
    - a. It promoted new bone formation in the distraction gap.
    - b. It eliminated complications of joint stiffness and joint dislocation.
    - c. It encouraged weight-bearing and patient activity throughout treatment.
    - d. It eliminated the need for bone grafting and plate application.
  3. Limb length inequality is commonly associated with all but one of the following conditions:
    - a. Noonan syndrome
    - b. Ollier's disease
    - c. Conradi-Hünermann chondrodysplasia punctata
    - d. Fibrous dysplasia
    - e. Neurofibromatosis
  4. Limb lengthening in short stature is controversial because:
    - a. It may negatively impact on ultimate joint function and cause early osteoarthritis.
    - b. There is little data in seemingly appropriate candidates, such as in patients with metaphyseal dysplasias or Turner syndrome.
  - c. Some popular limb lengthening techniques encourage loss of independence due to non-weight-bearing treatment requirements.
  - d. Ultimate functional outcomes and the effects on limb growth have not adequately been evaluated.
  - e. All of the above
5. Which of the following statements is/are true?
- a. Slow bone formation in fibrous dysplasia limits the utility of limb lengthening.
  - b. Despite abnormal fragility of the bone in Silencer types I and IV osteogenesis imperfecta, limb lengthening is appropriate if approached cautiously.
  - c. Intrinsic knee instability in congenital limb hypoplasia syndromes precludes the use of limb lengthening to a great extent.
  - d. Amputation is indicated for management of severe fibular hemimelia and tibial aplasia.
  - e. b and d

Answer Key: 1. C 2. B 3. A 4. E 5. E

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Drs. Stanitski, Lifshitz, Clarke, Horton, and Hall report no conflicts; Dr. Root serves on Genentech's National Cooperative Growth Study (NCGS) Advisory Committee; Dr. Blizzard is the President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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### Growth Hormone Secretagogues: Physiology and Function

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#### INTRODUCTION

The first growth hormone-releasing peptides (GHRPs) were engineered by Bowers and colleagues in 1976 while studying the growth hormone (GH)-releasing effect of the endogenous pentapeptide opioid enkephalin and its synthetic analogues. The initial GHRPs were active only *in vitro*.<sup>1</sup> By systematically altering amino acid composition, researchers developed the first compound that stimulated GH release both *in vivo* and *in vitro* in 1980. It was termed GHRP-6 because it was a hexapeptide. Subsequently, other GHRPs were prepared and denoted by the order in which they were identified (Table 1). Note that GHRP-6 and hexarelin are very similar.

In 1982, Guillemín et al determined the structure of hypothalamic GH-releasing hormone (GHRH), a 44 amino acid polypeptide that stimulates the synthesis

and secretion of GH through activation of a guanine triphosphate ( $G_s$ -protein) receptor, and consequent increase in somatotroph cytosolic levels of cyclic adenosine monophosphate (cAMP) and  $Ca^{++}$ .<sup>2,3</sup> The identification of GHRH resulted in its becoming the focus of research for the next several years.

In 1989, GHRP-6 was demonstrated to release GH. Utilizing structure-function data derived from analysis of GHRP-6 and the concept of a common "privileged structure" that permits specific chemical units to interact with diverse G-protein receptors, Smith et al in 1993 developed nonpeptidyl compounds that simulated the 3-dimensional spatial configuration of GHRP and had GH-releasing activity.<sup>4-6</sup> In 1996, these workers identified the endogenous  $G\alpha_{11}$  membrane receptor for these GH secretagogues that activates phospholipase C (PLC).<sup>7,8</sup> These data demonstrate the presence of an endogenous GH-regulating mechanism distinct from GHRH, although the endogenous ligand for this system and its site of origin have not as yet been described. In this presentation, we will review the chemistry and physiology of GHRP and nonpeptidyl GH secretagogues (GHSs) and assess their clinical potential.

#### CHEMISTRY OF GHRP AND NONPEPTIDYL GH GHSs

Table 1 presents the amino acid structures of several GHRPs in comparison to native met-enkephalin. These compounds were identified sequentially and

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| <b>CME Information.....</b>   | <b>page 48</b> |

Table 1  
Growth Hormone-Releasing Peptides

| Amino acid No. | 1    | 2      | 3     | 4   | 5                   | 6                   | 7                   |
|----------------|------|--------|-------|-----|---------------------|---------------------|---------------------|
| Met-Enkephalin | Tyr  | Gly    | Gly   | Phe | Met-NH <sub>2</sub> |                     |                     |
| GHRP-6         | His  | DTrp   | Ala   | Trp | DPhe                | Lys-NH <sub>2</sub> |                     |
| GHRP-1         | Ala  | His    | DβNal | Ala | Trp                 | DPhe                | Lys-NH <sub>2</sub> |
| GHRP-2         | DAla | DβNal  | Ala   | Trp | DPhe                | Lys-NH <sub>2</sub> |                     |
| Hexarelin      | His  | DMeTrp | Ala   | Trp | DPhe                | Lys-NH <sub>2</sub> |                     |

DβNal = D-2-naphthylalanine  
DMeTrp = D-methyl-tryptophan

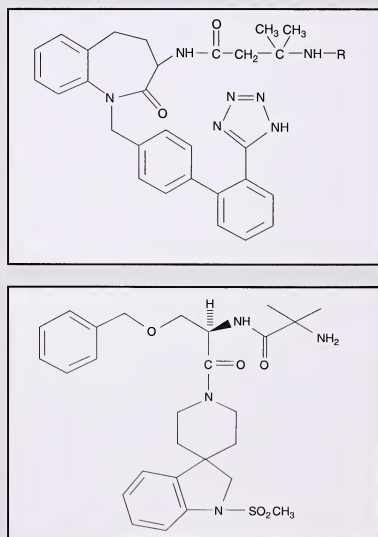
have increasing potency (GHRP-2 > GHRP-1 > GHRP-6 = hexarelin). Figure 1 depicts 2 nonpeptidyl GHSs, L-692,492, a benzolactam, and L-163,191 (MK-0677), a spiropiperidine, that have been most extensively studied. However, tetrahydroquinolone and isoindoline chemical structures with GH-releasing activity also have been designed.<sup>9</sup> Appreciating the relationship of these apparently very different chemical classes of compounds when depicted in 2 dimensions is difficult. However, computer-generated structural overlay maps clearly demonstrate their similarity in 3-dimensional conformation and, hence, their comparable biologic activity. The nonpeptidyl GHS MK-0677 is more potent and has a longer duration of action and greater oral bioavailability than do the GHRPs.

#### BIOLOGIC ACTIVITY OF GHRPs AND NONPEPTIDYL GHSs

The effects of GHRPs and nonpeptidyl GHSs are similar, as would be anticipated since both groups of secretagogues utilize the same receptor.<sup>7</sup> GHRPs and nonpeptidyl GHSs stimulate the secretion of GH in vitro and, in a variety of species, in vivo, including humans. They are active when administered intravenously, intramuscularly, subcutaneously, intranasally, and orally. In normal, short-statured, and obese children and in normal young and elderly adults, GHSs stimulate greater secretion of GH in vivo than does GHRH, but do so only in the presence of endogenous GHRH. The GH-releasing effects of combined GHRH and GHRP are synergistic. As with GHRH, the GH-releasing activity of GHSs is inhibited by somatostatin (SRIH). The amplitude, but not the frequency, of GH pulses is increased when GHSs are continuously infused over 24 to 36 hours; the acute GH secretory response to GHSs is attenuated, but that to GHRH is preserved.<sup>1</sup> These observations suggest that an as yet uncharacterized endogenous GHS influences the amplitude of GHRH-induced GH

secretion. Similarly, the continuous infusion of GHRH also increases GH pulse amplitude and desensitizes the pituitary to an acute bolus of GHRH but not to GHS. Thus, these GH secretagogues induce homologous but not heterologous desensitization. In prepubertal children, the GH-releasing effects of GHSs are reproducible and enhanced by

Figure 1

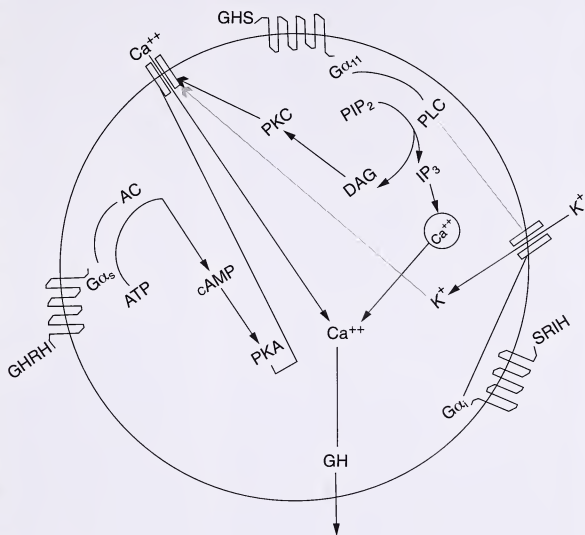


Chemical structures of 2 nonpeptidyl GHSs. L-692,492 (top) is a benzolactam. L-163,191 (MK-0677) is a spiropiperidine (bottom).

With permission of Root AW, et al.

Figure 2

Cellular action of GH releasing and GH-inhibiting agents. Acting through cAMP, GHRH increases PKA activity, leading to phosphorylation of L-type  $\text{Ca}^{++}$  channels and increased transport and IC  $\text{Ca}^{++}$ . SRIH increases transport of  $\text{K}^+$ , thus raising IC levels of  $\text{K}^+$  and inhibiting  $\text{Ca}^{++}$  transport. GHS increases IC  $\text{Ca}^{++}$  by activating PLC that (1) inhibits  $\text{K}^+$  transport, (2) mobilizes  $\text{Ca}^{++}$  from calciosomes through  $\text{IP}_3$ , and (3) enhances  $\text{Ca}^{++}$  transport through DAG activation of PKC. GHRH (GH-secreting hormone); GHS (GH-secreting hormone); SRIH (somatostatin); ATP (adenosine triphosphate); cAMP (cyclic adenosine monophosphate); PKA (protein kinase A); PLC (phospholipase C);  $\text{PIP}_2$  (phosphatidylinositol);  $\text{IP}_3$  (inositol triphosphate); DAG (diacylglycerol); PKC (protein kinase C); AC (adenylyl cyclase); IC (intracellular). Solid line represents (stimulatory effect); dotted line represents (inhibitory effect).



Adapted with permission from Smith RG, et al. Mechanism of action of GHRP-6 and nonpeptidyl growth hormone secretagogues. In: Barcu BB, Walker RF, eds. *Growth Hormone Secretagogues*. New York, NY:Springer-Verlag;1996:147-163.

pretest priming with estradiol or testosterone but not with oxandrolone.<sup>10</sup>

GHSs do not stimulate secretion of thyrotropin, luteinizing hormone, or follicle-stimulating hormone, but variably increase serum concentrations of prolactin and cortisol. GHSs stimulate release of GH in most short-statured children with GH deficiency (GHD), except those with interruption of the pituitary stalk or absence of the adenohypophysis.<sup>11-15</sup> In GHD children without evident anatomic insult to the hypothalamic-pituitary unit, GHS-induced GH secretion is quantitatively similar to that of GHRH and is synergistic with GHRH.

## THE GHS RECEPTOR

GHRH acts through a 423 amino acid  $\text{G}_s$ -protein receptor with 7 membrane-spanning domains whose gene is located at human chromosome 7p14. The GHRH receptor primarily activates adenylyl cyclase, leading to increased intracellular (IC) levels of cAMP and protein kinase A; activation of L-type  $\text{Ca}^{++}$  chan-

nels, resulting in increased cytosol  $\text{Ca}^{++}$  levels; immediate release of stored GH; and subsequent increase in GH synthesis (Figure 2).<sup>16,17</sup> GHS is unable to interact with the GHRH receptor. The functional G-protein receptor for GHS (Ia) is a 366 amino acid polypeptide with 7 transmembrane domains whose gene is located at human chromosome 3q26.2.<sup>7,18</sup> The second, nonfunctional isoform of the GHS receptor (Ib) is formed by alternative mRNA processing and is truncated at 289 amino acids; therefore, after the fifth transmembrane domain it terminates with an IC domain of 58 amino acids. GHS receptor Ib fails to bind or biologically respond to GHS, and its function is unknown.

Messenger RNAs for GHS receptor types Ia and Ib are expressed in very low amounts in both the anterior and posterior pituitary (their levels are 1/100th the receptor number of GHRH and SRIH receptors). They also are expressed in the arcuate, ventromedial, and supraoptic nuclei, infundibular hypothalamus (in neurons near the median eminence), hippocampus, and dentate gyrus.<sup>7,18</sup>



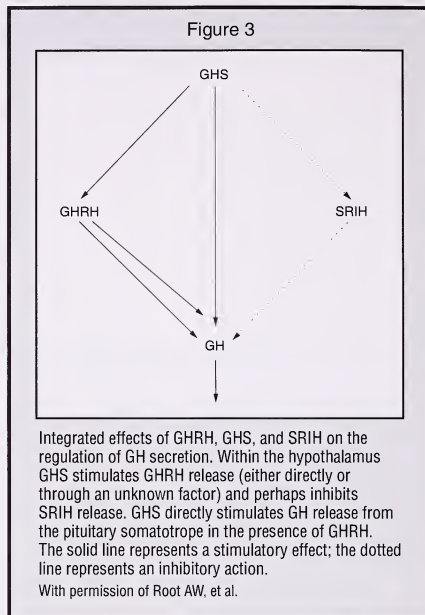
SRIH (acting through several G<sub>i</sub>-protein receptors) activates K<sup>+</sup> channels, leading to hyperpolarization and inhibition of Ca<sup>++</sup> channel function and thereby antagonizing the secretion of GH induced by GHRH and GHS (Figure 2). Through the G $\alpha_{11}$  subunit, type Ia GHS receptor activates PLC, which inactivates K<sup>+</sup> channels, leading to cellular depolarization and activation of Ca<sup>++</sup> channels. PLC also hydrolyzes membrane phosphatidylinositol, thus increasing cytosol concentrations of inositol triphosphate and diacylglycerol (Figure 2).<sup>8</sup> Diacylglycerol activates protein kinase C (PKC), which also activates L-type transmembrane Ca<sup>++</sup> channels and increases IC Ca<sup>++</sup> concentrations and GH secretion. Thus, one of the important functions of the GHS is to antagonize the inhibitory effects of SRIH on GH release stimulated by GHRH. These data explain, in part, the necessity for somatotroph exposure to GHRH in order for the GHS to act—that is, the latter works in large part by permitting GHRH to act. The PLC signal transduction system also can be utilized by GHRH and the adenyl cyclase system can be activated by GHRP-2.<sup>19</sup>

The presence of GHS receptors in the hypothalamus indicates that these agents also act centrally (Figure 3). Indeed, this may be their primary site of action, as they increase electrical activity and *c-fos* expression in GHRH and neuropeptide Y-containing neurons within the arcuate nucleus. Levels of GHRH in the pituitary portal vasculature also are increased.<sup>17,20,21</sup> GHS may act directly on GHRH neurons or indirectly through an as yet unknown factor. The dual site of GHS action permits these secretagogues to influence the amplitude of GHRH-mediated endogenous GH secretion. GHSs also stimulate release of arginine vasopressin, accounting for the transient increase in cortisol levels recorded after their administration.<sup>22</sup>

### CLINICAL SIGNIFICANCE OF THE GHSs

GHRH and GHSs have been tested to determine if they are of use in diagnosing the presence and/or the etiology of GHD. Since the most reliable way to identify the GHD patient with an anatomically intact hypothalamic-pituitary unit—and without a known insult to the central nervous system—is unclear,<sup>23,24</sup> the role of GHS testing in this process has not been defined. Bercu and Walker<sup>25,26</sup> proposed a scheme to distinguish between deficiency of GHRH, deficiency of endogenous GHS, and deficiencies of both by sequential administration of GHRH and GHS. Its utility has yet to be verified.

Mericq et al<sup>13</sup> reported that 15/22 children with idiopathic GHD responded to an acute injection of GHRH with a significant increase in GH levels; 12/22 responded to GHRP-1; a total of 19/22 responded to a combination of the 2 agents. Five children did not respond to either secretagogue alone, but did respond to the combination of agents. Three children did not respond to any single or combined stimuli.



Nineteen other children with clinical and hormonal findings consistent with idiopathic GHD have had normal GH secretion in response to GHRH and GHS.<sup>12,14</sup> These data confirm the difficulty in establishing the diagnosis of idiopathic GHD and assigning its pathophysiology to an absence of either GHRH or the endogenous secretagogue. Although a positive GH secretory response to GHS implies the presence of somatotropes and their exposure to GHRH, an absent or blunted response does not identify the site of error in GH secretion. Thus, testing with GHS cannot replace more standard studies that primarily establish the presence of deficient GH secretion in the appropriate clinical setting.

GHRH and GHSs have been and are being investigated as therapeutic agents. GHSs could be useful therapeutically only in patients with the ability to secrete GH in response to GHSs. Laron et al<sup>27,28</sup> administered hexarelin intranasally to 8 prepubertal, short, normal children for 8 to 10 months and recorded an increase in growth velocity from 5.3 to 7.4 cm/y with a parallel increase in bone maturation. After hexarelin was discontinued, growth rate stabilized or declined. Mericq et al<sup>29</sup> treated 6 GHD children with subcutaneous GHRP-2 for 6 months, and observed an increase in mean growth rate from 2.5 to 5.6 cm/y. Pihoker et al<sup>30</sup> administered GHRP-2 intranasally to 15 GHD children for 3 to 4 months and recorded an increase in mean growth velocity from 3.6 to 6.7 cm/y. In neither study did IGF-1

levels increase during treatment. Since these studies were of extremely brief duration, the effects of more prolonged administration of GHSs to children with GHD need to be studied to establish the role of GHSs in the management of this disorder. Whether the combined use of GHRH or its analogues or GHSs will be useful in the treatment of some GHD subjects is unknown at this point, but is a potential direction for further research. Given the disappointing results of GH treatment of normal short children,<sup>31</sup> it is unlikely that GHSs alone will prove effective in this group of children.

Consideration is being given to the therapeutic usefulness of GHRH and/or GHSs in older adults. GH secretion wanes between the third and fifth decades of life, possibly due to increase in somatostatinergic tone. Thorner<sup>32</sup> suggested alternatively that the decline in GH secretion with aging may be related to a decline in production of endogenous GHS with ensuing decreases in GHRH and GH secretion. In healthy elderly subjects, the GH secretory pulse amplitude can be increased by administration of GHRH or a GHS intravenously or orally.<sup>32-34</sup> Bach<sup>33</sup> reported that in elderly subjects with basal levels of IGF-1 <165 ng/mL, oral administration of 25 mg of MK-0677 for 6 months increased mean 24-hour GH concentrations, serum levels of IGF-1, and lean body and fat mass, and prevented decline in shoulder and knee strength when compared with placebo-treated subjects. Whether therapy with GH, GHRH, and/or a GHS will have long-term beneficial effects on the quality of life of the elderly subject without undue adverse effects has yet to be determined. If GH proves of value in the management of surgical wounds, burns, fractures, heart failure, or debilitating diseases such as AIDS, GHSs also might be useful in the management of these problems.<sup>35</sup>

## FUTURE DIRECTIONS

Future efforts will be focused on identifying the endogenous ligands that form this new system of GH regulation, such as localizing the cellular site of endogenous GHS production; determining the mechanisms

| CME CERTIFICATION   |
|---|
| <p>The <i>GGH</i> Editorial Board is pleased to announce Category 1 credit for <i>GROWTH, Genetics, &amp; Hormones</i> from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.</p> <p><b>Overview:</b> This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.</p> <p><b>Target Audience:</b> This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.</p> <p><b>Method of Physician Participation:</b> Physicians can study each issue of <i>GROWTH, Genetics, &amp; Hormones</i>, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.</p> <p><b>Learning Objectives:</b> Through participation in this enduring materials series, the participant will have the opportunity to:</p> <ol style="list-style-type: none"> <li>1. Apply current research and advances to the management of patient care for optimal clinical outcomes.</li> <li>2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.</li> <li>3. Conceptualize areas for future research in the field of growth and genetics.</li> </ol> |

by which its secretion is controlled; defining the physiologic role of GHS in normal and pathologic situations; establishing procedures to identify deficiency of the endogenous ligand; and determining the diagnostic and therapeutic utility of exogenous GHS in children with growth retardation, the elderly, patients with debilitating illnesses, and other appropriate clinical situations. Much illuminating investigation remains to be done.

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## Tyrosine Kinases: Their Role in Producing Endocrine and Other Cancers

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The history of tyrosine kinase oncogenes can be traced to 1911 when Francis Peyton Rous, of the Rockefeller Institute, demonstrated that a chicken tumor could be transplanted using a cell-free filtrate. Thus, the field of tumor virology was born. However, it was not until more recent advances in molecular biology that it was recognized that the Rous sarcoma virus transforms cells due to the presence of a tyrosine kinase, the *src* oncogene, in its genome. In 1976, Drs. Harold Varmus and J. Michael Bishop published the finding that the *src* oncogene in the virus was not a true viral gene but instead was a nor-

mal cellular gene that the virus had acquired during replication in the host cell. These normal cellular homologues are referred to as proto-oncogenes. However, the transforming version of the *src* oncogene was mutated, and constitutively active, as compared with the tightly regulated normal cellular SRC protein tyrosine kinase.

Since the discovery of the *src* oncogene as a tyrosine kinase, numerous tyrosine kinases have been found, and now number more than 100. Tyrosine kinases can be divided into 2 primary categories: receptor and nonreceptor tyrosine kinases (Figure 1). Receptor tyrosine kinases, such as the insulin receptor and the insulin-like growth factor receptor, contain extracellular ligand binding domains that bind to specific proteins, such as hormones or growth factors.<sup>1</sup> Ligand binding induces activation of the intracellular tyrosine kinase domain, leading to the initiation of signaling events specific for the receptor.

Nonreceptor tyrosine kinases are intracellular cytoplasmic proteins that are linked to a variety of transmembrane receptors, such as the growth hormone or prolactin receptors.<sup>2,3</sup> These nonreceptor tyrosine kinases are similarly activated after binding of ligand to their associated receptors (Figure 1).<sup>2,3</sup>



The list of tyrosine kinases also includes proteins that are involved in pathways that regulate cellular growth, activation, and differentiation. Despite the essential contribution of tyrosine kinase oncogenes to our current understanding of a variety of cellular signaling pathways and their historical importance to the field of tumor biology,<sup>4</sup> only a limited role for tyrosine kinases in human cancers has been identified. This article reviews the mechanisms by which protein tyrosine kinases may participate in malignant transformation and the tyrosine kinases that are known to play prominent roles in the production and growth of human cancer.

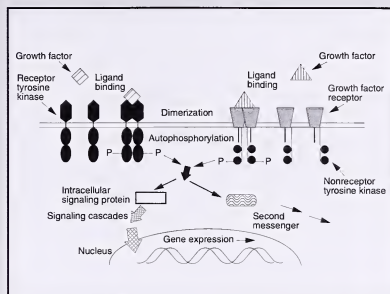
## ONCOGENIC ACTIVATION OF TYROSINE KINASES

Several mechanisms by which a tyrosine kinase might acquire transforming function exist, but in all cases the result is constitutive activation of a protein that is normally tightly regulated. To understand how constitutive activation of a tyrosine kinase might occur, the normal pathways that regulate kinase activity require review (Figure 1).

When a growth factor binds to its receptor, the receptor or its associated tyrosine kinase becomes transiently activated, which leads to the activation of other proteins in the growth-stimulatory pathway and the consequent production of small regulatory molecules called second messengers. These signals are ultimately transmitted to the nucleus, where expression of specific genes is induced and lead to cell division. At the same time, growth-inhibitory signals are generated normally to prevent cellular proliferation from continuing indefinitely. One of the functions of these inhibitory signals is the deactivation of the tyrosine kinase. Precise control of these positive and negative signaling events is necessary to maintain normal cellular growth.

Constitutive activation of tyrosine kinases occurs by several mechanisms (Figures 2 and 3). The first is by overproduction of a growth factor or by concomitant production of a growth factor and its receptor, which leads to abnormal receptor stimulation

**Figure 1**  
**Signaling Through Tyrosine Kinases**



Signaling through receptor (left) and nonreceptor (right) tyrosine kinases.

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and activation (Figure 2). A second mechanism is mutation of the tyrosine kinase, which leads to constitutive activity. Mutations may occur in either the catalytic or the regulatory regions of the protein, or mutations can occur in proteins that regulate the activity of tyrosine kinases (Figure 3).

Two tyrosine kinases have been clearly implicated in human diseases. These two, RET (REarranged during Transfection)<sup>5</sup> and ABL (isolated from the Abelson leukemia virus), are constitutively activated as the result of several different types of mutation. They serve as examples of the potential role of tyrosine kinases in human cancers.

## MULTIPLE ENDOCRINE NEOPLASIA AND THE RET TYROSINE KINASE

Multiple endocrine neoplasia type 2 (MEN 2) and familial medullary thyroid carcinoma (FMTC) are clinically distinct syndromes characterized by a predisposition to the development of endocrine tumors (Table 1). They have in common the occurrence of medullary thyroid cancer, which is the most common cause of death in affected families. Four distinct subtypes of MEN 2 exist: MEN 2A1, 2A2, 2A3, and 2B (Table 1).

FMTC is characterized by the development of bilateral medullary thyroid carcinoma at an average age of 40 to 50 years. To be classified as FMTC, neither patients nor their family members may have pheochromocytoma or parathyroid disease.

In contrast, patients with MEN 2A develop medullary thyroid carcinoma at an earlier age, typically between 20 and 30 years of age. Further, patients with MEN 2A also may develop pheochromocytoma and parathyroid hyperplasia (MEN 2A1), or only pheochromocytoma (MEN 2A2), or parathyroid adenomas or hyperplasia (MEN 2A3).

### In Future Issues

#### Insulin, the IGF System, and IDDM

Cheryl Deal, MD

#### Genetic Basis of Human Chondrodysplasias: A Review

William A. Horton, MD

#### How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?

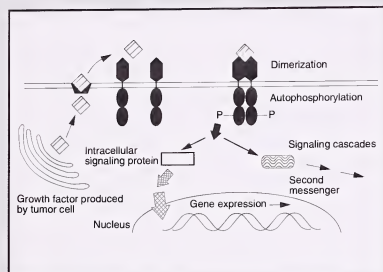
Arnold Slyper, MD

#### The Role of Leptin and Its Receptor in Obesity

Rudolph Leibel, MD



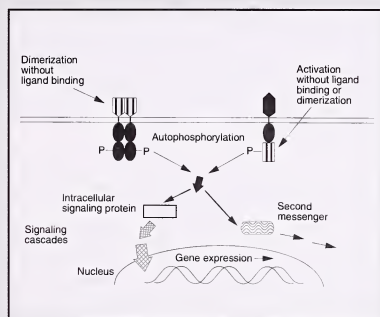
Figure 2  
Tyrosine Kinase Activation by an  
Autocrine Growth Loop



Constitutive ligand production resulting in tyrosine kinase activation.

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Figure 3  
Mechanisms Leading to  
Constitutive Kinase Activity



Mutations can produce constitutive tyrosine kinase activation. This might occur by a mutation in the extracellular domain (shown on the left) that causes receptor dimerization independent of ligand binding. Another possibility would be a mutation in the cytoplasmic domain that leads to kinase activation without either ligand binding or receptor dimerization (shown on the right).

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MEN 2B has a more complex phenotype. All patients have pheochromocytoma and medullary thyroid carcinoma, but parathyroid involvement is rare. In addition, there usually are consistent developmental abnormalities, including characteristic facies, a marfanoid habitus, thickened corneal nerves,

skeletal abnormalities, mucosal neuromas, and sometimes diffuse intestinal ganglioneuromas.

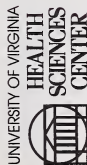
The genes responsible for each of the MEN 2 subtypes were identified using a combination of physical mapping and genetic linkage techniques.<sup>6,7</sup> These disorders were known to be inherited in an autosomal dominant fashion. Initial family studies localized the MEN 2A gene to a region of chromosome 10. Soon thereafter, linkage analysis mapped MEN 2B and FMTC to the same region as MEN 2A, suggesting that mutations in a single gene might be responsible for all 3 clinical phenotypes. Physical mapping of the region on chromosome 10 narrowed the MEN 2 locus to a 480-kb area in which the *ret* proto-oncogene was mapped, and *ret* emerged as the most likely candidate for the MEN 2 gene. In 1993, germline mutations of *ret* were identified in DNA from MEN 2A patients and in DNA from FMTC patients by 2 independent groups,<sup>8,9</sup> confirming the potential role of the RET tyrosine kinase in these disorders. A map of the RET tyrosine kinase and the mutations associated with specific disease phenotypes is depicted in Figure 4.

The *ret* proto-oncogene encodes a transmembrane receptor tyrosine kinase whose normal function and ligand remain unknown. In the adult, RET is expressed in cells and lineages derived from the branchial arches and neural crest, including the thyroid, parathyroid, adrenal medulla, enteric ganglia, brain, and autonomic nervous system.

The majority of *ret* mutations associated with both MEN 2A and FMTC involve any 1 of 5 cysteine residues located in the extracellular ligand-binding domain of RET. Both mutant and wild-type alleles are retained in tumor DNA, suggesting a dominant mechanism in the development of these malignancies. The most common mutation occurs at cysteine 634, accounting for 74% of *ret* mutations in families with FMTC and with all MEN 2A syndromes; the percentage increases to 92% in families with medullary thyroid carcinoma, parathyroid disease, and pheochromocytoma (MEN 2A1). Although it is clear that *ret* mutations are associated with medullary thyroid carcinoma, these data suggest that other factors contribute to the distinct disease phenotypes.

In MEN 2B patients, a single germline point mutation in the cytoplasmic tyrosine kinase domain of *ret* was reported by several groups in 1994.<sup>10,11</sup> About 95% of MEN 2B patients have the same point mutation, substituting a threonine for methionine at codon 918 in the RET tyrosine kinase domain. This mutation has been shown to be inherited with only this MEN disease phenotype and no other. As with MEN 2A mutations, both the wild-type allele and the mutant allele are present in tumor DNA, consistent with the autosomal dominant inheritance.

All the *ret* mutations examined lead to constitutive activation of the RET tyrosine kinase. However, the mechanism of RET activation differs for the mutations commonly associated with MEN 2A and



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4. a b c d

7. a b c d

2. a b c d

5. a b c d

8. a b c d

3. a b c

6. a b c d

9. a b c d

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1. Educational value of newsletter

5 4 3 2 1

2. Clinical relevance of articles

5 4 3 2 1

3. Newsletter style/format

5 4 3 2 1

4. Length of articles

5 4 3 2 1

5. Were the educational objectives met?

5 4 3 2 1

6. In your opinion, how could this newsletter be improved?

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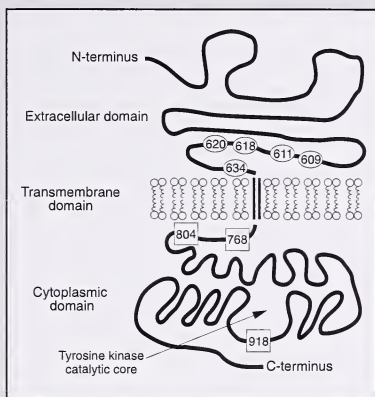
MEN 2B.<sup>7</sup> Normally, upon ligand binding, receptor tyrosine kinases dimerize, autophosphorylate, and as a result are activated. RET tyrosine kinases with amino acid 634 mutations characteristic of MEN 2A undergo dimerization and activation in the absence of ligand. In contrast, the single point mutation of methionine 918 in the tyrosine kinase domain of RET in MEN 2B leads to activation of the RET tyrosine kinase in the absence of dimerization. These 2 different mechanisms of activation suggest a potential explanation for the divergence in disease phenotypes.

Probably *ret* mutations are not sufficient for tumorigenesis in vivo. Cancer is typically thought to be a multistep process, resulting from an accumulation of defects in genes involved in the positive or negative regulation of cell proliferation and survival. As most patients with MEN 2 have thyroid C-cell hyperplasia, it is possible that the inherited activation of the RET tyrosine kinase leads to thyroid proliferation, with additional acquired somatic mutations leading to the development of cancer. If this scenario is correct, one might also expect to see *ret* mutations in sporadic cases of thyroid cancer. In fact, some of the same *ret* mutations seen in familial cases of medullary thyroid carcinoma are present in sporadic cases.<sup>6,7,12</sup> A somatic mutation of methionine 918 has been detected in 30% to 40% of sporadic medullary thyroid carcinomas.<sup>10,11</sup> A mutation at amino acid 768 of *ret*, present in 10% of cases of FMTC, also has been found in some sporadic cases of medullary thyroid carcinoma. Some cases of sporadic pheochromocytomas also contain somatic mutations of methionine 918 or in the MEN 2A region. These somatic mutations are distinguished from familial cases by comparing germline DNA to tumor DNA. In sporadic cases, *ret* mutations are seen in the tumor but not in normal tissues; in familial cases, *ret* mutations are seen in both.

Rearrangements of *ret* also have been found in 10% to 35% of human papillary thyroid carcinomas.<sup>7,12</sup> These rearrangements fuse the RET tyrosine kinase domain to sequences from another cellular protein. This results in a deletion of the extracellular ligand binding domain and leads to a constitutively activated RET tyrosine kinase. This activating RET rearrangement was observed in 60% of cases of papillary thyroid carcinoma in children from areas contaminated by the Chernobyl accident.<sup>13</sup> This same rearrangement of RET occurs in cell lines exposed to in vitro irradiation, suggesting that the RET rearrangement was induced by radiation exposure.<sup>14</sup>

Identification of germline mutations of the *ret* proto-oncogene in MEN 2A, MEN 2B, and FMTC and demonstration of *ret* mutations and rearrangements in sporadic medullary thyroid cancer and papillary thyroid carcinoma distinguishes *ret* as the most important gene involved in the development of thyroid cancer. A data base has been established to maintain and analyze *ret* mutations in MEN 2 families.<sup>15</sup> Discovering correlations of specific mutation

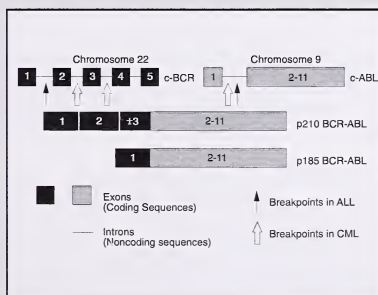
Figure 4  
RET Tyrosine Kinase



Schematic of the RET tyrosine kinase. Amino acid mutations frequently observed in MEN 2 in the extracellular domain of RET are portrayed as ovals. Rectangles denote amino acid mutations in the intracellular domain. Gray shaded mutations are found in MEN 2A or familial medullary thyroid carcinoma. The white rectangle depicts the MEN 2B amino acid mutation.

From Goodfellow PJ, Wells SA. RET gene and its implications for cancer. *JNCL*. 1995;87(20):1515-1523.

Figure 5  
The Molecular Consequences of the Philadelphia Chromosome Translocation



The Philadelphia chromosome translocation juxtaposes sequences of the breakpoint cluster region (BCR) on chromosome 22 with the gene encoding the c-ABL tyrosine kinase from chromosome 9. The BCR-ABL fusion proteins found in CML and ALL are depicted.

With permission of Druker BJ, et al.



with specific phenotypes would have substantial implications for screening MEN families.<sup>8,15,16</sup> Thus far, for example, mutations at amino acid 768 have been found only in FMTC, and not in any case of MEN 2A or MEN 2B. Data will need to be compiled from many more families to substantiate these findings.

DNA testing can now serve both to confirm the clinical diagnosis and predict the MEN 2 syndrome in individual family members. In the absence of a germline mutation, a patient with isolated medullary thyroid cancer can now be appropriately identified as a sporadic case. In a MEN 2A family, detection of a *ret* mutation in a family member can facilitate appropriate biochemical screening or consideration of prophylactic thyroidectomy, while mutation-negative individuals can be reassured. In MEN 2B, with its associated single germline mutation in amino acid 918, DNA testing offers a significant clinical advantage. Thyroid cancer in MEN 2B can present in childhood; therefore, *ret* mutation testing provides a true breakthrough in screening, providing opportunities for preventive management. Further correlation between mutation location and phenotype may permit streamlined surveillance of MEN 2 patients in the future. DNA testing has proven cost-effective in another inherited cancer syndrome, retinoblastoma, and may be similarly cost-effective in MEN 2.

## LEUKEMIA AND THE ABL TYROSINE KINASE

Chronic myelogenous leukemia (CML) accounts for approximately 20% of adult leukemia cases. CML is characterized clinically by an initial chronic phase, during which there are excess numbers of white blood cells in the peripheral blood and bone marrow. During the chronic phase of the disease, white blood cells mature normally and there is a full spectrum of white blood cells, from blasts to neutrophils, circulating in the peripheral blood. After a median of 4 years, there is a transition to an accelerated, or

blast, phase. This transition is accompanied by the loss of the capacity for terminal differentiation of white blood cells, resulting in an acute leukemia.

In 95% of CML cases, the Philadelphia chromosome is detectable. This chromosome abnormality is a somatic mutation that occurs in a hematopoietic stem cell as a result of a reciprocal translocation between chromosomes 9 and 22. This balanced translocation fuses sequences of the breakpoint cluster region (*bcr*) on chromosome 22 with the *c-abl* tyrosine kinase from the long arm of chromosome 9 (Figure 5).<sup>17</sup>

Specific BCR-ABL fusion proteins lead to distinct disease phenotypes. In CML, the BCR-ABL fusion protein contains 927 or 902 amino acids from BCR fused to the ABL tyrosine kinase. This fusion protein, termed p210 BCR-ABL, is found in 95% of patients with CML. However, it is also present in 5% to 10% of adults with acute leukemia for whom there is no evidence of antecedent CML. Another BCR-ABL fusion protein, p185 BCR-ABL, which contains only 426 amino acids from BCR, occurs in 10% of adult cases and 5% to 10% of pediatric cases of acute lymphoblastic leukemia, but is never seen in CML.<sup>18,19</sup> Various BCR-ABL fusion proteins are shown in Figure 5.

The BCR-ABL fusion proteins have severalfold elevation of tyrosine kinase activity over that in the normal *c-abl* kinase,<sup>20</sup> and the tyrosine kinase activity of the BCR-ABL protein correlates with the disease phenotype. For example, p185 BCR-ABL is more active as a tyrosine kinase than p210 BCR-ABL and is associated with a rapidly progressive acute leukemia. The p210 version of BCR-ABL, although activated as a tyrosine kinase as compared to *c-abl*, is less active than p185 BCR-ABL and is associated with a more indolent disease phenotype.

In many in vitro assays of tumorigenicity *bcr-abl* has transforming ability. However, the clearest

Table 1  
MEN 2 Subtypes and FMTC Clinical Features

| Category   | Medullary Thyroid Carcinoma | Pheochromocytoma | Hyperparathyroidism | Other Clinical Features  |
|------------|-----------------------------|------------------|---------------------|--|
| MEN 2A (1) | yes                         | yes              | yes                 |  |
| MEN 2A (2) | yes                         | yes              | no                  |  |
| MEN 2A (3) | yes                         | no               | yes                 |  |
| MEN 2B     | yes                         | yes              | no                  | Typical facies, mucosal neuromas, skeletal abnormalities, intestinal ganglioneuromas |
| FMTC       | yes                         | no               | no                  | Late onset, more indolent course   |

FMTC, familial medullary thyroid carcinoma; MEN, multiple endocrine neoplasia

evidence for the involvement of *bcr-abl* in leukemia comes from studies from the laboratories of Drs. Owen Witte and David Baltimore. In these studies, investigators expressed *bcr-abl* in murine bone marrow cells and used these cells to reconstitute lethally irradiated mice. These mice develop a syndrome resembling CML as well as other leukemias.<sup>21,22</sup> These data implicate *bcr-abl* as the cause of CML, and provide the strongest evidence for a protein tyrosine kinase as the etiologic agent in a human malignancy.

## PROTEIN TYROSINE KINASES AND OTHER CANCERS

Aside from the examples noted above, implication of tyrosine kinases in the etiology of other human malignancies has been difficult. Both *ret* and *abl* were identified in human cancers because of evident genetic abnormalities — heritable predisposition to cancer in the first instance, and characteristic cytogenetic abnormalities in tumor cells in affected patients in the second. These genetic abnormalities led to the discovery of activating mutations in tyrosine kinases and implicated the tyrosine kinase as the cause of the disease. Thus, the contribution of genetic abnormalities and mutations has been strong evidence for the involvement of ABL and RET tyrosine kinases in human diseases. Unfortunately, although most other cancers have associated genetic abnormalities, no other frequent mutations in tyrosine kinases have been found to suggest an etiologic role in a specific tumor.

As previously noted, there are several mechanisms by which tyrosine kinases can become constitutively activated in the absence of mutations in the tyrosine kinases. These mechanisms include autocrine production of a growth factor or mutations in proteins that regulate tyrosine kinase activity. There are numerous examples of possible autocrine growth loops involving tyrosine kinases in many human cancers.<sup>23</sup> However, in the absence of activating mutations, determining their contribution as a cause of the underlying malignancy has been difficult.

Tyrosine kinases also could have important roles in tumor progression. Activated tyrosine kinases might confer a proliferative advantage to subpopulations of cancer cells or they could potentiate tumor metastasis or tumor neovascularization. Expression

of certain tyrosine kinases in some human cancers has been shown to convey prognostic significance, supporting the concept that tyrosine kinases may be involved in disease progression. It remains to be determined if tyrosine kinases actually contribute to poor outcomes or are simply a reflection of a general dysregulation of cellular processes associated with advanced cancer. However, given the central role of tyrosine kinases in the control of cellular growth and differentiation, it is likely that tyrosine kinases are involved in many aspects of tumorigenesis.

Identifying molecular abnormalities in human tumors may lead to opportunities for therapeutic intervention. We have recently reported an ABL-specific tyrosine kinase inhibitor that kills BCR-ABL-expressing cells, but not normal cells, in vitro and in vivo.<sup>24</sup> Clinical studies with compounds such as these will be required to validate the concept that specific activated tyrosine kinases in human cancers can be targeted for therapeutic benefit.

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## Recombinant Human Growth Hormone and Recombinant Human Insulin-Like Growth Factor 1 in Patients With HIV-Associated Wasting

Previous open-label studies of short duration have demonstrated that rhGH or rhIGF-1 increases body weight and lean body mass and decreases body fat in adults with wasting (> 10% weight loss) associated with HIV infection and AIDS.

Schambelan et al report that in a 12-week, randomized, double-blind, placebo-controlled multicenter study of 178 HIV-infected patients, rhGH (0.1 mg/kg/d; average dose, 6 mg/d) increased body weight ( $1.6 \pm 3.7$  kg), lean body mass ( $3.0 \pm 3.0$  kg), and total ( $2.4 \pm 3.1$  L) and intracellular ( $1.3 \pm 2.9$  L) body water, while there were no changes in these values in the placebo group. Body fat declined ( $-1.7 \pm 1.7$  kg) in rhGH-treated patients, but did not decrease significantly in the placebo-treated patients. In the rhGH-treated group, treadmill work output increased an average of 13.2% after 12 weeks. The perceived health status or use of health facilities in rhGH-treated subjects did not change. rhGH was reasonably well tolerated, but many patients developed edema, arthralgia, and diarrhea. The authors concluded that rhGH could partially reverse the wasting associated with HIV infection, but this was not accompanied by a subjective improvement or alteration in disease status.

Waters et al conducted a double-blind study of the effect 12 weeks of administration of rhGH (1.4 mg/d, or one quarter of the dose utilized in the first study); rhIGF-1 (5 mg twice daily); rhGH with rhIGF-1; or placebo in 60 patients with AIDS-associated wasting. In part because of a large dropout rate, these workers noted only transient in-

creases in body weight and lean body mass and decline in fat mass in the groups receiving rhGH or rhIGF-1 alone; in the group receiving rhGH plus rhIGF-1 these changes persisted for 12 weeks. Increase in muscle strength and improvement in quality of life also were transient. In neither study was there any alteration in immune function or exacerbation of AIDS. Waters et al concluded that rhGH and rhIGF-1 at the doses employed in their study were not useful in the treatment of the wasting of AIDS.

Waters D, et al. Recombinant human growth hormone, insulin-like growth factor 1, and combination therapy in AIDS-associated wasting: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1996;125:865-872.

Schambelan M, et al. Recombinant human growth hormone treatment and HIV-associated wasting. *Ann Intern Med* 1996;125:873-882.

**Editor's comment:** Although rhGH, rhIGF-1, or a combination of the 2 agents can increase lean body mass and decrease body fat content in adults with HIV- and AIDS-associated wasting (at least transiently), beneficial effects on the course of the disease or the quality of life were not observed in either of the 2 studies. Despite such data, the Food and Drug Administration has approved a 12-week course of rhGH therapy for patients with HIV-associated wasting. The estimated cost per patient is \$12,000.

Allen W. Root, MD

## Adult Height in Growth Hormone Deficiency (GHD) Children Treated With Biosynthetic GH

This study consists of the largest number of patients (121) treated over the longest period with a constant dose (0.3 mg/kg/wk) of rhGH reported to date. One hundred six patients completed the study and attained their adult height. The chronologic age at initiation of therapy was  $11.3 \pm 2.1$  years for males and  $10.1 \pm 2.8$  years for females. The etiology of GHD was 102 idiopathic versus 19 organic. Eighty-four of the 121 developed puberty spontaneously. The total duration of GH treatment (with or without native pituitary GH) was approximately  $7.5 \pm 3.2$  years. The Bayley-Pinneau predicted adult height was  $163.2 \pm 7.4$  cm for males and  $150.0 \pm 7.0$  cm for females. The adult statistics are recorded in the table to the right.

Adult height was dependent on height (positively) and age (negatively) at the start of these protocols, duration of treatment on protocol, growth rate during first year, and sex.

The authors postulate that the significant increase in adult height over pretreatment predicted adult height may be due to larger doses of uninterrupted GH treatment than that used in previous studies. The authors also found, in contrast to previous studies, that spontaneous puberty or female sex did not adversely affect the adult height SDS, which improved significantly during puberty from

### Adult Height in Subjects Treated With rhGH

| Outcome Variable  | Males (n=72)          | Females (n=49)        |
|---|-----------------------|-----------------------|
| Adult height (cm) <sup>a</sup>  | 171.6 $\pm$ 8.2       | 158.5 $\pm$ 7.1       |
| Total height gained (cm) <sup>b</sup>   | 46.6 $\pm$ 11.8       | 41.6 $\pm$ 16.5       |
| Adult height SD score   | -0.7 $\pm$ 1.3        | -0.7 $\pm$ 1.1        |
| Adult height SD score minus midparental target height SD score <sup>c</sup>       | -0.6 $\pm$ 1.2 (n=66) | -0.4 $\pm$ 1.2 (n=45) |
| Age at onset of puberty <sup>a</sup>  | 14.0 $\pm$ 1.9        | 12.6 $\pm$ 2.2        |
| Height at start of puberty (cm) <sup>a</sup>                                      | 146.7 $\pm$ 10.5      | 139.1 $\pm$ 11.8      |
| Height SD score at start of puberty   | -1.9 $\pm$ 1.2        | -1.9 $\pm$ 1.5        |
| Adult height minus predicted adult height at start of treatment (cm) <sup>b</sup> | 8.5 $\pm$ 8.1         | 8.5 $\pm$ 7.1         |

Values are the mean  $\pm$  SD.

<sup>a</sup> By t test,  $P < 0.0003$ , males versus females.

<sup>b</sup> By paired t test, each sex,  $P < 0.0001$  (different from 0).

<sup>c</sup> By paired t test: females,  $P = 0.02$ ; males,  $P < 0.0001$  (different from 0)



$-1.9 \pm 1.3$  to  $-0.7 \pm 1.2$ , and maximum stimulated GH and more frequent GH injections were significant predictors of first-year growth rates. They were not predictive of adult height or adult height SDS.

Blethen SL, et al. *J Clin Endocrinol Metab* 1997;82:418-420.

**Editor's comment:** This is the most definitive study done to date to answer the questions posed when the study was first begun. Short GHD children attain normal heights when the diagnosis of GHD is made early and adequate GH therapy to produce catch-up growth is initiated early in life.

Robert M. Blizzard, MD

## Low-Dose Recombinant Human Growth Hormone Increases Body Weight and Lean Body Mass in Patients With Short Bowel Syndrome

A randomized, double-blind, placebo-controlled, crossover study using low-dose ( $0.17 \text{ mg/kg/wk}$ ) recombinant hGH in individuals with short bowel syndrome (SBS) due to Crohn's disease is reported. Ten adults with surgically created SBS for more than 1 year participated in the study. All had normal 24-hour growth hormone profiles. Their mean small intestinal length was  $1.3 \text{ m}$ ; 6 of the 10 had an ileojejunostomy. All required oral or parenteral fluids. One was maintained on parenteral nutrition. Lean body fat, bone mineral content, and bone mineral density were measured by dual-energy X-ray absorptiometry (DEXA). Fecal samples were collected on a metabolic ward and pooled in 4-day batches.

During treatment with rhGH, body weight increased by  $2.3 \pm 0.8 \text{ kg}$  ( $P=0.005$ ) (Figure 1). Lean body mass increased  $5.6 \pm 1.9\%$  ( $P=0.005$ ) while body fat did not change significantly. There were small but significant changes in bone mineral content but no significant changes were seen in total body bone mineral density. Mean daily energy intake from food was  $3,500 \text{ kcal}$ . Urinary nitrogen excretion did not change during this study, but nitrogen balance was significantly improved,  $4.8 \pm 2.9 \text{ g/d}$  versus  $2.3 \pm 2.9 \text{ g/d}$  ( $P=0.011$ ). The authors suggest that these studies demonstrate that short-term, low-dose GH therapy for as little as 8 weeks can increase body weight, lean body mass, total body water, and bone mineral content without clinical signs of edema or altered glucose metabolism. Thus, this may be a useful adjunct to nutritional support for patients with SBS.

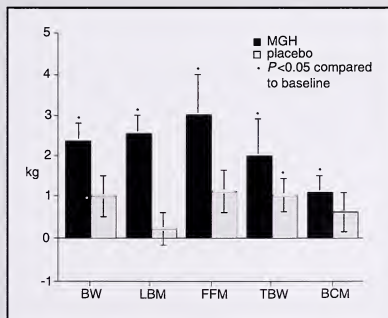
Ellegard L, et al. *Ann Surg* 1997;225:1:88-96.

**Editor's comment:** These interesting studies suggest ways in which the anabolic effects of GH may be useful in individuals with SBS secondary to Crohn's disease. Recently, growing adolescents with inactive Crohn's disease (*Digestive Diseases and Sciences* 1996;41:1754-1759) were reported to have increased energy expenditure as compared to both healthy growing adolescents and non-growing subjects with inactive Crohn's disease. Until we understand more about the pathophysiology of Crohn's disease, suggesting alternatives to increased nutrient intake as a means of improving growth in these individuals may not be possible. However, the studies by Ellegard et al suggest that individuals with Crohn's disease and SBS may

benefit significantly from the anabolic effects of rhGH. Byrne et al (*Annals of Surgery* 1995;222:243-255) studied rhGH in addition to a high-carbohydrate, low-fat diet with added glutamine in 17 adults with SBS and demonstrated significant improvement in absorption of protein and decrease in stool output. Although the studies performed by Byrne et al and those reported above by Ellegard and colleagues were performed in adults, the potential implications for children with congenital or acquired SBS are apparent. Randomized, multicenter trials are currently in progress using both pediatric and adult populations to evaluate this new therapeutic regimen. The estimated potential reduction in health-care costs associated with this treatment should be an incentive for industry support of these studies. There is reason to be optimistic that this could be an additional beneficial use of rhGH.

William L. Clarke, MD

Figure 1



Changes in body composition during 8 weeks of treatment with low-dose recombinant human growth hormone (rhGH) and placebo in 10 patients with short bowel syndrome. BW = body weight; LBM = lean body mass; FFM = fat-free mass; TBW = total body water; BCM = body cell mass.

From Ellegard L, et al. Low-dose recombinant human growth hormone increases body weight and lean body mass in patients with short bowel syndrome (SBS). Lippincott-Raven Publishers, NY. *Ann Surg* 1997;225(1):92.



## Trisomy 21: A Possible Molecular Basis

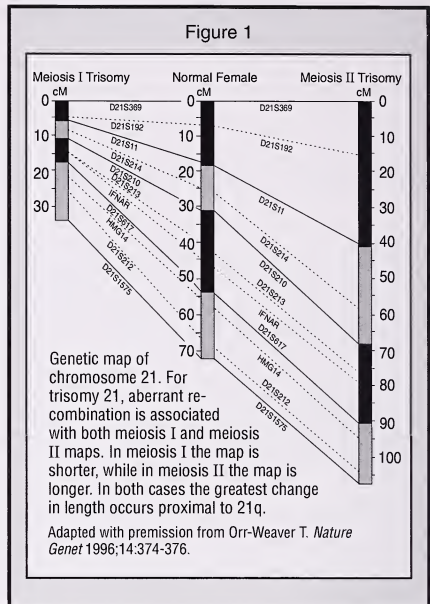
Trisomy 21 accounts for the vast majority of Down syndrome. It is the most common trisomy of newborns and the leading known cause of mental retardation. Trisomy 21 has long been known to result from failure of the 2 homologous chromosome 21s to segregate (nondisjunction) during meiosis, especially during maternal meiosis I. Recently, evidence has emerged that the genetic recombination that normally occurs between homologous chromosomes during meiosis is altered in trisomy 21. Indeed, reduced recombination confined primarily to the proximal region of chromosome 21q was found in cases due to meiosis I errors. Now, abnormal recombination also has been found in cases due to meiosis II errors. In the meiosis II errors, recombination is increased, and accounts for about 20% of trisomy 21.

Lamb et al studied 133 trisomy 21 cases of maternal meiosis II errors using a panel of chromosome 21 DNA markers that allowed them to examine genetic recombination as well as the parental and meiotic origin of the extra chromosome. They found increased recombination restricted mainly to the proximal q arm of the chromosome. Importantly, they detected no difference in the extent of recombination in chromosomes derived from older versus younger women.

The observations prompted speculation from these authors, as well as from Orr-Weaver in an invited editorial, about how decreased and increased recombination might contribute to chromosome segregation errors in meiosis I and meiosis II, respectively. The deviations from "normal" are depicted in the genetic map of chromosome 21, shown in Figure 1, in which the length of chromosome segments corresponds to the extent of recombination. It is suggested that physical attachments that exist between homologous chromosomes during recombination (so-called chiasmata, or sites of crossing over) and between sister chromatids are important for normal chromosome segregation. In the former instance, it is proposed that chiasmata that form near the end of the chromosome are less effective at promoting proper segregation than those formed proximally. Perhaps distal chiasmata are less stable than proximal ones. If so, a reduction in proximal chiasmata, which would be associated with the observed reduced recombination in this region, would predispose to missegregation at meiosis I.

To explain how increased recombination events in the proximal 21q might promote meiosis II errors, the possibility of chromosome entanglement is raised. In this scenario, some of the excessive proximal chiasmata are not resolved during meiosis I. This results in failure of the chromosome 21 homologues to segregate. If the homologues remain entangled after the first segregation, then their chromatids may not segregate properly during meiosis II. This implies that disturbances of meiosis I can adversely affect segregation at meiosis II—a new concept.

Since neither of these explanations addresses why trisomy 21 occurs more frequently in older mothers, a 2-step



process is proposed. In the first step, "susceptible" meiotic chromosome configurations are established prenatally in all female fetuses. In most instances, such configurations are resolved by normal meiotic processes. However, with increasing age, these processes become less effective and unresolved susceptible configurations result in nondisjunction and trisomy. Meiotic-specific proteins, such as spindle or microtubular motor proteins, that degrade with time are mentioned as candidates to explain the age-dependency of the meiotic errors.

Lamb NE, et al. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. *Nature Genet* 1996;14:400-405.

Orr-Weaver T. Meiotic nondisjunction does the two-step. *Nature Genet* 1996;14:374-376. Editorial.

**Editor's comments:** History has tended to keep the disciplines of cytogenetics and molecular genetics apart in many institutions. The work described above importantly demonstrates the value of integrating the 2 to address questions that have been around for decades. The results may not explain precisely why trisomy 21 occurs, but they provide hypotheses to test and molecular contexts in which to consider alternative possibilities.

William A. Horton, MD

## Dwarf Mice and the Aging Process

Brown-Borg et al have reported that Ames dwarf mice (*df/df*) live longer than their normal siblings of the same strain given the same environmental conditions. The authors followed 28 normal and 34 Ames dwarf mice born during July and August 1992 from the same litters. Both types of mice were maintained in a conventional environment and fed the same unrestricted lab chow and tap water. The male dwarf mice lived 350 days longer than normal male mice and the female dwarf mice lived 470 days longer than the normal female mice. Mean age at death for normal males is  $723 \pm 54$  days; for normal females  $718 \pm 45$  days; for dwarf males  $1,076 \pm 56$  days; and dwarf females  $1,206 \pm 32$  days.

Ames dwarf mice are characteristically normal size at birth but severely growth retarded after birth and are approximately one-third normal size as adults. They have primary pituitary deficiency, including absence or extreme reduction in GH, prolactin, and thyroid-stimulating hormone. The GH/IGF-1 axis is markedly depressed, and the mice exhibit reduced immune function.

The mechanisms suggested for longevity in these geneti-

cally dwarf mice are related to low GH and IGF-1 levels; low thyroid-stimulating hormone and thyroid hormone levels and hypogonadism; reduced metabolic rate, possibly due to reduced body size and underlying endocrine defects; reduced caloric intake; and failure of sexual maturation.

Brown-Borg HM, et al. *Nature* 1996;384:33. Letter.

**Editor's comment:** Aging is a complex process influenced by genetic and environmental forces. Genes and hormones, especially GH, IGF-1, and sex hormones, appear to play a role in longevity. It is well known that individuals with elevated GH levels resulting in acromegaly and pituitary gigantism have a shorter life span. The observation that female dwarf mice lived longer than male mice also suggests that female hormones play some role in the aging process. These animal models are important for studying the effects of hormones on growth, aging, and the aging process, and almost surely will improve our understanding of the aging process in both rodents and humans.

Judith G. Hall, MD

## Mechanisms and Treatment of Growth Retardation in Children With Liver Transplants

Sarna et al report on their experience with 18 months of rhGH treatment, beginning at least 18 months after liver transplant, in 8 children (5 boys, 3 girls). A total of 41 children have had liver transplants with a 70.2% graft survival after 1 year. The inclusion criteria for the study were: age > 2 years; liver transplant at least 18 months previously; height SD score (SDS) < -2.0 or growth velocity SDS < 0 for chronologic age and sex; bone age < 14 years in boys and ≤ 13 years in girls; and no serious complications due to transplantation. The patients were treated with 1.0 IU/kg/wk (approximately 0.3 mg) rhGH. They were measured at 2 weeks, 6 weeks, and 3 months, and at 3-month intervals thereafter using a Harpenden stadiometer. Height SDS was calculated.

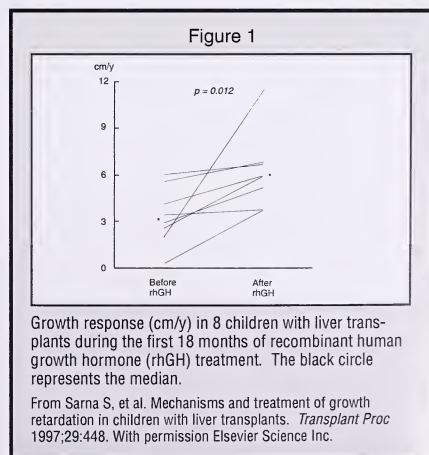
These 8 children received their liver transplants for a variety of causes, including hepatoblastoma (3), biliary atresia (4), and  $\alpha_1$ -antitrypsin deficiency (1). The median growth rate increased from 3.2 to 6.0 cm/y ( $P=0.012$ ) (Figure 1). The median height SDS increased from -3.9 to -3.0 ( $P=0.036$ ) during treatment. The individual growth responses did not correlate with baseline age, time elapsed after transplant, nocturnal GH secretion, serum IGF-1, or IGFBP-3. No rejection episodes were documented during treatment.

Sarna S, et al. *Transplant Proc* 1997;29:447-448

**Editor's comment:** The patients in this study had a significantly accelerated linear velocity despite receiving low doses of glucocorticoids (amount not specified). The authors state that traditional predictors of response to rhGH such as low GH secretory status and young age were not shown to be predictors of a good response in the current study. The study itself included too few subjects to be able to characterize individuals who might benefit the most

from GH therapy. This editor hopes that a larger, multicenter, multinational study could be performed so that such variables can be clearly identified. Since GH has some effects on the immune system, it is important to continue to monitor liver function tests closely during its administration. Although the authors conclude that "growth response is variable and difficult to predict," it is not unreasonable to expect that such information might be forthcoming from future studies.

William L. Clarke, MD



**GROWTH, Genetics, & Hormones Volume 13, Number 3**  
**Post Program Self-Assessment/CME Verification**

**Instructions:** The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. GHRP-6 was named this because:
- It was the 6th GH-releasing peptide developed.
  - It was 6 times the potency of GH-releasing hormone.
  - It was a hexapeptide.
  - It was active both in vivo and in vitro.
  - It was developed in 1986.
2. Which of the following substances cause the release of GH from the pituitary?
- MK-0677; a spiroperidine
  - Prolactin-releasing hormone
  - Hexarelin
  - GH-releasing hormone
3. GHRP and the nonpeptidyl GH secretagogues:
- Use the same receptor.
  - Both stimulate the secretion of GH in vitro and in a variety of species in vivo.
  - Both are active when given IV, IM, SC, intranasally, and orally.
4. The following are true statements:
- The GH-releasing activity of GHSs is inhibited by somatostatin.
  - The GH-releasing activity of combined GHRH and GHRP are synergistic.
  - GHSs stimulate greater secretion of GH than does GHRH.
  - Although a positive GH secretory response to GHS implies the presence of somatotrophs and their exposure to GHRH, an absent or blunted response does not identify the site of error in GH secretion.
5. Threonine is substituted for methionine at codon 918 in the RET tyrosine kinase in which disease phenotype?
- FMTG
  - MEN 2A
  - MEN 2B
  - MEN 2A & 2B
6. One possible explanation for the divergence of MEN 2A & 2B phenotype during RET activation may be:
- Dimerization and autophosphorylation of the receptor tyrosine kinase during ligand binding.
  - Dimerization and activation of tyrosine kinases in the absence of ligand which is characteristic of MEN 2A.
  - The fusion of the RET tyrosine kinase domain to sequences from different cellular proteins.
  - RET mutations along cell lines exposed to in vitro irradiation.
7. Which statement is false?
- BCR-ABL fusion proteins have severalfold elevation of tyrosine activity over that in the normal c-ABL kinase.
  - Tyrosine kinase activation of the BCR-ABL protein correlates with disease phenotype.
  - There is an inverse correlation between the activity level of the BCR-ABL protein and the rate of disease progression.
  - BCR-ABL has transforming ability in many in vitro assays of tumorigenicity.
8. By what mechanism can tyrosine kinase become constitutively activated?
- Autocrine production of growth factor
  - Mutation
  - Concomitant production of a growth factor and its receptor
  - All of the above
9. MEN 2A may be characterized by all but:
- Average age of onset in the second decade
  - Pheochromocytomas
  - Parathyroid disease
  - Developmental abnormalities, such as characteristic facies, marfanoid habitus, and skeletal abnormalities
- Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.
- Drs. Bercu, Kolibaba, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Diamond is President, Genentech Endowment for Growth Disorders, an independent foundation funded by Genentech to provide growth hormone to financially needy children. Dr. Druker reports grants from Ciba-Geigy, NIH, and The Leukemia Society for laboratory tests to investigate tyrosine kinase inhibitors. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

Answer Key: 1. c 2. a, b, c 3. a, b, c 4. a, b, c, d 5. c 6. b 7. b 8. c 9. d

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# GROWTH

## Genetics & Hormones

Vol. 13 No. 4

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### Molecular Genetics of Human Chondrodysplasias

**William A. Horton, MD**  
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Portland, Oregon*

#### INTRODUCTION

The human chondrodysplasias are a diverse and genetically heterogeneous group of disorders of skeletal development.<sup>1</sup> They especially affect linear bone growth and are due to mutations that disrupt endochondral ossification in the skeletal growth plate. The target events include the proliferation and differentiation of chondrocytes and the coincident production of cartilage that serves as a template for bone formation and gives rise to the articular surfaces of joints.

Defining the molecular genetics of the chondrodysplasias has been difficult because they are individually rare and because families large enough to be informative for linkage studies are uncommon. Nevertheless, a number of "chondrodysplasia" loci have now been identified.<sup>2</sup> This article provides a brief overview of recent progress in this field.

#### COL2A1

A mutation of the type II collagen gene (*COL2A1*) was first reported in 1989 in affected members of a family with spondyloepiphyseal dysplasia (SED) congenita. Since then, the number of *COL2A1* mutations in patients with the SED class of chondrodysplasias has risen to well over 30.<sup>3</sup> Examples of such mutations are shown in Figure 1, which also depicts the structure of collagen alpha chains, the product of the collagen genes. All mutations have been heterozygous, involving only 1 of the 2 *COL2A1* alleles. They map to the triple helical domain of the molecule. Most involve the substitution of glycine residues that are considered critical to proper assembly of the triple helix.

The clinical picture has ranged from achondrogenesis type II and hypochondrogenesis at the severe end of the spectrum to very mild SED with precocious osteoarthritis, often referred to as late-onset SED, at the other end. Clinical phenotypes of intermediate

severity include SED congenita and Kniest dysplasia. *COL2A1* mutations also have been found in Stickler dysplasia, in which the skeletal phenotype is typically dominated by degenerative arthritis rather than short stature as well as eye and inner ear abnormalities.

Several interesting observations have been made regarding the clinical features that result from particular *COL2A1* mutations, so-called genotype/phenotype correlations. For instance, there is a tendency for mutations that alter amino acids residing toward the C terminus of the collagen triple helix to produce more severe phenotypes compared with those altering amino acids toward the N terminus of the helix. This phenotypic gradient is similar to that observed for mutations of *COL1A1* and *COL1A2* in osteogenesis imperfecta.

#### Letter From the Editor

Our readers may be interested in knowing that the glossary concerning genetic terms, which was published in *GGH* (1997;13[2]), has now been put on a web page, with appropriate credit given to Drs. Judith Hall and William Horton, who are responsible for constructing this wonderful glossary. You may wish to make notations concerning this glossary, which can be brought up on the computer at your immediate command, and hang them in your clinic or other appropriate places. The URL is: <http://www.kumc.edu/gec/gloss.html>. Ms. Debra Stultz, Program Manager, Genetics Education Center, University of Kansas Medical Center, Kansas City, Kansas, is responsible for placing the glossary on the site, and we thank her for taking the initiative to do this.

Robert M. Blizzard, MD

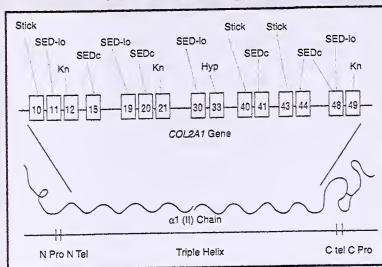
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Figure 1  
**COL2A1 MUTATIONS**



Schematic of *COL2A1* mutations. Type II collagen chain ( $\alpha 1$ (I) chain) near bottom with domains listed underneath. A portion of the *COL2A1* gene encoding the triple helical domain with exons numbered is drawn above. Representative chondrodysplasias with SED or SED-like clinical phenotypes are depicted above with lines indicating to which exons the mutations map. Abbreviations: C pro=carboxy propeptide; C tel=carboxy telopeptide; Hyp=hypochondrogenesis; Kn=Kniest dysplasia; N Pro=amino propeptide; N tel=amino telopeptide; SED-lo=SED-late onset; SEDc=SED congenita; Stick=Stickler dysplasia.

From Horton WA. Molecular genetic basis of the human chondrodysplasias. *Endocrinol Metab Clin North Am* 1996; 25:683-697.

However, as with osteogenesis imperfecta, there are many exceptions.

Three *COL2A1* mutations have been reported in patients with Kniest dysplasia that are predicted to interfere with splicing of mRNA transcripts. The consequence would be partial or complete deletion of exons that encode parts of the type II collagen triple helix. Such deletions are expected to disrupt assembly of collagen molecules; however, the reasons why they should produce the clinical features of Kniest dysplasia are not understood.

The mutations discussed until now are thought to act through a dominant negative mechanism at the molecular level. Since collagen molecules are comprised of 3 alpha chains, there are theoretically 8 possibilities for how the products of 2 *COL2A1* alleles can combine. If 1 allele is mutant, then half of the alpha chains will be mutant and 7 of the 8 possible combinations will contain at least 1 mutant chain. In general, the fate of collagen molecules containing mutant chains is not well understood. The most often proposed possibilities include premature degradation, which would reduce the abundance of type II collagen in cartilage matrix, and incorporation of the mutant molecules into cartilage collagen fibrils, adversely affecting their functions. Most likely, some combination of these and perhaps other events occurs.

The product of the *COL2A1* gene also is the alpha 3 chain of type XI collagen. Accordingly, phenotypic

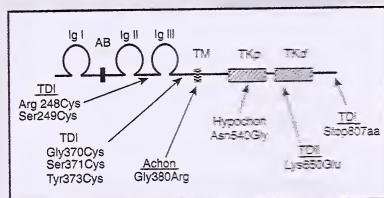
consequences of *COL2A1* mutations may be due in part to defects in the synthesis and functions of type XI collagen.

In contrast to the dominant negative action of most *COL2A1* mutations, those associated with Stickler dysplasia are believed to act by so-called haploinsufficiency. For example, 7 of the heterozygous mutations reported to date create premature translation stop signals, most often because the reading frame of the transcript is shifted so that an out-of-frame stop codon is encountered downstream. The result is that collagen chains synthesized from such transcripts are truncated. Most importantly, they lack the noncollagenous C propeptide, which is necessary for incorporation into triple helical molecules.

Somatic mosaicism has been found for *COL2A1* mutations. In one instance, the proband presented with the features of Kniest dysplasia. Somatic mosaicism was detected in the patient's mother, who exhibited mild skeletal abnormalities consistent with Stickler dysplasia. It also was found in the father of another patient with Kniest dysplasia. His features were compatible with late-onset SED.

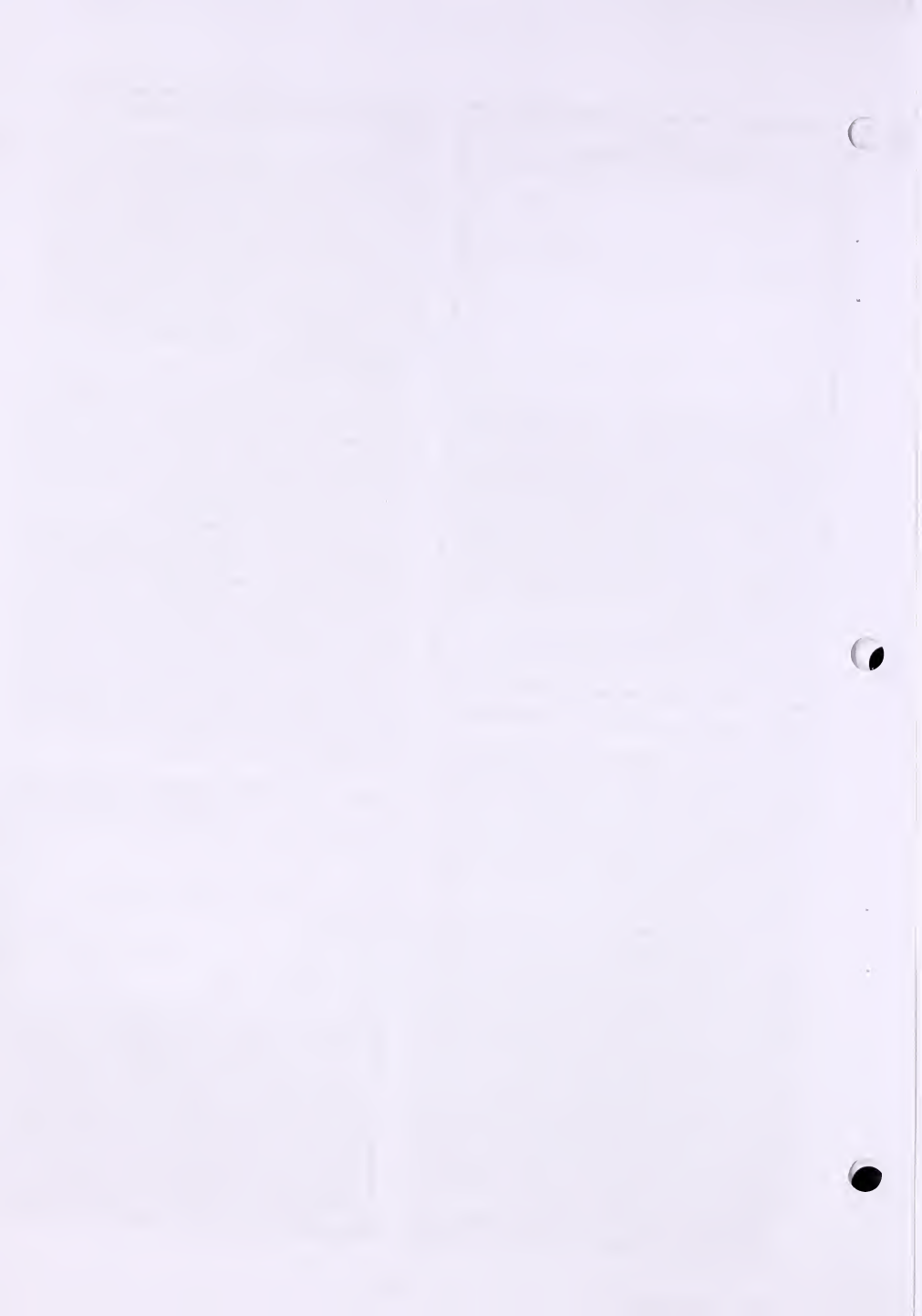
As noted above, heterozygous mutations of *COL2A1* are thought to act through dominant negative or haploinsufficiency mechanisms to introduce mutant collagen molecules in the extracellular matrix of cartilage in the former instance and/or to reduce the abundance of the protein in cartilage matrix in both circumstances. Precisely how either phenomenon disrupts skeletogenesis is not well understood. However, since cartilage serves as a template for endochondral ossification and since type II collagen is the principal structural protein of cartilage, it follows that cartilage containing abnormal or deficient type II collagen would not function properly as a template.

Figure 2



Schematic of *FGFR3* depicting structure of the receptor and sites of common mutations. Abbreviations: Ig I, II, and III = immunoglobulin domains I, II, and III; AB = acid box; TM = transmembrane domain; TKp = proximal tyrosine kinase domain; TKd = distal tyrosine kinase domain; TDI and TDI1 = thanatophoric dysplasia I and II; Achn = achondroplasia; Hypochon = hypochondroplasia; aa = translated amino acid.

Adapted from Horton WA. Molecular genetic basis of the human chondrodysplasias. *Endocrinol Metab Clin North Am* 1996;25:683-697.



## COL11A2

Mutations of *COL11A2*, the gene encoding the alpha 2 chain of type XI collagen, have been described in 2 families with a syndrome that closely resembles Stickler dysplasia.<sup>4</sup> Affected family members presented with mild SED, precocious osteoarthritis, and sensorineural hearing loss, all of which are characteristic of Stickler dysplasia; however, they lacked the eye abnormalities typical of the condition. The explanation for the latter exception lies in the fact that the alpha 2 chain of type XI collagen does not contribute to type XI collagen molecules in the eye as it does in other tissues.

After genetic linkage to this locus was established in 1 large family, subsequent analysis revealed a splice site mutation in 1 allele. In the second family, the Stickler-like syndrome presented as an autosomal recessive trait with 3 affected sibs and unaffected parents. The affected sibs had more extensive epiphyseal and hearing abnormalities than usual for Stickler dysplasia. It is of note that the sibs were fourth cousins and also that osteoarthritis was common on the paternal side of the family. The affected sibs were homozygous for a glycine mutation while both parents were heterozygous. Type XI collagen is a minor constituent of cartilage matrix, especially in the growth plate. It is thought to help regulate the size of cartilage collagen fibrils. How mutations disturb this or other functions is not understood.

## COL10A1

A number of heterozygous mutations have been reported in the gene for type X collagen (*COL10A1*).<sup>5</sup> They are all associated with the clinical features of the relatively mild Schmid-type metaphyseal chondrodysplasia. The mutations map to the region of the gene that encodes the C propeptide, and are of the types predicted to disrupt association of chains, the first step of helix formation. The effect is that half of the type X collagen chains cannot be incorporated into molecules, reducing the number of molecules by half. How a partial deficiency of type X collagen disturbs bone growth is not known. The protein is found only in the hypertrophic zone of the skeletal growth plate, where it is thought to facilitate endochondral ossification.

## FGFR3

Achondroplasia is by far the most common chondrodysplasia in humans. As a result, the research for the achondroplasia gene was more extensive than that for any other chondrodysplasia. In early 1994, the locus was mapped to a region of about 2.5 Mb of DNA at the tip of the short arm of chromosome 4 (4p16.3). Ironically, it mapped very near to another elusive disease gene locus, the Huntington disease locus. This was relevant because a number of genes in this region had been characterized as candidates for the Huntington gene, and one of these, the fibroblast growth factor receptor 3 gene (*FGFR3*), turned out to be the achondroplasia gene.<sup>6</sup>

Most remarkable about the achondroplasia mutations was that virtually every patient with typical achondroplasia had mutations at the same site: codon 380, which caused the normal glycine residue to be replaced by an arginine residue (Gly380Arg) (Figure 2). The Gly380Arg mutation has now been detected in several hundred patients.

The observations in achondroplasia prompted the search for *FGFR3* mutations in 2 disorders thought to be related: thanatophoric dysplasia (TD) and hypochondroplasia. These exhibit greater and lesser degrees of severity relative to achondroplasia, respectively. Mutations were quickly discovered in TD.<sup>7</sup> They clustered mainly to 4 locations in the *FGFR3* gene, as shown in Figure 2. The 2 forms of TD that had been implicated from skeletal X-ray studies, TDI and TDII, segregated genetically in that all TDI cases and no TDII cases had the Lys650Glu mutation. An interesting set of TD mutations removes the normal translation stop signal. The consequence of this mutation is that the *FGFR3* protein would be 141 amino acids longer than normal if translation continues to the next in-frame stop codon.

Heterozygous *FGFR3* mutations also were detected in hypochondroplasia.<sup>8</sup> As with the other *FGFR3* mutations, they cluster, in this case, to amino acid residue 540, where asparagine is replaced by lysine (Asn540Lys). On clinical grounds, 1 patient was thought to be a compound heterozygote for both achondroplasia and hypochondroplasia. The parents carried the respective diagnoses and the severity was intermediate between heterozygous and homozygous achondroplasia, as would be predicted for such a compound. Both the hypochondroplasia (Asn540Lys) and achondroplasia (Gly380Arg) alleles were found at this patient's *FGFR3* loci.

Thus, with few exceptions, human achondroplasia class mutations map to a limited number of codons within different regions of the *FGFR3* gene that correspond to domains of the mature protein. In other words, achondroplasia mutations map to the transmembrane domain, hypochondroplasia mutations to the proximal tyrosine kinase domain, TDII mutations map to the distal tyrosine kinase domain, and so on.

There has been much speculation about how *FGFR3* mutations interfere with skeletal development.

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Table 1  
Summary of Human Chondrodysplasia Mutations

| Locus          | Chromosomal Location | Gene Product Function                               | Clinical Phenotype   | Inheritance Pattern              | Proposed Effect of Mutation   |
|----------------|----------------------|---|--|----------------------------------|---|
| <i>COL2A1</i>  | 12q13.11             | Extracellular matrix protein                        | Achondrogenesis type II<br>Hypochondrogenesis<br>SED congenita<br>Kniest dysplasia<br>Last-onset SED<br>Stickler dysplasia | AD<br>AD<br>AD<br>AD<br>AD<br>AD | Dominant negative<br>Dominant negative<br>Dominant negative<br>Dominant negative<br>Dominant negative<br>Haploinsufficiency |
| <i>COL9A2</i>  | 1p33-p32.3           | Extracellular matrix protein                        | MED  | AD                               | Dominant negative   |
| <i>COL10A1</i> | 6q21-q22             | Extracellular matrix protein hypertrophic cartilage | Schmid-type metaphyseal chondrodysplasia   | AD                               | Haploinsufficiency  |
| <i>COL11A2</i> | 6p21.3               | Extracellular matrix protein                        | Stickler-like dysplasia  | AD<br>AR                         | Dominant negative<br>Loss of function   |
| <i>COMP</i>    | 19p12-13.1           | Extracellular matrix protein                        | Pseudoachondroplasia<br>MED Fairbanks<br>MED Ribbing   | AD<br>AD<br>AD                   | Dominant negative<br>Dominant negative<br>Dominant negative   |
| <i>FGFR3</i>   | 4p16.3               | Tyrosine kinase transmembrane receptor for FGfs     | Thanatophoric dysplasia<br>Achondroplasia<br>Hypochondroplasia   | AD<br>AD<br>AD                   | Gain of function<br>Gain of function<br>Gain of function  |
| <i>PTHrP</i>   | 3p21-p22             | G protein transmembrane receptor for PTH and PTHrP  | Jansen-type metaphyseal chondrodysplasia   | AD                               | Gain of function  |
| <i>DTDST</i>   | 5q31-q34             | Transmembrane sulfate transporter                   | Diastrophic dysplasia<br>Atelosteogenesis type II<br>Achondrogenesis type IB   | AR<br>AR<br>AR                   | Loss of function<br>Loss of function<br>Loss of function  |
| <i>ARSE</i>    | Xp22.3               | Arylsulfatase enzyme                                | Chondrodysplasia punctata  | XLR                              | Loss of function  |
| <i>SOX9</i>    | 17q24.1-q25.1        | Transcription factor                                | Campomelic dysplasia   | AD                               | Haploinsufficiency  |

Adapted from Horton WA. Molecular genetics of the human chondrodysplasias. *Eur J Hum Genet* 1995;3:357-373.

The bulk of the evidence supports the idea that they activate receptor signaling in the absence of ligand (constitutive activation) and that the extent of activation varies according to the mutation and correlates with the severity of the clinical phenotype. For example, hypochondroplasia mutations activate the signaling pathway to a small extent while TD mutations activate it much more. The downstream elements of the *FGFR3* pathway are not well defined. Presumably, they influence the proliferation and differentiation of growth plate chondrocytes.

Given that virtually all TD mutations and most achondroplasia mutations arise de novo, the mutation rate of the *FGFR3* locus must be very high. Most remarkable, however, is the mutation rate at the codons most often involved, especially the 380 codon, for which the mutation rate is much higher than other highly mutable human genes. There is no satisfactory explanation for this extremely high mutation rate.

## COMP

As its name implies, pseudoachondroplasia resembles achondroplasia. However, its clinical features differ substantially, and it is a genetically distinct autosomal dominant chondrodysplasia. It was linked to chromosome 19p12-13.1 about 3 years ago. The

gene for cartilage oligomeric matrix protein (*COMP*) was mapped to this region in late 1994, and mutations in *COMP* were found in pseudoachondroplasia soon afterwards.<sup>9</sup> *COMP* is a member of the thrombospondin family of proteins. It is found in the extracellular matrix of cartilage and, to a lesser extent, other connective tissues, including ligament and tendon. Its function in these tissues is not well defined.

*COMP* mutations have been identified in a number of patients with pseudoachondroplasia. They are heterozygous and map mainly to regions of the gene encoding calmodulin repeats. These repeats are a distinctive element of the molecule; they are thought to bind calcium, which is necessary for the correct folding of the molecule.

At the same time that pseudoachondroplasia was mapped to chromosome 19p12-13.1, linkage to this site also was established for the Fairbanks type of multiple epiphyseal dysplasia (MED) in 1 large family. The locus was termed EDM1. Subsequent analysis of genomic DNA from MED patients revealed a mutation in the *COMP* gene, establishing that pseudoachondroplasia and some forms of MED are allelic disorders and presumably share common pathogenetic features. *COMP* mutations have subsequently been detected in the Ribbing form of MED.



*COMP* mutations are believed to act through a dominant negative mechanism. Indeed, the molecule normally exists in extracellular matrix as a pentamer. If one considers the ways in which the products of a mutant and a normal allele can combine into pentameric molecules, only 1 of 32 possible combinations contains 5 normal *COMP* monomers; 31 contain at least 1 mutant monomer. It has been proposed that this dominant negative effect disrupts *COMP* synthesis and secretion, leading to accumulation of abnormally folded molecules inside cells and/or a deficiency of *COMP* outside cells of relevant tissues.

## COL9A2

MED was genetically mapped in a large family to chromosome 1 in 1994. The locus was named EDM2 to distinguish it from the EDM1 locus on chromosome 19, which is now known to be the *COMP* locus. When the *COL9A2* locus was located in the chromosome 1 region of interest, it was considered a strong candidate gene for this family. A heterozygous mutation was subsequently detected in several members.<sup>10</sup> Type IX collagen is a quantitatively minor cartilage collagen thought to participate in the regulation of collagen fibril assembly in cartilage matrix. The mutation is assumed to behave in a dominant negative fashion. It should be noted that genetic linkage to both the *COMP* (EDM1) and *COL9A2* (EDM2) loci has been excluded in 1 family with the clinical picture of MED.

### CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

## DTDST

The autosomal recessive chondrodysplasia, diastrophic dysplasia (DTD), was mapped to chromosome 5q in 1990, and a gene encoding a sulfate transporter was identified as harboring DTD mutations 4 year later.<sup>11</sup> The gene was designated *DTDST* for diastrophic dysplasia sulfate transporter. Several mutations have now been described for DTD. In addition, mutations of *DTDST* also have been detected in 2 other autosomal recessive chondrodysplasias: the more severe atelosteogenesis type II and the much more severe achondrogenesis type IB.<sup>12</sup> Interestingly, some of the same mutant alleles have been found in DTD and the other disorders. In fact, it appears that different combinations of mutant alleles determine the severity of the clinical phenotype.

The nature of *DTDST* mutations as well as the recessive inheritance of the disorders suggest that the phenotypes result from different degrees of loss of function of the sulfate transporter protein. Compared with other tissues, cartilage is very rich in proteoglycans, such as aggrecan, whose glycosaminoglycan side chains are heavily sulfated, ie, chondroitin sulfate and keratan sulfate. There is evidence that cartilage glycosaminoglycans are poorly sulfated in *DTDST* disorders.

## PTHrPR

The Jansen type of metaphyseal chondrodysplasia is a distinctive autosomal dominant chondrodysplasia that shares many phenotypic features with the acquired vitamin D deficiency disease, rickets. Since many of the manifestations of rickets reflect overactivity of parathyroid hormone (PTH), investigators searched for abnormalities in PTH and its receptor, which also serves as a receptor for another hormone called PTH-related protein, or PTHrP. While the hormone studies were unremarkable, the PTHrP receptor analysis revealed a mutation in a patient with Jansen-type metaphyseal chondrodysplasia.

The heterozygous mutation causes a histidine-to-arginine substitution in the PTHrP receptor protein.<sup>13</sup> This histidine is highly conserved among members of the G protein-coupled transmembrane receptor family to which the PTHrP receptor belongs. Substantial evidence suggests that this mutation as well as one other are activating mutations, ie, the receptor is activated in a ligand-independent fashion.

Recent studies indicate that PTHrP signaling serves as a "brake" on terminal differentiation of chondrocytes in the growth plate. Activating mutations of the PTHrP receptor would be expected to enhance this braking effect, presumably slowing bone growth.

## ARSE

Chondrodysplasia punctata (CDP) refers to a heterogeneous group of skeletal dysplasias in which abnormal calcium deposits form in cartilage tissues to pro-





duce stippled epiphyses on X-ray films. An X-linked recessive form of CDP has been mapped to the short arm of the X chromosome (Xp22.3), near the boundary of the pseudoautosomal region of the X chromosome. When this region was searched for genes, 3 adjacent genes were found that encoded previously unrecognized sulfatase enzymes. Because of predicted structural similarities to arylsulfatases A, B, and C (ARSA, ARSB, and ARSC), the novel sulfatase genes were named *ARSD*, *ARSE*, and *ARSF*. Mutations in the *ARSE* gene were found in several boys with X-linked recessive CDP. Some of the patients exhibited severely reduced *ARSE* enzyme activity in a cell culture assay.

Defects in sulfate metabolism might be expected to be expressed in cartilage, given the high degree of sulfation of matrix constituents (above). However, the mechanism by which they cause calcium in deposits in cartilage is not evident.

### SOX9

Campomelic dysplasia is a chondrodysplasia associated with sex reversal, ranging from minor abnormalities of external genitalia to complete sex reversal. Previous reports of chromosomal rearrangements in campomelic dysplasia with sex reversal localized the gene(s) responsible to chromosome 17q24.1-q25.1. Recently, the candidate region was placed near the *SOX9* locus. *SOX9* is a member of a family of transcription factor genes structurally related to the *SRY* (sex-determining region Y) gene, which encodes a factor necessary for testicular development in mammals. The mature protein contains a high mobility group (HMG) domain and a proline- and glutamine-rich domain believed to confer DNA-binding and transcription-activating properties, respectively.

Mutations of *SOX9* have now been reported in a number of patients with campomelic dysplasia, including those with and without sex reversal.<sup>14</sup> Several of the mutations predict that translation would be stopped prematurely, truncating the transcription factor so that the activation domain is missing. The vast majority are heterozygous, supporting autosomal dominant inheritance. Evidence of somatic mosaicism for a mutant allele in maternal lymphocytes was found in one instance. The mother had no overt signs of campomelic dysplasia and had given birth to a normal XX girl previously.

Given the inactivating nature predicted for the mutations, they most likely act through haploinsufficiency. It has been suggested that transcription regulation functions of *SOX9* are dose-dependent. *SOX9* transcripts have been demonstrated in growth plate chondrocytes of growing bones; however, the functions are unknown.

### CONCLUSIONS

Several conclusions can be drawn from these observations, as summarized in Table 1. First, the concept of chondrodysplasia families, which was originally

based on finding qualitative similarities in clinical phenotypes, has been confirmed at the molecular level. Mutations of *COL2A1* and *FGFR3* in the SED and achondroplasia classes of disorders demonstrate this well. The characteristics of the mutations, however, differ substantially.

Mutations of *COL2A1* are dispersed throughout the gene, and mutations in genes whose products interact functionally with type II collagen, ie, *COL11A2*, cause similar phenotypes. Genetic heterogeneity is the rule. The evidence to date suggests that the "generic" SED phenotype results from dysfunction of cartilage collagen fibrils during bone growth and maintenance of articular cartilage. Since such fibrils are comprised of types II, IX, and XI collagen molecules, mutations in any of the contributing genes could potentially give rise to an SED or SED-like phenotype. Subtle differences in the functions that constituent molecules have and in the consequences of specific mutations account for particular SED phenotypes. The MED phenotype seems to show similar genetic heterogeneity, with mutations having been found at the *COMP* and *COL9A2* loci and linkage to another locus implicated in other MED families.

In contrast, mutations of *FGFR3* display the opposite phenomenon of genetic homogeneity; they cluster to only a few codons, and there is a remarkable fidelity with regard to particular mutations giving rise to particular clinical entities. This implies a very high degree of specificity with regard to the biologic sequelae of signaling through mutant *FGFR3* receptors. Subtle differences in the degree of constitutive activation probably determine the particular clinical phenotypes. A similar phenomenon may be found in *PThrPR* mutations as more are identified.

The extremely high rate of spontaneous mutation of certain *FGFR3* codons is of considerable interest. Given the high degree of sequence conservation in the vicinity of the mutations in mammals, one would expect to find comparable mutations in other species, especially the mouse, where such phenotypic variants have been carefully monitored. However, there appears to be no such mutant mouse, nor is there a satisfactory explanation for the discrepancy.

Allelism within the chondrodysplasias deserves comment. In several instances, suspected allelism has been confirmed, as in the disorders associated

#### In Future Issues

**Insulin, the IGF System, and IDDM**  
Cheryl Deal, MD

**How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?**  
Arnold Slyper, MD

**The Therapeutic Use of Growth Hormone in Turner Syndrome and Other Non-GH-Deficient States**  
Ron Rosenfeld, MD



with *COL2A1* and *FGFR3* mutations. In some cases, allelism came as a surprise, as for pseudoachondroplasia and MED on the one hand and DTD, atelosteogenesis type II, and achondrogenesis type IB on the other. On the contrary, the Schmid and Jansen types of metaphyseal chondrodysplasia, which share at least enough phenotypic similarity to be classified together, turn out to result from mutations of quite different genes: one encoding an extracellular matrix protein, the other a transmembrane receptor. These findings indicate that clinical phenotypes are not always good predictors of genotypes.

Finally, the number of chondrodysplasia loci seems to be shrinking. A decade ago, when little was known about the loci, it was predicted that the number would be large, given the complexity of skeletal development. The recent advances, however, have demonstrated that mutations at the *COL2A1* and *FGFR3* loci alone account for a great majority of patients with chondrodysplasias. Thus, despite the complexity, the number of genes critical for skeletal growth for which

redundancy cannot compensate for mutations is not as large as originally suspected.

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#### Abstracts From the Literature

### Growth Hormone in Combination With Anabolic Steroids in Patients With Turner Syndrome: Effect on Bone Maturation and Final Height

In an uncontrolled trial of the efficacy of rhGH and androgens on growth of girls with Turner syndrome (TS), the authors report the final heights of 20 patients treated for 4.0 to 7.7 years (see Figure 1). Initially, all children received rhGH alone for 12 to 30 months (either 12 or 18 IU/m<sup>2</sup>/wk, or approximately 4 to 6 mg/m<sup>2</sup>/wk); an androgen in the form of oxandrolone (0.0625 mg/kg/d) or testosterone (5 mg IM every 2 weeks) was then introduced; after 12 to 24 months the testosterone-treated children were changed to oxandrolone until the end of the therapeutic trial. Estrogen was introduced after a bone age  $\geq$  12.5 years was achieved. (This ranged from 13.0 to 19.6 years; mean age, 16.3  $\pm$  1.7 years.) A progestin was added after approximately 15 months. At final height (growth rate < 2.0 cm/y), the children were 15 to 23 years of age. The mean projected adult height (PAH; derived from Austrian data on untreated patients with TS) was 143.7  $\pm$  4.0 cm (range, 137.5 to 151.0 cm); the mean achieved final height was 152.9 cm (range, 145.0 to 158.9 cm); the mean increment above PAH was 9.3  $\pm$  4.9 cm (range, 1.4 to 21.4 cm). Relative to target height (target height minus achieved height), mean final height was -9.6  $\pm$  5.3 cm lower, but the range of final heights relative to target heights was -2.4  $\pm$  18.7 cm; one patient reached and one exceeded target height. Treatment before or after 11.5 years did not affect outcome. The authors concluded that rhGH and androgen can result in a substantial increase in adult stature in girls with TS.

Haeusler G, et al. *Acta Paediatr* 1996;85:1408-1414.

**Editor's comment:** The data indicate that the combination of rhGH and androgen can increase final height in girls

with TS. The use of rhGH in girls with TS has been approved by the Food and Drug Administration (FDA). The results of the present study, however, were achieved with postponement of estrogen therapy until an average age of 16 years. There is no discussion by the authors of the impact of this delay in initiation of puberty on the psychosocial well-being of these patients. It is this writer's practice to introduce estrogens into the treatment program of girls with TS at 13 to 14 years of age, depending on individual circumstances. Other investigators<sup>1,2</sup> have not recorded comparable achievements in final heights in girls with TS treated with rhGH alone.<sup>1-4</sup> Until long-term data on the quality of life (educational achievement, psychosocial well-being, career attainment) of patients with TS are available, the efficacy of rhGH therapy on this important point remains conjectural.

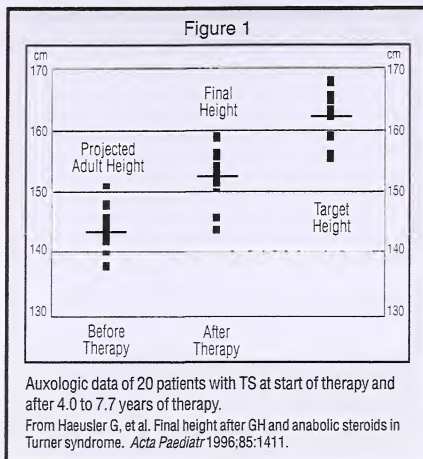
Allen W. Root, MD

1. Hindmarsh PC, et al. *Lancet* 1996;348:13-16.
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**2nd Editor's comment:** Members of the Editorial Board do not always agree, but such disagreement can lead to constructive contemplation. My personal view is that this article considers at least 4 issues: (1) Does rhGH alone significantly increase the ultimate height of patients with TS? (2) Does oxandrolone enhance the action of rhGH in patients with TS? (3) Does the timing of estrogen administration alter the ultimate height of TS patients if given early during the teen years? (4) If estrogen is given in the late







teens instead of in the early teens, is this delay psychologically handicapping to TS patients?

The FDA recently has approved the use of rhGH for the long-term treatment of short-stature associated with TS. Thus, it has been recognized as an agent that increases the average ultimate height of TS patients. Whether this improvement is significant is in the eyes of the beholders, which is not necessarily the same in the eyes of TS patients and non-TS patients. The data prompting approval by the FDA are presented in part in an article by Rosenfeld et al, published in the *Journal of Pediatrics*. These authors studied 70 TS children, verified by karyotype. A prospective study was used. In an initial phase lasting 12 to 24 months, subjects were randomized to either (1) observation alone, (2) oxandrolone, (3) GH, or (4) GH plus oxandrolone. After completion of the first phase, subjects on GH alone continued to receive only GH. All other subjects were treated with GH plus oxandrolone. Data from this trial were compared with growth characteristics of 25 American historical control subjects with TS—matched

for age, height, parental target height, and karyotype—who never received GH or androgens.

Of the 70 subjects enrolled, 60 completed the clinical trial. The 17 subjects receiving GH alone all completed the trial and reached a mean height of  $150.4 \pm 5.5$  cm. This was  $8.4 \pm 4.5$  cm taller than their mean PAH at enrollment (95% confidence interval, 6.3 to 10.6 cm). The 43 subjects receiving GH plus oxandrolone attained a mean height of  $152.1 \pm 5.9$  cm; this was  $10.3 \pm 4.7$  cm taller than their mean PAH. The historical controls had a mean adult height of  $144.2 \text{ cm} \pm 6.0$  cm, precisely matching their original PAH of  $144.2 \pm 6.1$  cm.

The authors concluded that GH, either alone or with oxandrolone, is capable of both stimulating short-term growth and augmenting adult height in TS girls. With early diagnosis and initiation of treatment, an adult height > 150 cm is a reasonable goal for most girls with TS.

In respect to the second question: Having been involved with the combined use of rhGH and oxandrolone in GH deficiency and in TS over many years, I have a strong impression that not only is growth accelerated with the combination but also, possibly, ultimate height, providing oxandrolone is not used before the bone age is 9 years and the dose does not exceed 0.1 mg/kg/d. The data of Rosenfeld et al and Haeusler et al support this impression.

In respect to the third and fourth questions: I believe whether estrogen is administered early or late in the teen years should be a personal decision of the TS patient. Unequivocally there is a trade-off: Early sexual development hastens epiphyseal fusion and decreases ultimate height. Some girls prefer sexual development to greater ultimate height and some do not.

Finally, the cost of rhGH, which is expensive, must be taken into account. This cost cannot be ignored and must be factored into the decision making of the patient, the family, and others.

Again, my opinion is that of only one member of the Editorial Board. The use of rhGH will be considered further in a future article to be published in GGH, entitled, "How Safe and Effective Is hGH at Pharmacologic Dosing?" In the meantime, Letters to the Editors are most welcome.

Robert M. Blizzard, MD

## SHOX, Short Stature and Turner Syndrome

Turner syndrome is one of the most common and most widely studied forms of short stature. It classically results from absence of an X or a Y chromosome, leaving the patient with a 45,XO karyotype. However, a subset of patients have only deletions of the X or Y. Many of these deletions map to the pseudoautosomal region (PAR1) at the tips of the chromosome short arms. From analysis of short stature in such patients, a 700-kb interval of PAR1 has recently been implicated to contain the gene(s) involved in short stature in Turner syndrome.

With this as a starting point, Rao et al did further deletion mapping to narrow the interval associated with short stature

to 170 kb. This critical region was deleted in 36/36 patients with short stature and rearrangement of Xp22 or Yp11.3. From extensive analysis of cosmids covering this interval, they identified 3 exons of a novel homeobox-containing gene, which they named *SHOX*. *SHOX* appears to be alternatively spliced to produce transcripts (*SHOXa* and *SHOXb*) that yield proteins 292 and 225 amino acids in length, respectively. Expression studies revealed that *SHOXa* is widely expressed, whereas *SHOXb* is more restricted, and is predominantly expressed in bone marrow fibroblasts.

These results strongly suggest that haploinsufficiency for *SHOX* proteins causes short stature in Turner syndrome.



If so, isolated mutations of *SHOX* should produce short stature in the absence of other Turner syndrome features. Accordingly, screening of 91 unrelated male and female patients with idiopathic short stature yielded 1 patient with a heterozygous missense mutation predicted to truncate the protein such that the functions of both the 225 and 195 amino acid proteins would be potentially disturbed. The mutation segregated with short stature in the family. In an accompanying News and Views report, Zinn cautioned that evidence for haploinsufficiency of *SHOX* causing short stature in Turner syndrome is not definitive even though it is substantial.

*SHOX* has a very similar sequence to the mouse gene called *OG-12a*, which encodes a protein of unknown function. *OG-12a* does not map to the mouse X chromosome. The authors speculate that if *OG-12a* is the mouse *SHOX* homologue, its non-X chromosome location may explain why the XO-deficient "Turner syndrome" mouse does not exhibit short stature.

Rao E. et al. *Nat Genet* 1997;16:54-63.

Zinn AR. *Nat Genet* 1997;16:3-4.

**Editor's comment:** This article would appear to settle the argument over whether short stature in Turner syndrome is caused by loss of 1 or loss of several genes that influence stature, although as Zinn points out, the evidence is not definitive since there could still be other growth-influencing genes whose expression is affected by chromosomal rearrangements. Of interest is what *SHOX* gene products do in the growing bone. Unfortunately, *SHOX* expression was not studied in cartilage, most notably in the growth plate. Nevertheless, the high level of expression of *SHOXb* in bone marrow fibroblasts, which are physically in close proximity to the growth plate, is intriguing. It will be interesting to see if expression of the putative *SHOX* transcription factors affects expression of growth factors that might diffuse into and influence growth plate function.

William A. Horton, MD

**2nd Editor's comment:** Dr. Jay Ellison of the University of California-San Francisco wrote in a Letter to the Editor: "Our group has cloned the gene termed 'SHOX' independently, which we named 'PHOG' (pseudoautosomal homeobox-containing osteogenic gene). We have demonstrated significantly higher levels of expression in certain bone-derived cells. Its deletion in patients with short stature, the predicted altered dosage in 45,X individuals, and the nature of the encoded protein and its expression pattern make PHOG an attractive candidate for involvement in the short stature of Turner syndrome. We also have found that the mouse homologue of PHOG is autosomal, which may help to explain the lack of growth abnormality in mice with monosomy X."

Readers need to be aware of the dual nomenclature. This gene, no matter what it is called, will be an important consideration in exploring the various causes of short stature. A number of significant questions remain, including what is the growth mechanism that is controlled by *SHOX*/*PHOG*?

Robert M. Blizzard, MD

**3rd Editor's comment:** Normal growth is undoubtedly regulated by many genes, including genes others than *SHOX* on the Y chromosome, as discussed by Zinn. In the paper by Rao et al, the authors have defined a homeobox gene (*SHOX*) in PAR1 of the sex chromosomes (Xp22 and Yp11.3) that seems to influence growth, since it is absent in short patients who lack this portion of one sex chromosome and a mutation leading to truncation of its gene product has been identified in all but 1 of 91 individuals with intrinsic short stature. The genetic mechanisms through which *SHOX* regulates growth have yet to be determined. It will be of interest to determine if polymorphic variations of this gene influence the range of heights characteristic of a population.

Allen W. Root, MD

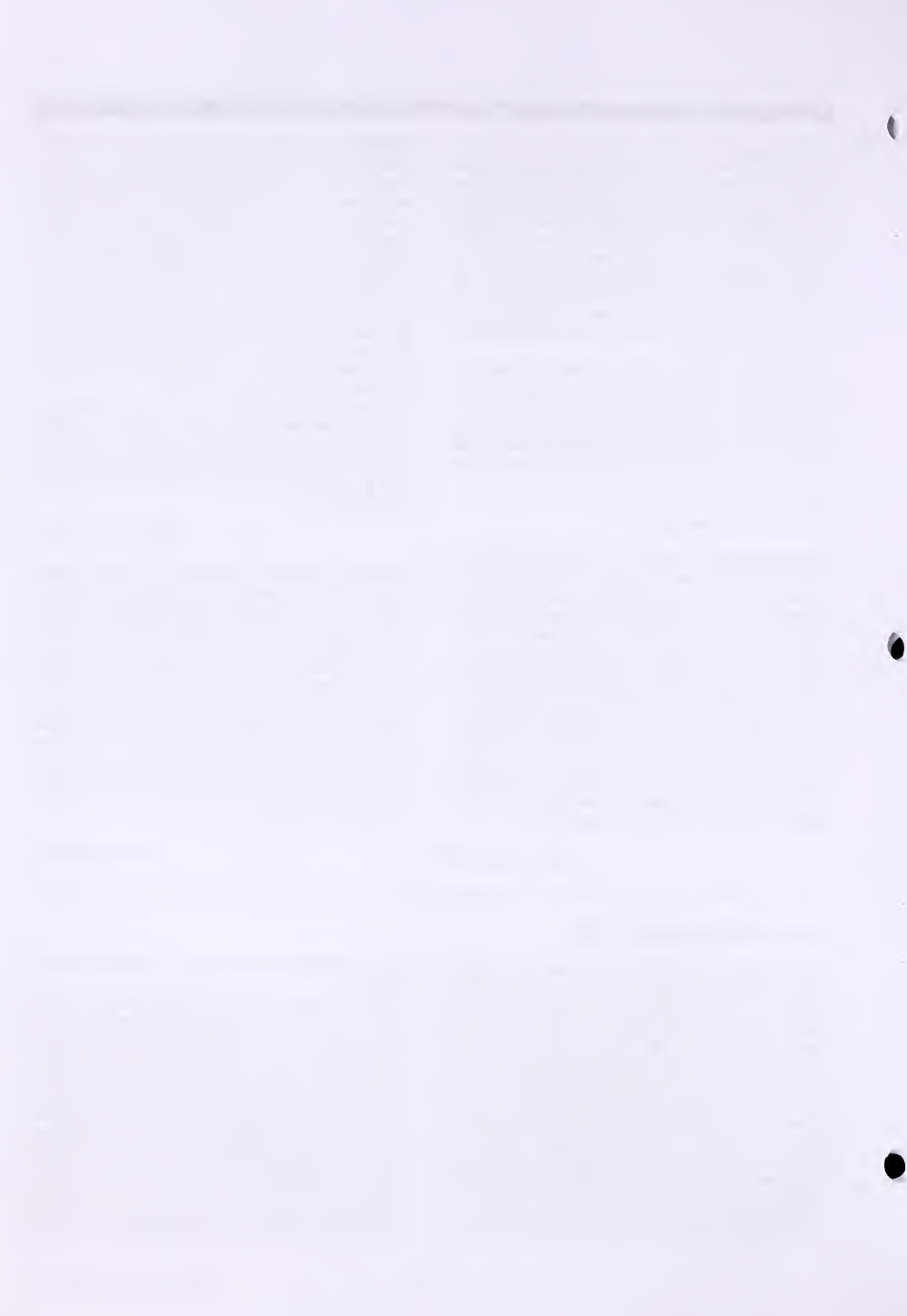
## Human Chromosomes in Mice

The introduction of foreign DNA into the mouse genome, ie, transgenesis, is an extremely powerful tool for elucidating molecular and cellular disturbances that contribute to human diseases. The limiting factor in this technology has been the size of foreign DNA that can be incorporated into the host genome. In the early days of transgenic mice, only small fragments of genes could be transferred. This has improved steadily with successes in introducing entire genes, including local regulatory sequences, and more recently in transferring substantial amounts of DNA in the form of yeast artificial chromosomes (YACs). Interest has developed in generating human artificial chromosomes (HACs) that could be introduced like YACs. However, a report from a Japanese group headed by Isao Ishida indicates that this may not be necessary since human chromo-

somes themselves can function as vectors in transgenic mice.

The group successfully introduced human chromosomes or chromosome fragments from fibroblasts into mouse embryonic stem (ES) cells via microcell-mediated chromosome transfer. The ES cells were transferred to preimplantation mouse embryos, where they populated a wide variety of developing tissues to generate chimeric mice. The foreign chromosomes, which potentially contained thousands of human genes, survived repeated cell divisions as well as differentiation of cells into many cell types. Several of the human genes were shown to be expressed under tissue-specific regulation in adult chimeric mice. Most notable were immunoglobulin genes, which were found to undergo rearrangement of V, J, and D





segments to generate functional immunoglobulins that were detected in the mouse serum for more than a year. Finally, the group demonstrated transmission of a human chromosome 2 fragment to offspring through both the male and female germlines.

Tomizuka K, et al. *Nat Genet* 1997;16:133-143.

Rastan S. *Nat Genet* 1997;16:113-114.

**Editor's comment:** There are several implications from this work. The most remarkable is that human chromosomal elements can interact with mouse mitotic, meiotic, and transcriptional machinery to ensure mendelian transmission and appropriate expression of genes carried by

the human chromosome vectors. The authors refer to such mice as transchromosomal mice. This technology will allow investigations not previously possible, such as studies of how distant regulatory elements influence gene expression or how functionally related, contiguous genes, such as globin or hox genes, are regulated. Phenomena that involve interactions between neighboring genes, such as imprinting, might be studied in such mice, as might aneuploid states such as trisomy 21. Finally, it is often debated whether science drives technology or vice versa. This seems to be an example of the latter, although there is an abundance of scientific questions to be answered by this new technology.

William A. Horton, MD

## "Master Gene" for Bone Formation

The discovery of the transcription factor MyoD as a master gene for muscle development several years ago prompted a search for similar genes for other tissues. Four papers in the May 30, 1997, issue of *Cell* describe a gene of comparable importance to osteoblast differentiation and bone growth. The findings were nicely reviewed by Rodan and Harada.

The story begins with efforts by Komori and colleagues to disrupt T-cell function by knocking out a gene encoding the transcription factor *Cbfa1* (core-binding factor). The factor is a member of the runt-domain gene family of developmentally important transcription factors originally described in *Drosophila*. It was known to bind to promoters of genes expressed in T cells. When the knockout occurred, however, the most dramatic features had more to do with skeletal development than with T-cell function. The null mice died shortly after birth. They were slightly smaller than their normal littermates and had shorter limbs, but were normally proportioned. Most remarkable was a complete absence of bone. Both membranous and endochondral bones of the cranium and endochondral bones elsewhere were absent. In their place was partially calcified cartilage. No osteoblasts were detected and osteoclasts were reduced in number. There was no obvious problem with hematopoiesis.

*Cbfa1* null mice generated independently by Otto et al showed essentially the same findings. They also demonstrated that *Cbfa1* is normally expressed in areas destined to become bone in mouse embryos. They noticed that mice heterozygous for the knockout displayed ossification defects of the clavicles and membranous bones of the skull similar to that seen in humans with the autosomal dominant disorder, cleidocranial dysostosis (CCD). A mouse with a similar phenotype resulting from a radiation-induced deletion had been studied by the Olsen group<sup>4</sup> as a model for CCD. It was quickly determined that *Cbfa1* mapped to the region of interest and that it was disrupted by the radiation-induced deletion.

The next step was to test CCD patients for *CBFA1* mutations. Mundlos et al studied 39 patients and found large and small deletions, insertions, and missense mutations that inactivated *CBFA1*. The mutations were heterozygous in all of the patients.

As these studies were being done, the Karsenty lab (Ducy et al) had started from a different perspective: identifying osteoblast-specific transcription factors (OSFs) that bind to the osteocalcin promoter. Osteocalcin is a bone-specific protein. They had cloned such a factor, which they named *Osf2*. Its expression was restricted to the early stages of osteoblast differentiation, and it was shown to bind to several genes expressed by osteoblasts. They also showed that forced expression of *Osf2* in nondifferentiated cells induced expression of osteoblast-specific genes and that the presence of antisense mRNA downregulated expression of these genes in osteoblastic cells.

As it turned out, *Osf2* and *Cbfa1* (*CBFA1*) are the same. Thus, this transcription factor seems to be required for differentiation of osteoblasts and for normal skeletal development and growth. Haploinsufficiency for *CBFA1* causes CCD.

Ducy P, et al. *Cell* 1997;89:747-754.

Komori T, et al. *Cell* 1997;89:753-764.

Mundlos S, et al. *Cell* 1997;89:773-779.

Otto F, et al. *Cell* 1997;89:765-771.

Rodan G, Harada S. *Cell* 1997;89:677-680.

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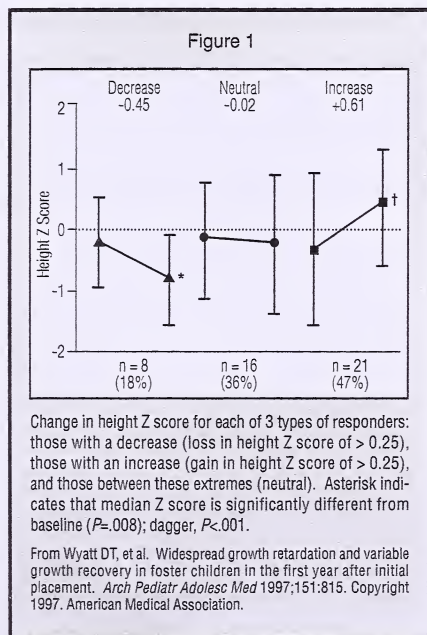
**Editor's comment:** This is a fascinating story with important implications for understanding bone growth. The fact that the skeletal structures formed and "grew" to close to normal length in the newborn mice null for *Cbfa1* suggests that conversion of the cartilage template to bone is not as essential for bone lengthening as many would guess. The photomicrographs suggest that new cartilage is deposited at the ends of lengthening "bone" regardless of the fate of hypertrophic cartilage in the center of the structure. This implies that the signals that drive chondrocyte proliferation and at least early hypertrophy in the growth plate are

not derived from subchondral bone, ie, from osteoblasts or other cell types that normally reside in bone marrow. However, such signals may be necessary to complete the terminal differentiation process that occurs in the growth plate, since this seemed to be lacking in the null mice. These mice should be valued for studying the events that occur at the interface between cartilage and bone in a growing bone. One wonders what the status of *Cbfa1* is in the shark skeleton.

William A. Horton, MD

### Widespread Growth Retardation and Variable Growth Recovery in Foster Children in the First Year After Initial Placement

The authors hypothesized that the actual prevalence of pre-placement growth failure may be greater than that defined by a single cutoff percentile, eg, the 5th percentile, at placement. The objective was to determine the growth pattern of 45 children, 1.5 to 6.0 years of age, in the first year of foster care placement. Height, weight, weight for height, and annual growth velocity Z scores at 1 year after placement, as compared with baseline values, were used as outcome measures. All children received comprehensive medical care.



The changes in height Z scores are plotted in Figure 1. Forty-seven percent experienced catch-up growth (gain in height Z = +0.61) that equaled that seen in the first year of GH therapy in children with classic GH deficiency. The authors interpreted these data as reflecting prior growth retardation.

The authors drew the following conclusions: (1) Growth retardation is widespread in children placed in foster care, with almost half showing marked catch-up growth after placement; (2) initial height is not a good predictor of future growth; (3) the use of cutoff percentiles (only 5 of the 45 were  $<5$ th percentile by actual measurement at baseline) will miss the great majority of children who will show catch-up growth; and (4) the response of any given child is variable and cannot be accurately predicted by any baseline auxologic feature. In the authors' experience, 1 in 5 children in foster care experiences a significant loss in height Z score after placement, which may indicate ongoing medical or nutritional problems, unmet psychosocial needs, or failure of the foster care family. The causes of growth failure preplacement and subsequent catch-up growth are unclear, but they may be related mainly to psychosocial factors that are corrected for in some children with foster care placement.

Wyatt DT, et al. *Arch Pediatr Adolesc Med* 1997;151:813-816.

**Editor's comment:** These data and the conclusions reached are well documented. The authors are to be congratulated for an important clinical investigative study. The use of Z scores is essential to analyses of the data and the conclusions. The authors state that future analyses of data in this study will examine the relationships between growth and other medical, developmental, and psychologic diagnoses; the amount and type of health-care services received; changes in health and mental status; and the quality of the foster home environment. The editors of GGH encourage the authors to expedite these analyses and reports. The study of this group is exceedingly important.

Robert M. Blizzard, MD





## Growth Hormone Treatment in Growth-Retarded Children With End-Stage Renal Failure: Effect on Free/Dissociable IGF-1 Levels

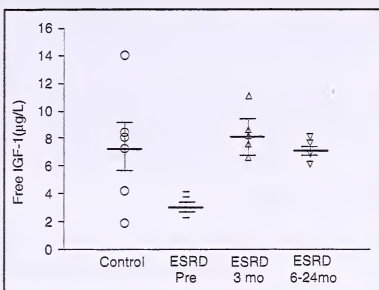
One of the causes for growth retardation in children with end-stage renal disease (ESRD) is thought to be an abnormality in the biologic effects of GH. Despite high serum levels of hGH in ESRD and usually normal values of insulin-like growth factor 1 (IGF-1), somatomedin biologic activity is low. This has been attributed to binding of IGF-1 by an excess of IGF-binding proteins (IGFBPs), leading to decreased free IGF-1 concentrations.

Berek et al tested this hypothesis by measuring free IGF-1 by direct immunoradiometric assay (IRMA) in 5 children with ESRD. In 2, free IGF-1 also was measured after centrifugal ultrafiltration of serum. Free IGF-1 concentrations were one third to one half those measured by direct IRMA, suggesting that the IRMA measured both free IGF-1 and that fraction that was easily dissociable from IGFBPs. In basal specimens, the mean free/dissociable IGF-1 levels were lower in ESRD patients than in body mass index-, age-, and pubertal status-matched control subjects ( $3.0 \pm 0.3 \mu\text{g/L}$  vs  $7.3 \pm 2.1 \mu\text{g/L}$ ;  $1.24 \pm 0.05\%$  vs  $2.12 \pm 0.7\%$ , respectively). The mean free/dissociable IGF-1 peaked at  $8.5 \pm 1.0 \mu\text{g/L}$  after 3 months of treatment with rhGH, declining to  $6.9 \pm 1.4 \mu\text{g/L}$  between 6 to 24 months of therapy (Figure 1). Growth rate and total IGF-1 values also rose during rhGH administration. Thus, the increase in growth rate during rhGH administration was associated with a rise in free/dissociable IGF-1 levels.

Berek A, et al. *J Pediatr Endocrinol Metab* 1997;10:197-202.

**Editor's comment:** These data support the concept that the growth-promoting effects of rhGH in children with ESRD is related to an increase in free IGF-1 concentrations. In this paper, the authors did not report a relationship between the

Figure 1



Serum free IGF-1 concentrations in control children and children with chronic renal failure before, at 3 months, and at 6 through 24 months of GH treatment. The horizontal lines and vertical bars indicate the mean and SEM in each group.

From Berek A, et al. Growth hormone treatment in growth retarded children with end stage renal failure: effect on free/dissociable IGF-1 levels. *J Pediatr Endocrinol Metab* 1997;10:200. Freund Publishing House Ltd.

basal or incremental growth rate and the concentration or incremental increase in free/dissociable IGF-1 values. Additional studies will be helpful in clarifying fully the mechanisms by which rhGH increases growth in ESRD.

Allen W. Root, MD

## Pancreatic Agenesis Attributable to a Single Nucleotide Deletion in the Human *IPF1* Gene Coding Sequence

IPF1 is a homeodomain protein critical for development of the pancreas in mice and is a key factor for the regulation of the insulin gene in the beta cells. Disruption of this gene in transgenic mice produces failure of pancreatic development. In this report, a single nucleotide deletion within codon 63 in a patient with pancreatic agenesis apparently does the same. The patient was homozygous for the point deletion and both parents were heterozygous, in contrast to the normal allele structure in 184 individuals. The cytosine deletion was in codon 63. A frameshift beginning at the C-terminal border of the transactivation domain of *IPF1* was consistent in all cells. The data indicated that a truncated protein lacking the homeodomain (and nuclear localization signal) is produced from the mutation. If the parallel between humans and affected mice holds, the pancreatic buds do form, but they undergo only limited ductal outgrowth and branching, with a blockage of both pancreatic endocrine

and exocrine differentiation. Although there was no clear history of consanguinity, the studies strongly suggest that the abnormal alleles are likely to have been derived from a single common ancestor.

In addition to pancreatic agenesis, 3 cases of severe pancreatic hypoplasia and 1 case of complete absence of the islets have been reported. The authors are tempted to speculate that the phenotypes of pancreatic hypoplasia and selected agenesis of the islets might represent a spectrum of less severe mutations that may impair but not abolish *IPF1* functions. Alternatively, these disorders may be a consequence of mutations and other factors that are essential for full development of the pancreas. Most intriguingly, the authors postulate that abnormal *IPF1* function also may be a candidate factor in the development of insulin-dependent diabetes mellitus.

Stoffers DA, et al. *Nat Genet* 1997;15:1-50. Letter.



**Editor's comment:** An intriguingly rare condition is probably explained by these investigators. Recently, a white female infant was diagnosed with pancreatic agenesis shortly after birth, and with pancreatic exocrine insufficiency at 18 days of age. Neonatal diabetes mellitus was the working diagnosis initially. Ultrasound examination demonstrated pancreatic agenesis. Normal development has continued until 5 years of age with replacement of insulin and pancreatic enzymes. A strong family history of noninsulin-dependent diabetes mellitus existed and supports the possibility of partial affection.

Pancreatic agenesis needs to be considered in the differential diagnosis of neonatal diabetes and also with

the observation of malabsorption in the newborn period. A similar study of the IPF1 gene coding sequence might be revealing in the Johanson-Blizzard syndrome, which is characterized by pancreatic insufficiency in addition to other anomalies such as congenital deafness, poor formation of teeth, corneal atresia, and urogenital anomalies.

With time it becomes more and more apparent that one mis-substitution of an amino acid at a critical place on a gene can totally change the life of the host far beyond what we ever could have believed 10 years ago.

Robert M. Blizzard, MD

### Gonadal Function After Bone Marrow Transplantation for Acute Leukemia During Childhood

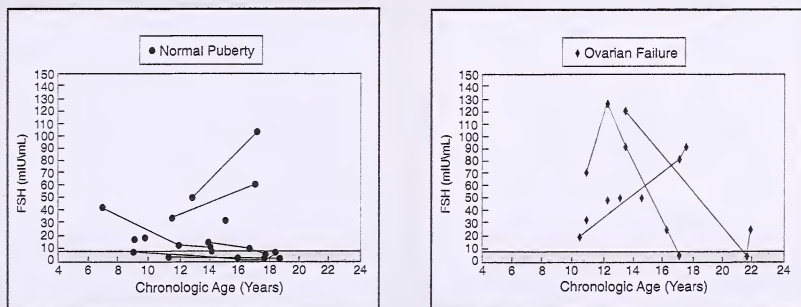
Bone marrow transplantation (BMT) is a major advancement in the treatment of childhood leukemia and in other disorders, as many children are surviving for long periods. Consequently, Sarafoglou et al examined the impact of BMT on gonadal function following high-dose chemotherapy and hyperfractionated total body irradiation (radiation given 3 times daily for several days) in 33 surviving children treated for acute leukemia. All patients were prepubertal and less than 12 years at the time of BMT. The median age at last examination for boys was 14 years (10.4 to 17.1 years) and 16.9 years (9.5 to 21.9 years) for girls.

Of the 17 boys, 14 (82%) had spontaneous puberty, 13 (76%) had age-appropriate plasma concentrations of

testosterone; 2 (11%) remained clinically and hormonally prepubertal; and 1 (6%) had overt Leydig cell failure requiring androgen replacement therapy, although this individual also received testicular irradiation. Thirty-six percent of pubertal boys had increased levels of luteinizing hormone (LH), reflecting evidence of Leydig cell damage; and 64% had increased levels of follicle-stimulating hormone (FSH), reflecting germ cell damage. Pubertal boys with increased LH were significantly younger at BMT ( $5.4 \pm 0.8$  years vs  $7.8 \pm 0.8$  years).

Of 16 girls, 9 (56%) had spontaneous puberty with onset of menarche at a median age of 13 years (9.5 to 15.8 years). Six of these 9 girls (67%) had increased LH and in-

Figure 1



Plasma concentrations of FSH in girls after BMT with normal puberty/menarche (left panel) and in girls with ovarian failure (right panel). Solid lines connect serial determinations in the same patient. Shaded area represents the range for the normal population (follicular phase of the menstrual cycle). BMT, bone marrow transplantation; FSH, follicle-stimulating hormone.

From Sarafoglou K, et al. Gonadal function after bone marrow transplantation for acute leukemia during childhood. *J Pediatr* 1997;130:214.





creased FSH. Seven of 16 girls (44%) required hormone replacement because of clinical and biochemical evidence of ovarian failure (Figure 1). One of the 16 (6%) recovered ovarian function after 5.5 years. Girls with ovarian failure were significantly older at BMT compared with those with spontaneous puberty/menarche ( $8.6 \pm 2.3$  years vs  $6.1 \pm 1.8$  years).

The authors concluded that most prepubertal boys undergoing BMT with chemotherapy and hyperfractionated total body irradiation can expect to have normal puberty. For prepubertal girls, approximately 50% retained sufficient ovarian function to enter puberty and menstruate regularly. Increased age at transplantation was associated with decreased ovarian function in girls.

Sarafoglou K, et al. *J Pediatr* 1997;130:210-216.

**Editor's comment:** Two factors seem to be associated with gonadal dysfunction in these youngsters: (1) age at BMT, and (2) pubertal status. Many children who are younger at BMT seem to retain or recover gonadal function and are able

to enter spontaneous puberty. The study documented that spontaneous puberty occurred in 82% of boys and 56% of girls, which is quite encouraging for males but less reassuring for females, almost half of whom fail to attain spontaneous puberty. Therefore, the ovaries appear to be more prone to damage with irradiation and high-dose chemotherapy. Not surprisingly, there is an association with direct testicular irradiation and gonadal failure. With improvement in medical technology, the availability of BMT, and the ability to control side effects, more and more children are now undergoing the procedure for many hematologic, oncologic, and metabolic diseases. It will be important to follow each subset of these individuals and see what percentage are fertile and what problems arise related to germline mutation (change in genetic information) after recovering gonadal function following irradiation. Similarly important will be the incidence of abortion or congenital abnormalities in the offspring. The outstanding success of some types of therapy frequently leads to new sets of questions.

Judith G. Hall, MD

## Longitudinal Assessment of Hormonal and Physical Alterations During Normal Puberty in Boys, V: Rising Leptin Levels May Signal the Onset of Puberty

Leptin is a recently described hormone that currently is receiving much attention in relation to both obesity and hypogonadotropic hypogonadism. The authors have studied the changes in leptin concentration with the onset of puberty in 8 normal male children progressing from the prepubertal to adult male state. Comparisons of serum leptin, dehydroepiandrosterone sulfate (DHEAS), testosterone levels, and body mass index (BMI) were carried out every 4 months.

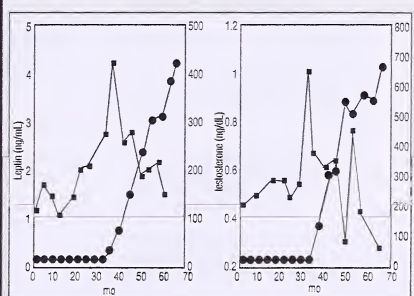
Interestingly, the baseline prepubertal leptin levels were very variable among patients (0.4 ng/mL to 6.0 ng/mL), but in all instances a definite spike in leptin concentration of approximately 2 times base level was noted in the immediate prepubertal period just prior to testosterone rising  $\geq 25$  ng/dL, which marks the onset of puberty in the male. By midpuberty the levels had fallen to nearly the prepubertal levels and fell slightly thereafter (see Figure 1). No direct correlation was observed in relation to DHEAS or BMI.

The authors speculate that leptin might be responsible for the nocturnal surges in luteinizing hormone secretion

observed with the onset of puberty. They state that there may be a relationship to neuropeptide Y, a target of leptin action in the arcuate nucleus, which positively regulates gonadotropin hormone-releasing hormone secretion in vitro and in vivo.

Mantzoros CS, et al. *J Clin Endocrinol Metab* 1997; 82:1066-1070.

Figure 1



Leptin and testosterone concentration changes over the study period.

From Mantzoros CS, et al. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys, V: rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab* 1997;82(4):1066-1070. ©The Endocrine Society.

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**Instructions:** The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Mutations of the type II collagen gene (*COL2A1*) act through a (an) \_\_\_\_\_ mechanism.

- a. loss of function
- b. gain of function
- c. haploinsufficiency
- d. dominant negative
- e. epigenetic

2. Schmid metaphyseal chondrodysplasia is caused by mutations of:

- a. *COL2A1*
- b. *COL11A2*
- c. *COL10A1*
- d. *FGFR3*
- e. *COMP*

3. Mutations in the diastrophic dysplasia sulfate transporter gene (*DTDST*) are associated with which disorder(s):

- a. achondrogenesis type IB
- b. achondrogenesis type II
- c. atelosteogenesis type II
- d. hypochondrogenesis
- e. a and c

4. Which of the following disorders are caused by mutations that activate transmembrane receptors:

- a. Jansen metaphyseal chondrodysplasia
- b. pseudoachondroplasia

- c. campomelic dysplasia
- d. Kniest dysplasia
- e. a and c

5. Which of the following describes *FGFR3* mutations:

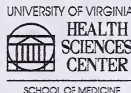
- a. They tend to be dispersed throughout the gene.
- b. They reflect a very high mutability of the gene.
- c. They block signal transduction through this transmembrane receptor.
- d. They account for a small proportion of human chondrodysplasias.
- e. They predispose to mutations of transcription factor genes.

Answer Key: 1. d 2. c 3. e 4. a 5. b

#### CME Accreditation Statement

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Drs. Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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### The Spectrum of Uses for Growth Hormone in Children

Ron G. Rosenfeld, MD

Chairman

Department of Pediatrics

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#### INTRODUCTION

This is a reasonable time for a reassessment of the use of growth hormone (GH) in childhood, as pituitary-derived human GH (hGH) was first tested in children 40 years ago and recombinant DNA-derived hGH has been available for more than a dozen years. Multiple controlled and uncontrolled clinical trials have been performed and tens of thousands of children worldwide are receiving therapy. GH has received Food and Drug Administration (FDA) approval in the United States for 3 pediatric indications: childhood GH deficiency (GHD), growth failure associated with chronic renal insufficiency (CRI); and short stature associated with Turner syndrome (TS). Treatment also has been approved for AIDS-associated wasting and adult GHD. The ability to produce recombinant GH in essentially unlimited quantities has allowed higher doses to be employed for both conventional and less conventional uses. Provocative GH testing, for years the definitive diagnostic method for GHD, has come under renewed criticism; alternative diagnostic strategies, such as 24-hour GH sampling, quantitative excretion of urinary GH, and determinations of serum concentrations of insulin-like growth factor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3) and, possibly, the acid labile subunit (ALS), have been proposed. Although Creutzfeldt-Jakob disease is not a complication of

#### Letter From the Editor

The two lead articles in this issue are published primarily for nonendocrinologists who read *GROWTH, Genetics, & Hormones*, although endocrinologists also will undoubtedly appreciate the authors' opinions. The purpose of these articles is stated in the opening sentence of Dr. Rosenfeld in his article entitled "The Spectrum of Uses for Growth Hormone in Children." Specifically, Dr. Rosenfeld states: "This is a reasonable time for a reassessment of the use of growth hormone (GH) in childhood." Dr. Rosenfeld is eminently qualified as an expert both in studying the clinical and laboratory aspects of the GH axis and in caring for patients with disturbances of this axis. Dr. Slyper also qualifies as a significant contributor, as is evident from his thoughtful and cautious approach to the use of GH as a pharmacologic agent. He published his thoughts initially in 1995 in *Medical Hypothesis* in an article entitled "How Safe and Effective Is hGH at Pharmacologic Dosing?" He has updated his presentation for publication in this issue of *GGH*.

Other recent important articles concerning the use of human GH (hGH) that our readers may wish to consult include (1) an article from the Committee on Drugs and the Committee on Bioethics of the American Academy of Pediatrics, published in *Pediatrics* (1997;99:122-129), that is entitled "Considerations Related to the Use of rhGH in Children"; (2) an article edited by Cuttler et al, entitled "Short Stature and GH Therapy: A National Study of Physician Recommendation Patterns," published in *JAMA* (1996;276:531-537); and (3) an article from a committee appointed from the Lawson Wilkins Pediatric Endocrine Society, entitled "Guidelines for the Use of GH in Children With Short Stature," published in *J Pediatr* (1995;127:857-867). The first two are abstracted in this issue of *GGH*; however, reading the articles in their entirety is highly recommended.

Letters to the Editors expressing the thoughts and opinions of our readers regarding the lead articles of this issue of *GGH* and related topics are both welcome and encouraged. These can be sent c/o Robert M. Blizzard, MD, 1224 West Main Street, Suite 701, Charlottesville, VA 22903 or faxed to 804-977-9450.

For the Editorial Board,  
Robert M. Blizzard, MD  
Editor-in-Chief

#### In This Issue

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rhGH administration, new safety issues have been raised, such as tumor recurrence and leukemia. Finally, new therapeutic options may become available—for example, GH-releasing hormone (GHRH), GH-releasing peptide (GHRP), and IGF-1—and the use of GH must be evaluated in light of such proposed alternative treatments.

## APPROVED INDICATIONS FOR GH THERAPY IN CHILDHOOD

As stated above, there are 3 FDA-approved indications for the use of GH therapy to promote growth during childhood: childhood GHD, growth failure associated with CRI, and short stature associated with TS. Each of these diagnostic categories is worthy of comment.

### Childhood Growth Hormone Deficiency

On the surface, this would appear to be the least controversial indication for GH treatment. Children with classic GHD have severe growth failure, and GH **replacement** should lead to catch-up growth and the potential for achieving normal adult height. The problem here lies not in the therapy but in the diagnosis.<sup>1</sup> The potential pitfalls in provocative GH testing have been described in detail<sup>2</sup> and are presented in Table 1. These pitfalls have made the diagnosis of childhood GHD less clear-cut.

While measurement of serum concentrations of GH-dependent peptides such as IGF-1, IGFBP-3, and ALS has certain advantages, currently it is difficult to determine whether testing these factors is superior to provocative GH testing in identifying partial GHD and in predicting the clinical response to GH treatment.<sup>3</sup> Biochemical diagnostic strategies are further strained by the testing of children who do not have clear evidence of growth failure. Accordingly, it is proposed that auxologic criteria be employed as working guidelines when considering a diagnosis of GHD (Table 2).

Thus, the diagnosis of GHD should integrate auxologic and biochemical strategies, which need not be limited to provocative GH testing but can include other

aspects of the GH/IGF axis, such as GH-dependent IGF-1, IGFBP-3, and ALS. Even an integrated diagnosis, however, should be considered provisional, especially for partial GHD, and the diagnosis must be reconsidered in the child who does not respond appropriately to conventional GH therapy. It is worth pointing out that even though GH has been used in the treatment of GHD for 40 years, there still has never been a **controlled trial** demonstrating the impact of therapy on adult height.

Even when the appropriate diagnosis of GHD has been established, controversies remain, such as what the appropriate starting dose of GH should be. While studies do demonstrate a dose-response for GH,<sup>4</sup> the slope of this correlation is relatively shallow, with only modest increases in growth rate seen when the GH dosage is increased, for example, from 0.025 to 0.05 mg/kg/d or from 0.05 to 0.1 mg/kg/d. On the other hand, it has been argued that there are psychosocial benefits to returning a short child to the normal growth curve as rapidly as possible, and that treatment at the larger dose best assures the eventual attainment of normal adult height.<sup>5</sup> Given the significant cost of GH and the potential for dose-related side effects,<sup>6</sup> it seems best to individualize the therapeutic approach. For example, in children who are diagnosed early in life or when short stature is mild, beginning treatment with a dosage of 0.025 mg/kg/d and reserving the option of increasing the dose if at any time the growth response attenuates is most logical. The young child with "true" or severe GHD should respond well initially to the lower dosage (0.025 mg/kg/d). However, for the older child with GHD or the younger GHD child with severe short stature, a higher initial dosage of GH (0.05 mg/kg/d) is appropriate. It is important to recognize, however, that high doses of GH may carry an increased risk of side effects or of accelerated entry into puberty. In general, it is always best to individualize the therapeutic approach, with careful assessments of clinical responsiveness and potential adverse effects.

### Growth Failure Associated With Chronic Renal Insufficiency

In the United States, growth failure associated with CRI was the second FDA-approved indication for GH. This approval was based entirely upon short-term data. While a control group was employed, neither long-term studies nor controlled investigations carried out until attainment of adult height have been performed to date.<sup>7</sup> The physiologic basis for growth failure secondary to CRI remains unknown, and consequently, the rationale for GH therapy must be considered pharmacologic rather than physiologic. Although several studies have demonstrated increased serum concentrations of IGF-inhibitory binding proteins, it is not clear that GH works in these patients by normalizing IGFBP levels or increasing free IGF-1.<sup>8</sup> Long-term safety issues surrounding the use of GH in children with CRI and/or following kidney transplantation still need to be addressed.

Table 1  
**Potential Pitfalls in Provocative GH Testing**

- The nonphysiologic nature of pharmacologic testing
- Difficulty in resolving conflicting data from 2 or more tests
- The inconsistencies in reproducing the response to the same pharmacologic tests
- The arbitrary definition of a "normal" response
- Age variability and sex steroid effect of the GH response
- Interassay variations among various GH radioimmunoassays
- The impact of nutrition, adiposity, and emotional state on the GH response
- Cost of tests
- Risks of testing, eg, hypoglycemia induction



## Short Stature Associated With Turner Syndrome

There is little evidence to support the concept of an endocrine etiology for the characteristic growth failure of patients with TS. Serum concentrations of GH and IGF are normal for age in prepubertal girls with TS, despite the fact that growth failure is typically demonstrable by midchildhood or earlier.<sup>9</sup> Considering TS as a form of skeletal dysplasia is probably appropriate, and, accordingly, GH therapy should be recognized as being pharmacologic rather than physiologic. A large number of studies have demonstrated growth acceleration with GH treatment in TS, although the growth response does not match that observed in naive GHD children.<sup>10</sup> Furthermore, several studies have shown a positive effect of GH on adult height.<sup>11-13</sup> In the Genentech-sponsored clinical trials, GH-treated patients had an 8.4-cm increase over their baseline projected adult height; subjects receiving both GH and oxandrolone had a 10.3-cm increase.<sup>13</sup> While this study did not include a placebo control group followed to adult height, a matched historical control group had a mean adult height identical to that of the treated patients' baseline projected adult height. This salutary effect of GH on adult height has been demonstrated in several other studies,<sup>11,12</sup> although another reported a more modest improvement in adult height.<sup>14</sup>

However, most subjects in the latter studies<sup>11-13</sup> generally have been characterized by a relatively advanced chronologic and bone age and/or a relatively early introduction of estrogen for feminization. Given the effect of estrogen on epiphyseal fusion, estrogen therapy unequivocally will compromise the net growth response to GH. From the aspect of maximizing the adult height of each TS patient, the critical issue appears to be the **number of estrogen-free years that GH is administered**. Ideally, therapy should be individualized for each patient in an effort to both normalize growth and adult height and allow pubertal development at a relatively normal age. This approach requires early diagnosis of TS and initiation of GH treatment by midchildhood or earlier, before the patient's height falls below the 10th percentile on the normal female growth chart.

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Table 2  
**Auxologic Guidelines for the Diagnosis of GH Deficiency**

- Severe growth retardation (height  $>3$  standard deviations [SD] below the mean for chronologic age) in the absence of an alternative explanation
- Moderate growth retardation (height  $-2$  to  $-3$  SD below the mean for age) plus growth deceleration (height velocity  $<25$ th percentile for age) in the absence of an alternative explanation
- Severe growth deceleration (height velocity  $<5$ th percentile for age) in the absence of an alternative explanation
- A predisposing condition, eg, cranial irradiation, plus growth deceleration
- Other evidence of pituitary dysfunction, eg, other pituitary deficiencies, neonatal hypoglycemia, micropallus

## Transition From Childhood to Adult GHD

Treatment of adult GHD is now an FDA-approved indication for GH. While the efficacy of GH in correcting the metabolic consequences of and improving quality-of-life issues associated with long-standing adult GHD has been demonstrated, the benefit and/or effect of continuing GH treatment in childhood-onset GHD following epiphyseal fusion have not been demonstrated. Although it may appear logical to continue therapy without a hiatus, the benefits, if any, of this approach require analysis through randomized, controlled trials. Unfortunately, significant compliance issues are inevitable in an adolescent population to whom no immediately obvious benefit of continued parenteral medication is apparent. Additionally, continuing GH treatment in adult patients with childhood-onset GHD requires standardization of the retesting of these patients, since as many as 60% to 70% of them will have normal provocative GH results when reevaluated as adults.<sup>15</sup>

## THE USE OF GH FOR UNAPPROVED INDICATIONS

GH has been used for treatment of short stature associated with skeletal dysplasias, dysmorphic syndromes, metabolic conditions such as hypophosphatemic rickets, idiopathic intrauterine growth retardation (IUGR), and idiopathic short stature (ISS). In the absence of demonstrable GHD, the growth response to GH treatment is generally modest. None of these conditions has been studied with sufficient numbers of subjects to allow adequate evaluation of the impact of therapy on adult height; in particular, no concurrent control group has been employed in long-term investigations. This is of particular importance in those conditions for which there are insufficient historical data to allow comparison of the observed growth response with the natural history of the disorder and in those disorders that are by nature heterogeneous, as in ISS.

The issue of GH treatment for ISS is particularly complex.<sup>16-19</sup> Undoubtedly, some of these patients



have constitutional delay of growth and maturation and can be expected to attain normal adult heights, even in the absence of therapy. Furthermore, the limitations in our ability to accurately diagnose GHD in children, as discussed above, may make it difficult to distinguish between some patients with ISS and others with partial GHD. There are some children who have been diagnosed as GHD who are in fact endocrinologically normal, just as there are some children categorized as having ISS who have either partial GHD or IGF deficiency. An additional concern with GH treatment of ISS is the issue of whether GH therapy results in an earlier onset of puberty than might have otherwise occurred, resulting in early epiphyseal fusion and the forfeiture of whatever height gain might have been attained during the early years of GH treatment.<sup>20</sup> These issues make it difficult to strongly recommend GH treatment for patients in these categories. On the other hand, there are undoubtedly ISS or IUGR patients who respond effectively to GH treatment, sometimes in as robust a manner as GHD patients. Accordingly, it is recommended that such patients be treated as part of prospective clinical trials or on a case-by-case basis, following a full discussion of the potential benefits and risks of therapy.

## THE FUTURE OF GH THERAPY

It is anticipated that GH will remain the treatment of choice for children with classic GHD, at least for the near future. Improved formulations, easier methods of reformulation and routes of administration, and long-lasting GH preparations should all enhance compliance with and, ultimately, the success of GH treatment. As more data are accumulated on the cost:benefit ratio of higher GH doses and as more experience is gathered on the adverse effects of high GH doses, a

better rationale for dosing should be developed. Therapy for non-GHD short children, such as those with CRI or TS, will continue to entail pharmacologic doses of GH to obtain short-term increases in height. Ultimate adult heights will be increased in TS patients, but whether the same will apply to patients with CRI or those with ISS and other causes of short stature remains to be determined. It is incumbent upon the endocrine community, pediatricians, and internists to continue careful monitoring of GH recipients for both short-term and long-term side effects. As experience accumulates with GHRH and other GH secretagogues, such as the GHRPs, we will be able to determine whether such therapeutic options provide any benefits over GH, even if only to a subset of patients.

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# How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?

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Until 1985, cadaveric growth hormone (GH) was the sole source of human GH (hGH). The recommended dosage of between 0.24 and 0.3 IU/kg/wk or approximately 0.1 mg/kg/wk represented a compromise between the limited hormone supply and obtaining an optimal growth response. Since the introduction of biosynthetic hGH, the recommended dosage has increased to 0.3 mg/kg/wk (0.78 to 0.9 IU/kg/wk). This is roughly triple that used in the past and about 3 times the rate of endogenous GH production, except at ado-

lescence.<sup>1</sup> Use of this dose has led to growth acceleration in conditions in which GH may be partially deficient, namely idiopathic short stature,<sup>2</sup> as well as conditions in which GH secretion is normal, such as Turner syndrome,<sup>3</sup> bone dystrophies,<sup>4</sup> and intrauterine growth retardation.<sup>5</sup> Irrespective of their endogenous GH status, many short children will experience growth acceleration on currently recommended hGH doses. It is not surprising, therefore, that conditions other than classic (severe) GH deficiency (GHD) now account for more than 40% of patients treated in this country with biosynthetic hGH.

It is clear from these studies, however, that currently recommended doses of hGH often have gone beyond providing physiologic replacement and are now phar-

macologic. In Turner and other syndromes, for example, GH treatment is clearly aimed at producing a supraphysiologic effect, namely, a final height beyond that dictated by genetic potential.<sup>3</sup> This is a unique venture. Never before in the history of medicine has a biologic agent been used in an attempt to produce such widespread and permanent physical changes. However, to anticipate that this can be achieved without undesirable side effects also would be unprecedented. With this in mind, issues of safety should be of paramount concern. Unfortunately, there is a limit to which the available data from long-term replacement dosing or short-term pharmacologic dosing can be used to guarantee the ultimate safety of pharmacologic GH therapy.

There is a general consensus that over the short term pharmacologic GH dosing is reasonably safe.<sup>6</sup> An increased risk of intracranial hypertension and slipped capital femoral epiphysis has been documented, but the incidence of these complications is not large.<sup>6</sup> Of all potential complications, however, it is the risk of malignancy that should be of greatest concern. To date, 44 patients have developed leukemia following GH treatment, 12 of them from Japan.<sup>6</sup> The malignancies reported have been stem cell malignancies, such as leukemias, lymphomas, or thymomas.<sup>7</sup> Many of the patients had received pituitary-derived GH at moderate doses, and some had been off thera-

py for several years at the time of diagnosis. Of the 12 cases from Japan, 8 had idiopathic GHD and none of the usual risk factors for leukemia such as chemotherapy, radiation therapy, or preexisting malignancy. The data from Japan, therefore, remain unexplained.<sup>8</sup> It is generally agreed that there is insufficient evidence to incriminate GH therapy as a cause of leukemia, leukemic relapse, or tumor recurrence.<sup>6</sup> Nevertheless, a high index of suspicion needs to be maintained, since GH has the potential for being carcinogenic.<sup>7</sup> Acromegalic patients are at increased risk for developing benign and malignant tumors, particularly colon polyps and adenocarcinoma.<sup>9</sup> In a small group of acromegalic patients with active disease, 53% had colonic polyps.<sup>10</sup> In rats, both hypophysectomy and large doses of GH influence the effect of carcinogens.<sup>7</sup> Intraperitoneal injection of large doses of purified pituitary-derived GH into rats for up to 485 days resulted in rapid growth as well as neoplasia in multiple organs: lymphosarcomas of the lung, adrenocortical and adrenomedullary carcinomas, solid ovarian tumors, and breast tumors.<sup>7</sup> Such toxicology studies may have little relevance when considering physiologic replacement dosing, but the situation may be otherwise for pharmacologic dosing.

Of possible relevance to this issue are observations that melanocytic nevi of children with hypopituitarism and Turner syndrome show increased growth, increased proliferative activity, and atypical signs of differentiation during GH therapy, although there is no evidence of neoplasia.<sup>11,12</sup> Increased chromosome fragility also has been demonstrated in lymphocytes obtained 3 to 12 months into the treatment of normal short children, in addition to an increase in spontaneous chromosome rearrangements and a significant increase in bleomycin-induced aberrations.<sup>13</sup>

Hyperinsulinemia and insulin resistance have been noted in Turner syndrome and normal short children during GH therapy.<sup>14,15</sup> The absence of diabetes in all but a few patients in no way excludes the possibility of sequelae from a childhood spent in a state of hyperinsulinemia and insulin resistance.<sup>7,14</sup> Doubtless, most children will suffer no long-term effects, but this may not be the case for patients who already have a predisposition to atherosclerosis, diabetes, or hypertension. We have to admit that our knowledge of the natural history of these diseases is limited, and it may be decades before we can say with certainty that treatment has no influence on the development of these conditions.

Between 53% and 76% of patients with acromegaly develop joint problems, with a delay of approximately 10 years between the onset of acromegaly and the appearance of arthropathy.<sup>16</sup> Typical joint changes include widening of the joint spaces, osteophyte formation, joint capsule calcification, and mineralization of ligamentous insertions.<sup>17</sup> These changes are irreversible. Whether joint disorganization also occurs in developing joints as a consequence of higher-dose GH therapy will not be known for years. Reports of

#### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.



avascular necrosis of the femoral head and slipped capital femoral epiphyses in a few treated children may or may not be the tip of the iceberg.<sup>18</sup> The potential for joint disturbances following high-dose GH treatment could be a particular concern in conditions with preexisting abnormalities of the growth cartilage. Modest short-term growth acceleration has been achieved in some patients with achondroplasia using pharmacologic GH dosing.<sup>4</sup> The basic defect in this condition is in the fibroblast growth factor receptor 3 gene and relates in some manner to abnormal cartilage growth and endochondral ossification. A bone dysplasia also contributes to the short stature of Turner syndrome, and an abnormality of cartilage cannot be excluded.<sup>19,20</sup> GH-treated children with chronic renal failure could be another group at high risk for bone and joint complications.<sup>18</sup> The extent to which abnormal joints and bones can be increased beyond their genetic potential and yet retain complete functional integrity throughout adulthood is not known.

Even if we follow a safety-first approach to pharmacologic GH treatment, the benefits of therapy are another important issue. We should not be placing any child at *unnecessary* risk. While higher-dose GH has improved the height prognosis for children with classic (severe) GHD, the situation is far more ambiguous with respect to children with other forms of GH insufficiency. This is a relevant concern, since these children now constitute a large proportion of children being treated with GH.

Neurosecretory GH dysfunction was first observed in children who had undergone prophylactic cranial irradiation for leukemia. Frequent sampling of endogenous GH over 24 hours demonstrated diminished GH secretion; GH pulses were attenuated in size and diminished in number.<sup>21</sup> Similar secretory patterns were subsequently found in other short, poorly growing children who had not undergone cranial irradiation and who had "passed" provocative GH stimulation testing.<sup>22</sup> The concept arose of a spectrum of GH insufficiency, ranging from short but normally growing children at one end of the spectrum, and children with classic GHD at the other, and a group of children with borderline to subnormal growth and partial GHD in between. At the same time there was a loosening of the "pass-fail" criteria for GH stimulation testing and a cutoff of 10 ng/mL rather than 5 to 7 ng/mL was adopted.<sup>23</sup>

Despite wide acceptance of neurosecretory GH dysfunction as a distinct clinical entity, there is much about this syndrome that is extremely ambiguous. There are, for example, no objective criteria for its diagnosis. Twenty-four-hour GH monitoring is expensive and labor-intensive, and has remained primarily a research tool. It also seems to be no better at diagnosing GHD than stimulated GH levels.<sup>24</sup> The diagnostic cutoff levels used during GH stimulation testing are recognized as arbitrary.<sup>25</sup> The finding of substantial discrepancies between one GH assay and the next, to the point that the diagnosis of GHD may depend on which assay is used, has highlighted the inadequacies of provocative testing.<sup>26</sup>

By default, therefore, a subnormal growth velocity often becomes the decisive factor in the decision to initiate GH treatment. However, the measurement of short-term growth velocity is itself subject to biases and inaccuracies. Growth velocities in the autumn and winter may be more than 2 cm/y lower than during the rest of the year, and a growth velocity of less than 2.5 cm/y during these seasons may be normal.<sup>27</sup> One study found that growth velocity was significantly higher after GH testing than before testing (3.4 cm/y versus 5.1 cm/y for prepubertal children and 3.4 cm/y versus 6.3 cm/y for pubertal children). An explanation for this odd finding may be that growth velocities prior to testing were transiently low, leading to a selection bias in referral.<sup>28</sup> The 95% confidence limits of a single height measurement performed by skilled personnel is  $\pm 0.5$  cm.<sup>29</sup> There is a similar lack of precision for measuring yearly growth velocities. For a short normal child growing along the 25th percentile, the confidence limits for yearly growth velocity span the 8th to 52nd percentiles. In general, the lower limit would be considered abnormal while the upper limit would be within the normal range. For measurements taken by inexperienced personnel or at less than 12 months apart, confidence limits would be even greater. Over 2 years, there is no correlation between year to year growth velocities, suggesting that short-term growth velocity is an unreliable means of predicting future growth.<sup>29</sup> The ambiguities surrounding the diagnosis of neurosecretory dysfunction no doubt account for some of the discrepancies in GH prescribing practices between one pediatric endocrinologist and another.

Recent interest in treating GHD adults has focused attention on the question, "What percentage of patients diagnosed with GHD in childhood truly have this condition?" The answer should give pause for thought. Tauber et al<sup>30</sup> found that 71% of 98 adults previously diagnosed as having partial GHD (peak GH response between 5 to 10 ng/mL) and 36% of 33 adults diagnosed with complete GHD (peak GH response <5 ng/mL) had normal stimulated GH peaks of greater than 10 ng/mL on a single stimulation test.

Not only is the diagnosis of partial GHD ambiguous, but the results of GH treatment also are unclear. For any child receiving GH, puberty appears to be an important dividing line in terms of therapeutic response. Testosterone and estrogen increase the amplitude of GH pulses, and a pubertal increase in GH accounts in part for the growth spurt of puberty.<sup>31</sup> This is, however, a 2-way relationship, as GH also influences pubertal

#### **In Future Issues**

##### **Molecular Physiology of Leptin and Its Receptor**

Yiyi Zhong, PhD, and Ron L. Leibell, MD

##### **Growth Hormone Replacement In Adult GHD Patients**

Peter Sonksen, MD

##### **Insulin, IGF-1 and IDDM: Recently Implicated Genetic Loci**

Cheryl L. Deal, PhD, and Constantin Polychronakos, MD

sex steroid secretion.<sup>32</sup> GH treatment of GHD and non-GHD children to final height results in accelerated pubertal progression and a pubertal decrease in height standard deviation scores (SDS) for bone.<sup>33-36</sup> The acceleration in pubertal progression is dose-dependent. In a large group of male children with isolated GHD, a doubling of GH dose from 15 IU or 5 mg/m<sup>2</sup>/wk to 30 IU or 10 mg/m<sup>2</sup>/wk increased the rate of pubertal maturation but had no effect on growth velocity.<sup>37</sup> An earlier onset of puberty also was noted in a controlled study of non-GHD children, and this study concluded that therapy may actually have led to a decrease in final height.<sup>34</sup>

The implication of these observations appears to be that for children who are not truly GHD, the closer to puberty that GH treatment is initiated the less likelihood of a gain in final height. If treatment is started early in childhood, there is a greater chance of exceeding genetic potential. However, to accomplish this, supraphysiologic doses of GH need to be administered throughout childhood, resulting in a greater potential for long-term complications.

### Recommendations

Based on this discussion, I propose the following recommendations, appreciating that many of these points are out of line with the current practices of many pediatric endocrinologists:

1. The recommended dosage of GH treatment of 0.3 mg/kg/wk is a high one for the initial treatment of children with severe (classic) GHD. Treatment should be started at a lower dose and further dose changes titrated against the observed growth effect.

2. Families of short children who pass provocative testing and in whom pharmacologic GH treatment is contemplated should be informed that negative short-term data provide no assurance as to the ultimate safety of pharmacologic GH therapy and that the benefits of treatment in terms of final height are unknown. Families of children with Turner syndrome who are about to be placed on the newly recommended GH dosage of up to 0.375 mg/kg/wk also should be informed that there is little information on the short-term safety of this dose and none on its long-term safety.

3. For poorly growing peripubertal children, GH testing should be accompanied by sex steroid priming so as to exclude the transient, physiologic GHD present in many youngsters with constitutional delay of puberty. Sex steroid priming has gone out of favor in recent years, but could be used far more extensively.

4. A multicenter controlled trial should be organized to follow to final height children specifically with neurosecretory GH dysfunction or partial GHD treated with currently recommended doses of GH. It can no longer be taken for granted that these children benefit from therapy. Noncontrolled studies using estimated heights or historical controls are incapable of demonstrating conclusively the benefits of treatment. In my opinion, a study of this nature should take priority over other contemplated growth studies investigating new indications for GH treatment with ever increasing doses.

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### Editorial Board

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## Short Stature and Growth Hormone Therapy: A National Study of Physician Recommendation Patterns

The objective of this study was to learn the attitudes of pediatric endocrinologists (PEs) regarding prescribing growth hormone (GH) to short children. Of 534 anonymous surveys, 434 (81.3%) were returned. Extensive planning of the questionnaire permitted the collection and analysis of data revealing the attitudes of 340 of the 434 respondents who currently manage short stature in children. Of the children currently being treated, 58% were GH deficient (GHD) and 15% had Turner syndrome (TS). The remaining 27% had other causes for short stature. Eight case histories, differing only in physiologic growth variables (extent of short stature, growth velocity, normal or abnormal bone age) were presented and the respondents were asked whether they were likely to recommend GH for each case. Three additional sets of decisions focusing on the contingency variables of price and family wishes also were included in the questionnaire. The first 2 contingencies proposed that the price of GH therapy fell from approximately \$13,000 per year to \$2,000 per year or \$100 per year. In the third contingency, physicians were asked their recommendation if the family strongly desired GH therapy, assuming that the price remained at current levels.

Analyses of the data revealed 3 noteworthy patterns in the responses. First, 68.1% agreed that GH use for non-GHD short stature has increased in the past 5 years and that the physician's knowledge about family finances is marginal in the overall decision-making process whether to prescribe GH. Second, PEs believe that short stature matters and has dysfunctional emotional impact on many children and adults. Third, a lack of consensus existed among the PEs regarding the perceived efficacy (adult height and long-term adverse effects) of GH therapy for non-GHD children.

In applying a logistic model to physicians' decisions to recommend GH, 3 sets of predictors were used: (a) the physiologic growth variables previously discussed; (b) contingency variables, ie, treatment cost and family wishes; and (c) physicians' beliefs about short stature and GH treatment. The growth rate was very important, as the likelihood of GH being prescribed increased 3.4-fold for a growth rate below the 3rd percentile versus the 3rd to 10th percentiles. A height falling below -3 standard deviations (SD) increased by 2.8-fold the likelihood of GH being prescribed than if the patient was between -2 and -3 SD below

the mean. A normal bone age instead of a delayed bone age increased the recommendation to use GH. Also, boys with comparable shortness to girls, corrected for sex, were 1.3-fold more likely to receive GH than girls. Physicians were sensitive to cost and would have significantly increased recommending the use of GH if it cost \$2,000 per year instead of \$13,000 per year. A cost of \$100 per year would have further significantly increased recommendations. Family wishes clearly influenced the recommendations made by many physicians. In addition, the odds of a positive recommendation increased 13% if the physician believed GH would add at least 1 inch to the ultimate height of non-GHD children. The authors concluded from analyses of the data that physiologic factors, contingency factors, and belief factors exert independent and additive effects on recommendations for GH therapy.

The conclusions that can be drawn from this study have several implications for GH and analogous interventions such as treatment of attention deficit disorder, in vitro fertilization, and genetic testing. Like GH therapy, these analogous interventions hold promise for increasing the quality of life for a targeted patient population, but with uncertain risks and benefits and often at considerable financial cost. As HMOs try to limit the use of GH because of a lack of consensus concerning its use, even referrals for short stature may be limited, which would be unfortunate since referral not only should address whether GH is recommended but also elucidate whether a cause for short stature requiring therapies other than GH is present.

Cuttler L, et al. *JAMA* 1996;276:531-537.

**Editor's comment:** This article demonstrates the optimal use of scientific methodology in constructing a questionnaire that will yield interpretable data. Our readers are encouraged to review the entire article for its multiple contributions, particularly the insight it yields into the factors that may prompt the prescribing of other agents that may improve the patient's quality of life but not necessarily significantly improve the ultimate height of the patient.

Although originally published in 1996, this article seemed worthy of abstracting in GGH as it is an excellent corollary to the 2 lead articles in this issue.

Robert M. Blizzard, MD

## Considerations Related to the Use of Recombinant Human Growth Hormone in Children

This is an excellent overview of different aspects of the use of growth hormone (GH) by the Committee on Drugs and the Committee on Bioethics of the American Academy of Pediatrics. Some important points are summarized here, but the reader is encouraged to review the complete article published in *Pediatrics* 1997;99:122-129.

### BACKGROUND Recombinant GH Products

The biosynthetic process involves a chemical synthesis of the DNA fragment encoding the first 24 amino acids and complementary DNA copies of messenger RNA prepared from human pituitary cells. The entire DNA sequence is intro-

duced into a bacterium, *Escherichia coli*, which enables the synthesis of GH. The products currently available in the United States differ in that somatrem (Protropin®, Genentech, Inc.) contains an additional methionine group whereas somatropin (Nutropin® and Nutropin AQ®, Genentech, Inc., and Humatrope®, Eli Lilly & Co.) are identical to human GH.

Optimal dosing strategies have not been developed fully for any product. Most pediatric endocrinologists recommend 0.18 to 0.30 mg/kg/wk depending on the product, given in equally divided daily doses 6 or 7 times a week.

### Problems in the Diagnosis of Growth Hormone Deficiency

Because random fasting serum GH levels do not differ between GH-deficient (GHD) and non-GHD patients, other physiologic and pharmacologic tests have been developed to identify GHD patients. Classic severe GHD can be diagnosed if the peak stimulated value on 2 tests is  $\leq 10$   $\mu$ L or less in association with delayed bone age and slow growth rate. Other forms of GHD, ie, partial GHD, cannot be fully diagnosed by these pharmacologic tests. Thus, GH treatment should be considered primarily on clinical grounds for those patients who present with slow growth velocity and delayed bone age.

### Goals for rhGH Administration to Children With Short Stature

Much of the controversy surrounding the use of GH is related to the absence of well-defined goals for therapy. Most physicians agree that GH therapy should be reserved for patients with either classic GHD or some other conditions that exhibit a demonstrable benefit with GH treatment, such as Turner syndrome (TS) or chronic renal insufficiency (CRI). The goal of GH treatment is to attempt to maintain age-appropriate growth and to attain a final adult height that is consistent with the patient's genetic potential. In contrast, other physicians argue that all patients with short stature (SS) may be given a trial because of the physical and psychosocial handicaps associated with this condition. However, there is no universal consensus regarding the definition of SS. Some physicians define SS as a height below a certain percentile on a standard longitudinal height chart; others believe that height velocity for age and sex is the more appropriate indicator.

Unfortunately, there is an absence of generally accepted criteria for diagnosing "inadequate secretion," partial GHD, or GH dysregulation. Also, some children with various forms of SS respond to GH therapy with accelerated growth velocity. For these reasons, some pediatric endocrinologists believe that GH should be made available for a therapeutic trial to all children with SS regardless of its etiology as long as they have a decreased growth rate.

A survey conducted by the American Academy of Pediatrics revealed that abnormal GH levels during provocative testing followed by decreased growth velocity in SS patients were the criteria used most frequently by pediatric endocrinologists.

### Risks Associated With GH Therapy

Short-term treatment with GH has been associated with few

side effects, but long-term risks are still unknown. The most frequent side effects are as follows:

- antibody formation at an overall level of 10% or lower with no clinical effects.
- pseudotumor cerebri related to the use of GH and/or insulin-like growth factor 1. Benign intracranial hypertension with papilledema has been reported rarely. Cessation of GH therapy has reversed the symptoms in reported cases. In some cases, spontaneous resolution has occurred in spite of continued treatment.
- unusually lean and inappropriately muscular appearance due to increased cellular metabolism.
- potential physiologic and psychologic trauma related to years of regular injections.
- theoretical concerns that GH therapy might be related to an increased risk of malignancy. Although an increased incidence of leukemia among patients treated with GH has been reported in Japan, a recent US/Canadian survey did not show an increased risk for leukemia or brain tumor unless other risk factors were present, ie, previous radiation therapy or chemotherapy.

### ETHICAL ISSUES

Since there are no data demonstrating improvement in ultimate height in patients with nonclassic GH, one question should be addressed: Is GH therapy acceptable for children who do not fulfill the criteria for classic GHD?

It also is well known that being tall is indisputably viewed as a benefit in our culture, and is associated with multiple advantages, including higher income, academic achievement, self-esteem, and social status. The concepts of normality and abnormality are difficult to define, and they incorporate many sociocultural variables. In most cases, a better alternative may be to help children and their families to achieve pride and fulfillment on their own terms.

There also are important economic issues regarding GH. Some children who do not have classic GHD now receive GH therapy, and these children are likely to come from the more financially well-off sector of society. On the other hand, many children lack access to the most basic health care, and it would be ethically inappropriate to spend scarce monetary resources to provide GH therapy to anyone who is not classically GHD or truly GH-resistant.

### CONCLUSIONS AND RECOMMENDATIONS

1. Therapy with GH is medically and ethically acceptable for:
  - children with classic GHD
  - children with CRI who are awaiting kidney transplantation
  - girls with TS
  - children whose extreme SS keeps them from participating in basic activities of daily living and who have a condition for which the efficacy of GH therapy has been demonstrated.
2. Two key considerations argue against widespread administration of GH therapy to other short children:
  - There could be unknown long-term risks.
  - The treatment could result in either no increase or only an insignificant increase in final adult height.

3. Therapy may be justified for children whose height could prevent them from participating in the basic activities of daily living.
4. Pediatricians should be alert to commercial efforts to

stimulate parental interest in GH therapy as an avenue for improving athletic ability and other forms of social "success" for their children.

Fima Lifshitz, MD

### Increased Height in Patients With Medulloblastoma

Robertson et al report their surprising findings from chart reviews of 85 patients with medulloblastomas seen at the University of Iowa College of Medicine from 1963 to 1995. These patients (64 children and 21 adults) had their height and weight documented on standardized growth charts before treatment of their tumors. The data show that 22.4% of these patients were above the 95% curve (see Table) in height. In a comparison group of patients with cerebellar astrocytomas, only 7.1% were above the 95% curve for height. Thus, there is a clear difference between linear growth in the 2 different groups. Most of the increased height was in male patients; however, 56 of the 85 patients were male. Interestingly, patients who presented as adults also were taller than expected at diagnosis.

The authors related that medulloblastoma cell lines can express different levels of growth factors, including epidermal growth factor, platelet-derived growth factor, transforming growth factor, and insulin-like growth factor (IGF). They note that since the adults in the study also were above normal height, something must have occurred that predated the development of neoplastic cells.

Robertson SC, et al. *Neurosurgery* 1997;41:561-566.

**Editor's comment:** These authors have presented some very interesting and intriguing data. Since the study was retrospective, there are no carefully collected hormonal data from the individuals, ie, testosterone, growth hormone, IGF-1, etc. Nor do the authors present any data regarding the pubertal status of the children at the time of diagnosis. It is conceivable that some of the children with medulloblastoma may have had early puberty, which would account for their being taller than expected. Postoperatively, growth failure is the rule rather than the exception in these individuals. Although we have no data on final heights in the children, one would anticipate that those patients diagnosed and treated as children would not end up being tall adults. The data do suggest, however, that it is important for pediatric endocrinologists to continue to encourage their neurosurgical colleagues to evaluate the hormonal status of their patients preoperatively as well as after treatment.

William L. Clarke, MD

**Preoperative Height and Weight of Patients With Medulloblastomas<sup>a</sup>**

| Medulloblastoma<br>Preoperative Curves (%) | Height |    | All Patients<br>Total (%) | Weight |    | Total (%) |
|--|--------|----|---------------------------|--------|----|-----------|
|  | M      | F  |                           | M      | F  |           |
| >95  | 14     | 5  | 19 (22.4)                 | 6      | 0  | 6(7.1)    |
| >90  | 18     | 8  | 26 (30.6)                 | 9      | 1  | 10(11.8)  |
| >75  | 36     | 11 | 47 (55.3)                 | 15     | 2  | 17(20.0)  |
| >50  | 46     | 18 | 68 (80.0)                 | 32     | 10 | 42(49.4)  |
| >25  | 55     | 25 | 80 (94.1)                 | 44     | 14 | 58(68.2)  |
| >10  | 55     | 29 | 84 (98.8)                 | 50     | 18 | 68(80.0)  |
| >5   | 55     | 29 | 84 (98.8)                 | 54     | 23 | 77(90.6)  |
| >0   | 56     | 29 | 85 (100)                  | 56     | 29 | 85(100)   |

<sup>a</sup>Numbers in each column represent the total number of patients above or equal to the percentile group listed.

From Robertson SC, et al. Increased height in patients with medulloblastomas. *Neurosurgery*. 1997;41:561-566.

### Prenatal Diagnosis From Fetal Cells in Maternal Circulation

Detection of fetal aneuploidy by noninvasive means has been a long-term goal of the prenatal diagnostician. Screening procedures based on measuring substances in maternal serum, for example, maternal serum  $\alpha$ -fetopro-

tein, detect many instances of aneuploidy. However, many are missed, and this deficit has prompted the search for other strategies, including analyzing fetal cells circulating in maternal serum. Indeed, it has been known for many



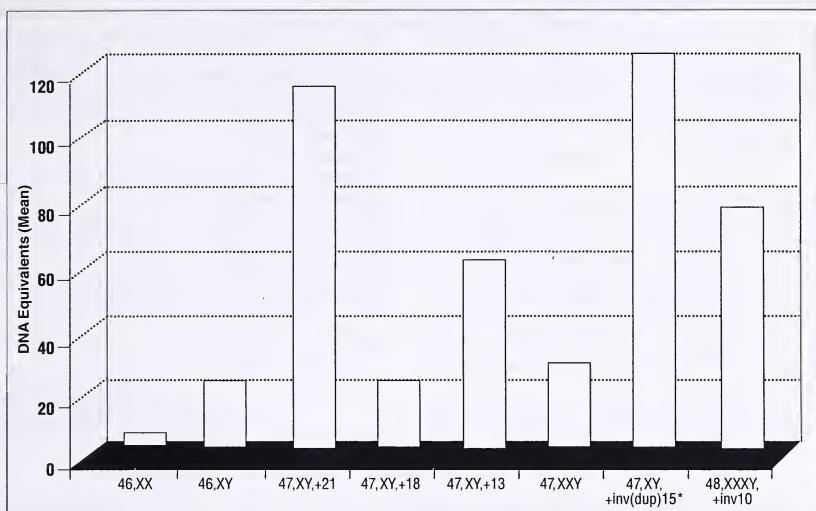


Figure. Bar graph demonstrating mean number of male fetal-cell DNA equivalents detected in maternal samples, stratified by fetal karyotype. Note that the highest number of male fetal-cell DNA equivalents is detected when the fetus has 47,XY,+21 or 47,XY,+inv(dup)15. The asterisk (\*) indicates that the values for 47,XY,+inv(dup)15 are off the scale, with a mean value of 230.

From Bianchi DW, et al. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. *Am J Hum Genet* 1997;61:822-829. Published by University of Chicago. ©1997 by the American Society of Human Genetics.

years that a limited number of fetal trophoblasts, lymphocytes, granulocytes, nucleated erythrocytes, and platelets reach the maternal circulation. Several studies have explored various aspects of this issue, but differences in patient populations, cells studied, and methods used to enrich and analyze the cells have made it difficult to draw definite conclusions about the efficacy of this approach for prenatal diagnosis.

An article by Bianchi et al sheds light on the issue. In a large, multicenter clinical trial, PCR-amplified Y-chromosome sequences were obtained from 16-mL peripheral blood samples of 199 women carrying chromosomally normal fetuses and from 31 women with male aneuploid fetuses. They sought to determine the number of male cells, or their equivalent, that could be detected in maternal serum under different clinical conditions. Results were expressed as male fetal-cell DNA equivalents. There was no enrichment of cells so that values reflected the number of cells in the original sample and were not artifacts of enrichment; no distinction was made between the different cell types.

The mean number of male fetal-cell DNA equivalents from 90 women bearing 46,XY fetuses was 19, and more than 80% had values over 2 cell equivalents. There was no difference if the specimen was taken before or after amniocentesis. Surprisingly, Y-specific DNA sequences were found in about one fourth of women carrying female fetuses, although the values were lower than when male fetuses

were being carried. Possible explanations for the presence of male cells in the maternal circulation were that the male cells had come from a male twin who had been lost early in the current pregnancy, or were from a previous transfusion from a male donor, or were from a previous pregnancy with a male fetus.

Most remarkable, Bianchi et al found a substantial increase in the number of male fetal-cell DNA equivalents if the fetus was aneuploid. There was a 6-fold increase in fetal cells detected in the maternal circulation when the fetus had trisomy 21 (see Figure). Lesser increases were observed for trisomy 13 and 18, but fewer cases were assessed. The authors pointed out that this finding is consistent with pathologic observations of placental abnormalities in trisomies.

The authors concluded that a small but consistent number of fetal cells are normally transfused across the placenta into the maternal circulation. The number is increased substantially for aneuploid fetuses, especially for trisomy 21, which should make feasible detection of at least trisomy 21 from maternally circulating fetal cells.

Bianchi DW, et al. *Am J Hum Genet* 1997;61:822-829.

Goldberg JD. *Am J Hum Genet* 1997;61:806-809. Invited Editorial.

**Editor's comment:** This technology has evolved from little more than wishful thinking 2 decades ago to an almost fea-



sible prenatal diagnostic approach for trisomy 21 and potentially other aneuploidies. As pointed out in an accompanying editorial by Goldberg, there are problems that still must be resolved, such as the persistence of fetal cells from previous pregnancies. He notes 2 issues that must be addressed before widespread testing of fetal cells in maternal circulation is undertaken. The first issue is whether it should be offered to all pregnant women. The second issue is whether it should be used for gender selection.

William A. Horton, MD

**Guest Editor's comment:** Advanced maternal age, variously defined as 35 to 40 years of age at the time of delivery, has long been a standard indication for prenatal diagnosis by chorionic villus sampling or amniocentesis. However, because the majority of infants with autosomal trisomies are born to women under 35 years of age, a number of approaches are used to screen for high-risk pregnancies among younger women. Clinical trials currently under way involving analysis of fetal cells in maternal circulation offer prospects for yet an additional approach.

Maternal serum triple screening, ie,  $\alpha$ -fetoprotein, HCG, and estriol, which assist in detecting neural tube defects,

Down syndrome, and trisomy 18, provides a high detection rate for these entities. However, its use at 15 to 20 weeks of gestation, followed by the subsequent requirement for amniocentesis if the screening is suspicious for definitive diagnosis, is too late in pregnancy for use by many women. Transvaginal ultrasonography also is used to detect birth defects. Taiplae et al recently published their experience in detecting increased nuchal translucency in 10,000 unselected pregnancies, reporting a sensitivity of 54% for the detection of trisomy 21.

It is reasonable to assume that for the foreseeable future a combination of maternal serum triple screening, ultrasonography, and very possibly, analysis of fetal cells in maternal circulation will be used for testing pregnancies of younger women. However, none of these techniques is currently sufficiently sensitive or specific enough to obviate standard cytogenetic analysis of the fetus to arrive at a confident prenatal diagnosis of an autosomal aneuploidy.

Taiplae P, et al. *N Engl J Med* 1997;337:1654-1658.

Thaddeus E. Kelly, MD, PhD  
Professor of Pediatrics and Genetics  
University of Virginia School of Medicine

## Gene Therapy: Promises, Problems, and Prospects

Gene therapy is a concept with which most of us are familiar. We know of its potential and that it has not lived up to this potential. However, few of us understand the biology that underlies gene therapy or appreciate the obstacles that gene therapists face. Fortunately, Verma and Somia have come to the rescue with a timely and concise review of the subject.

First, they point out that despite more than 200 clinical trials currently under way worldwide, there has been no clear success story yet. They consider the primary obstacles to be the lack of an efficient delivery system, the lack of sustained expression, and often a host immune response to therapy.

To Verma and Somia, the Achilles' heel of gene therapy is the delivery system. The properties of currently used gene therapy vectors, including retroviral, lentiviral, adenoviral, and adeno-associated viral vectors, are compared. Each has certain advantages, but each also has disadvantages. For example, retroviral vectors, which have been employed

most widely in clinical trials, integrate well into host genomes and there are few immunologic problems; however, expression of the therapeutic gene is short lived. In contrast, adeno-associated viruses support long-term expression, but the logistics of producing large quantities of virus needed for therapy is difficult. As for adenoviral vectors, many patients have preexisting immunity to adenoviral proteins. Lentiviral vectors, which are related to HIV, show considerable promise. The authors conclude that the ideal vector will be constructed from elements of different viral vectors.

Regarding clinical trials, Verma and Somia note that more than half the trials initiated to date involve cancer; nearly 30 involve monogenetic disorders as listed in the Table (page 13). They also point out that most of the trials are Phase I (safety) studies, and that for the most part, no major toxicity problems have been encountered with the existing delivery systems.

Finally, the authors are optimistic about the future of gene therapy, basing their optimism on the steady progress being made in vector design.

Verma IM, Somia N. *Nature* 1997;389:239-242.

**Editor's comment:** This is a short but informative review of the current status of gene therapy. It is written to be understood by the nongeneticist, yet provides a broad overview of the field.

William A. Horton, MD

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## Candidate Diseases for Gene Therapy

| Disease   | Defect  | Incidence  | Target Cells   |
|---|---|--|--|
| <b>Genetic</b>  |   |  |  |
| Severe combined immunodeficiency (SCID/ADA)             | Adenosine deaminase (ADA) in ~25% of SCID patients                    | Rare   | Bone marrow cells or T cells   |
| A<br>Hemophilia   | Factor VII deficiency   | 1:10,000 males   | Liver, muscle, fibroblasts, or bone marrow cells                     |
| B   | Factor IX deficiency  | 1:30,000 males   |  |
| Familial hypercholesterolemia                           | Deficiency of low-density lipoprotein (LDL) receptor                  | 1:1 million  | Liver  |
| Cystic fibrosis   | Faulty transport of salt in lung epithelium. Loss of <i>CFTR</i> gene | 1:3,000 whites   | Airways in the lungs   |
| Hemoglobinopathies: thalassemias/sickle cell anemia     | Structural defects in $\alpha$ - or $\beta$ -globin gene              | 1:600 in certain ethnic groups                                 | Bone marrow cells, giving rise to red blood cells                    |
| Gaucher disease   | Defect in the enzyme glucocerebrosidase                               | 1:450 in Ashkenazi Jews  | Bone marrow cells, macrophages                                       |
| $\alpha_1$ -Antitrypsin deficiency: inherited emphysema | Lack of $\alpha_1$ -antitrypsin                                       | 1:3,500  | Lung or liver cells  |
| <b>Acquired</b>   |   |  |  |
| Cancer  | Many causes, including genetic and environmental                      | 1 million/y in US  | Variety of cancer cell types; liver, brain, pancreas, breast, kidney |
| Neurologic diseases                                     | Parkinson's, Alzheimer's, spinal cord injury                          | 1 million Parkinson's and 4 million Alzheimer's patients in US | Direct injection in the brain, neurons, glial cells, Schwann cells   |
| Cardiovascular  | Restenosis arteriosclerosis   | 13 million in US   | Arteries, vascular endothelial cells                                 |
| Infectious diseases                                     | AIDS, hepatitis B   | Increasing numbers   | T cells, liver, macrophages  |

From Verma IM, Somia N. Gene therapy: promises, problems, and prospects. *Nature* 1997;389:240.

## Changes in Bone Mineral Density, Body Composition, and Lipid Metabolism During Growth Hormone (GH) Treatment in Children With GH Deficiency

Adults with childhood-onset growth hormone deficiency (GHD) have reduced bone mass, increased fat mass, and disorders of lipid metabolism. The aim of the present study was to evaluate bone mineral density (BMD), bone metabolism, body composition, and lipid metabolism in GHD children before and during 2 to 3 years of GH treatment. The mean age of the 40 children participating in this study of bone metabolism and body composition was 7.9 years. An additional 17 GHD children participated in the study of lipid metabolism. Lumbar spine BMD, total body BMD, and body composition were all measured with dual energy X-ray absorptiometry. Volumetric BMD (or bone mineral apparent density [BMAD]) was calculated to correct for bone size. Standard deviation scores (SDS) were used to compare with normative data.

Lumbar spine BMD, total body BMD, and BMAD were all decreased at baseline. All these BMD variables increased significantly during treatment. The Table (page 14) presents the effects at various time points. Lean tissue mass SDS

increased continuously. Fat mass SDS decreased markedly during the first 6 months and remained stable thereafter. The chemical parameters of bone formation and resorption at baseline did not differ from those of normals and then increased during the first 6 months of treatment. Serum 1,25 dihydroxyvitamin D increased continuously during treatment, whereas parathyroid hormone and serum calcium remained stable. The lipid profile was normal at baseline.

The authors conclude that children with GHD have decreased bone mass. BMD, together with height and lean tissue mass, increased during treatment, which also had a beneficial effect on lipid metabolism.

Boot A, et al. *J Clin Endocrinol Metab* 1997;82:2423-2428.

**Editor's comment:** This interesting paper adds further data supporting the importance of GH treatment for GHD children, promoting linear growth and regulating different metabolic pathways. All patients presented in this study

## Mean of Different Variables at Baseline and During Growth Hormone Therapy

| Variable                 | Baseline<br>n = 38 | At 6 Months<br>GH Therapy<br>n = 37 | At 1 Year<br>GH Therapy<br>n = 33 | At 2 Year<br>GH Therapy<br>n = 33 |
|--------------------------|--------------------|-------------------------------------|-----------------------------------|-----------------------------------|
| Lumbar spine BMD SDS     | -1.62              | -1.33 <sup>a</sup>                  | -0.98 <sup>a</sup>                | -0.64 <sup>a</sup>                |
| Lumbar spine BMAD SDS    | -0.51              | -0.50                               | -0.37                             | -0.19 <sup>a</sup>                |
| Total body BMD SDS       | -0.94              | -1.35 <sup>b</sup>                  | -1.02                             | -0.61 <sup>b</sup>                |
| Bone mineral content SDS | -2.29              | -2.36                               | -1.52 <sup>a</sup>                | -1.24 <sup>a</sup>                |
| Lean tissue mass SDS     | -2.72              | -1.86 <sup>a</sup>                  | -1.53 <sup>a</sup>                | -1.14 <sup>a</sup>                |
| Fat mass SDS             | -0.02              | -0.59 <sup>c</sup>                  | -0.31 <sup>c</sup>                | -0.59                             |
| % Body fat SDS           | 0.93               | -0.39 <sup>a</sup>                  | -0.10 <sup>a</sup>                | -0.45 <sup>c</sup>                |
| Height SDS               | -2.98              | -2.32 <sup>a</sup>                  | 1.86 <sup>a</sup>                 | -1.63 <sup>a</sup>                |
| Body mass index SDS      | 0.45               | 0.24                                | 0.39                              | 0.37                              |

<sup>a</sup>  $P < 0.001$ ; <sup>b</sup>  $P < 0.02$ ; <sup>c</sup>  $P < 0.01$  compared to baseline.

BMAD, bone mineral apparent density

BMD, bone mineral density

SDS, standard deviation score

From Boot A, et al. Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. *J Clin Endocrinol Metab* 1997;82:2425. ©The Endocrine Society.

showed evidence of the anabolic effect of GH, as demonstrated by the increase in BMD, the increase in lean body mass, and the decrease in body weight. Some of these metabolic effects may be considered direct effects of GH replacement. An increase in the serum 1,25 dihydroxyvitamin D level has been reported during GH treatment due to renal inactivation induced by insulin-like growth factor 1, an indirect effect resulting in the beneficial increase in BMD.

The authors concluded that treatment had a beneficial effect on lipid metabolism. However, there were no significant changes found in lipid metabolism as baseline values were all normal. In my opinion, no conclusions can be drawn from the present study regarding the beneficial effects on lipid metabolism. Long-term studies in children need to be done since adults with GHD are at risk of hypercholesterolemia and coronary heart disease.

Fima Lifshitz, MD

## Growth Hormone Therapy in Prepubertal Children With Noonan Syndrome: First Year Growth Response and Comparison With Turner Syndrome

The authors report that during the first year of administration of recombinant human growth hormone (rhGH; 0.15 U/kg/d given by daily injection) to 23 prepubertal subjects with Noonan syndrome ( $9.4 \pm 3.0$  years), the increase in height velocity was 8.5 cm, approximately twice the pre-treatment growth rate. In a group of females with Turner syndrome of similar age at initiation of rhGH, the mean height increment was 8.1 cm during the first year of treatment. Four of 23 Noonan syndrome subjects had no significant change in height standard deviation scores (SDS) during rhGH administration. In Noonan patients, the in-

crement in height velocity during rhGH administration was directly related to birth weight, suggesting that low-birth-weight children with Noonan syndrome responded less well to treatment. The changes in bone age, growth velocity, and height SDS were similar in Turner and Noonan syndrome groups. The authors conclude that the linear growth response to short-term administration of rhGH is comparable in patients with Noonan and Turner syndrome.

De Schepper J, et al. *Acta Paediatr* 1997;86:943-946.



**Editor's comment:** Although phenotypically similar, patients with Noonan syndrome have growth patterns distinct from those of patients with Turner syndrome. The mean adult height of male patients with Noonan syndrome is 162.5 cm, and the mean adult height of female patients is 152.7 cm; the latter is almost 10 cm greater than the mean adult height of untreated subjects with Turner syndrome.<sup>1</sup> Romano et al<sup>2</sup> reported that 3/6 males with Noonan syndrome treated with rhGH achieved final heights greater than predicted, but specific data were not provided. In view of the minimal positive effect of rhGH on final height of normal short children,<sup>3</sup> assessment of the role of rhGH treatment in children with Noonan syndrome must be deferred until adult height data are available.

Incidentally, the spontaneous growth pattern of Northern European patients with Turner syndrome recently has been reported.<sup>4</sup> The mean adult height of these subjects was 146.9 cm, approximately 4 cm greater than that reported by other investigators, underscoring once more the importance of ethnic as well as familial genetic factors on growth.

Allen W. Root, MD

1. Ranke MB, et al. *Eur J Paediatr* 1988;148:220-227.
2. Romano AA, et al. *J Pediatr* 1996;128:S18-S21.
3. Schmitt K, et al. *Eur J Pediatr* 1997;156:680-683.
4. Rongen-Westerlaken C, et al. *Acta Paediatr* 1997;86:937-942.

## The Duration of Puberty in Girls Is Related to the Timing of Its Onset

The authors serially took the history of and examined 163 normal girls from age 10 to 15 years, determining the ages at which thelarche developed and menarche occurred. The mean age at menarche was 12.62 years (see Table). The younger the age at thelarche the more prolonged was the interval between thelarche and menarche. There was an inverse relationship between age at thelarche and interval to menarche.

Marti-Henneberg C, et al. *J Pediatr* 1997;131:618-621.

**Editor's comment:** The investigators defined menarche not as the first episode of vaginal bleeding, but as the first menses that was followed by "regular cycles." While this definition is different than the usual one used in the United States, the data are of interest because they address the issue of the tempo of pubertal development and suggest that the later its onset, the more rapid is the progression of sexual maturation. The manuscript utilizes the term "duration of puberty" as the interval between thelarche and menarche. This is misleading as the duration of puberty extends well past this point.

Allen W. Root, MD

**Age at Menarche and the Duration of Puberty in the Overall Study Sample and the Subgroups Assigned by Age-of-Onset of Puberty**

| Study Subjects  | Menarche Age      |               | Duration of Puberty |             |
|-----------------|-------------------|---------------|---------------------|-------------|
|                 | Mean $\pm$ SEM    | Range (y)     | Mean $\pm$ SEM      | Range (y)   |
| Total (n = 163) | 12.62 $\pm$ 0.06  | 10.25 - 14.41 | 1.96 $\pm$ 0.06     | 0.25 - 4.25 |
| 9 y (n = 22)    | 11.77 $\pm$ 0.15* | 10.25 - 12.91 | 2.77 $\pm$ 0.15*    | 1.25 - 4.25 |
| 10 y (n = 53)   | 12.27 $\pm$ 0.10  | 11.00 - 13.91 | 2.27 $\pm$ 0.10     | 1.00 - 3.91 |
| 11 y (n = 54)   | 12.77 $\pm$ 0.07  | 11.59 - 14.25 | 1.78 $\pm$ 0.07     | 0.59 - 3.25 |
| 12 y (n = 27)   | 13.44 $\pm$ 0.10  | 12.42 - 14.41 | 1.44 $\pm$ 0.10     | 0.42 - 2.41 |
| 13 y (n = 7)    | 13.65 $\pm$ 0.09  | 13.25 - 13.92 | 0.65 $\pm$ 0.09     | 0.25 - 0.92 |

Menarche is defined as "regular cycles." Duration of puberty is defined as the period between thelarche and regular cycles.

Correlation (age at onset versus age at menarche)  $r = 0.66$ ;  $P < 0.001$

Correlation (age at onset versus duration of puberty)  $r = 0.62$ ;  $P < 0.001$

\* Stepwise analysis of variance  $P < 0.001$  between groups

From Marti-Henneberg C, et al. The duration of puberty in girls is related to the timing of its onset. *J Pediatr* 1997;131:618-621.



**GROWTH, Genetics, & Hormones Volume 14, Number 1**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Which of the following are potential pitfalls in provocative GH testing?
  - a. The arbitrary definition of a "normal" response.
  - b. Interassay variations among various GH radioimmunoassays.
  - c. The impact of nutrition, adiposity, and emotional state on the GH response.
  - d. Age variability and sex steroid effect of the GH response.
  - e. All of the above.
2. Auxologic guidelines for the diagnosis of GHD include which of the following?
  - a. Severe growth retardation ( $-3$  SD for chronological age) in the absence of an alternative explanation.
  - b. A predisposing condition, eg, cranial irradiation.
  - c. Other evidence of pituitary dysfunction, eg, other pituitary hormone deficiencies.
  - d. Amblyopia.
3. Dr. Rosenfeld suggests that the starting dose of GH in a younger GHD child usually should be
  - a. 0.025 mg/kg/d.
  - b. 0.05 mg/kg/d.
  - c. 0.075 mg/kg/d.
4. Which of the following statements is/are true?
  - a. The characteristic short stature in TS is of endocrine etiology.
  - b. Serum concentrations of GH and IGF-1 are normal for age in prepubertal girls with TS.
  - c. GH therapy in TS should be considered pharmacologic, not physiologic.
  - d. All of the above.
5. In Dr. Rosenfeld's opinion, which of the following conditions have been adequately studied to be treated therapeutically with GH when short stature is present?
  - a. Hypophosphatemic rickets.
  - b. Idiopathic GHD.
  - c. Idiopathic short stature.
  - d. Dysmorphic skeletal dysplasia.
  - e. Prader-Willi syndrome.
6. The recommended dose of hGH on many package inserts for treatment of GHD is
  - a. 0.3 mg/kg/wk.
  - b. 0.1 mg/kg/wk.
  - c. 0.5 mg/kg/wk.
7. The physiologic replacement dose of hGH in a child of prepubertal age is approximately
  - a. 0.3 mg/kg/wk.
  - b. 0.1 mg/kg/wk.
  - c. 0.5 mg/kg/wk.
8. In Dr. Slyper's opinion, which of the following complications is the one of most significant concern when using pharmacologic GH treatment?
  - a. Malignancy.
  - b. Slipped capital femoral epiphysis.
  - c. Hyperinsulinemia and insulin resistance.
9. It is generally agreed that there is insufficient evidence to implicate GH
  - a. As a cause of leukemia.
  - b. As a cause of colonic polyps.
  - c. As etiologic in developing atherosclerosis.
10. Dr. Slyper recommends which of the following?
  - a. Treatment with hGH should be less than 0.3 mg/kg/wk.
  - b. Families of individuals receiving hGH should be informed that negative short-term data provide no assurance as to the ultimate safety of pharmacologic GH therapy.
  - c. Families of children with TS who are to receive treatment should be told there is no safety information on the long-term treatment of children with pharmacologic doses of hGH.
  - d. All of the above.

Answer Key: 1. e 2. a,b,c 3. a 4. b,c 5. b 6. a 7. b 8. a 9. a,c 10. d

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**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

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# GROWTH

## Genetics & Hormones

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### Molecular Physiology of Leptin and Its Receptor

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#### INTRODUCTION

Many years of experimental physiology in rodents and humans\* suggested the existence of a humoral signal "reporting" body fat mass to the brain.<sup>1-3</sup> Physiologic studies of rodent single-gene obesities, "obese" (*ob*) and "diabetes" (*db*) mice and Zucker fatty (*fa*) rats, indicated that the respective gene products play an important role in such a signaling system. The recent cloning of the *ob* (leptin; gene symbol, *Lep*) and *db/fa* (the leptin receptor; gene symbol, *Lepr*) genes have now identified some of the specific molecular components of this system for regulation of body weight. This brief review summarizes our current understanding of the molecular physiology of leptin and its receptor.

\* Capital letters (*LEP* and *LEPR*) designate human genes.  
Small letters (*Lep* and *Lepr*) designate rodent genes.

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#### PHENOTYPE OF *ob* AND *db* MICE

The *obese* (*Lep<sup>ob</sup>*) and *diabetes* (*Lepr<sup>db</sup>*) mutations are autosomal recessive mutations located on mouse chromosomes 6 and 4, respectively. The *ob* mutation, identified at the Jackson Laboratory in 1950, arose originally on stock V and was later bred to the C57BL/6J line, a diabetes-resistant strain.<sup>4</sup> The

#### Letter From the Editor

Dear Colleagues:

The Editorial Board very much needs your assistance. In planning for the future we need to explore with you alternative ways of making this journal available to you.

Specifically, if we go on-line will you continue to read *GROWTH, Genetics, & Hormones (GGH)* each quarter, or will *GGH* more likely be read if you continue receiving it as you currently do?

In this issue you will find a stamped self-addressed card that we need returned by September 1, 1998, but preferably now so we are certain to receive and consider your wishes as we move into the 1999 world of high-tech communication.

Please remove the card and respond now whether the time you are reading this letter is June, July, or August.

We will appreciate your quick response. We pledge to continue to bring you a valuable informative publication in 1999.

*For the Editorial Board,  
Robert M. Blizzard, MD  
Editor-in-Chief*

mutation was named "obese" because of the severe obesity of the homozygous mutant C57BL/6J mice. The *diabetes* (*db*) mutation was identified at the Jackson Laboratory in 1966.<sup>5</sup> The *db* mutation arose spontaneously on C57BL/KsJ, a diabetes-susceptible strain. The C57BL/KsJ *db/db* mice developed severe diabetes; thus, the mutation was given the name of "diabetes." *Ob/ob* and *db/db* animals are phenotypically indistinguishable when the mutations are maintained in the same strain background.<sup>6,7</sup> Both mutations result in hyperphagia, impaired thermoregulatory thermogenesis, and hypothalamic infertility. Homozygous affected animals develop severe and early onset obesity with reduced lean body mass, shortened axial skeleton, and decreased brain size.<sup>3</sup> The mutant animals also are physically less active and develop obesity even in the absence of hyperphagia.<sup>3,8</sup> Thus, over a period of 6 weeks, *db/db* mice pair-fed to lean controls accumulated 42% more body weight and approximately 5 times more fat mass than their lean littermates, indicating an increase of energy efficiency and preferential partitioning of calories to fat in the *db/db* mice.<sup>8</sup> When maintained in C57BL/6J, both *ob/ob* and *db/db* animals develop transient hyperglycemia early in life (8 to 10 weeks), but later are euglycemic and hyperinsulinemic because of compensatory pancreatic beta-cell hyperplasia and hypertrophy. When these mutations are maintained on a diabetes-susceptible strain, such as C57BL/KsJ, the mutant animals become severely diabetic with insulinopenia due to subsequent pancreatic beta-cell atrophy.<sup>6,7</sup> The genes that mediate such strain-specific differences in diabetes susceptibility are not known, but are clearly of great interest for the insight they will provide into the molecular bases for diabetes susceptibility.<sup>9-11</sup>

The functional nature of the *ob* and *db* gene products was first suggested by Coleman's parabiosis (cross-circulation) experiments, in which he joined *ob/ob* and *db/db* mice to lean partners and to each other.<sup>3</sup> Coleman's experiments suggested that the *ob* animal was mutant in a gene for a circulating suppressor of food intake while the *db* animal was mutant in a receptor for this hormone.

## MOLECULAR CLONING AND CHARACTERIZATION OF LEPTIN

The complexity of the metabolic and endocrine phenotype of *ob* and *db* mice made it extremely difficult to identify the primary molecular defects. Hence, in the mid-1980s, projects were initiated to clone *ob* and *db* genes by positional genetic strategies.<sup>12-15</sup> This technique enables the cloning of genes based on their physical location on a chromosome, without

prior knowledge of their precise function, and has been successfully employed for cloning cystic fibrosis, muscular dystrophy, and Huntington's disease genes.<sup>16-18</sup>

The mouse *ob* gene was cloned by this technique in 1994. The gene encodes a 167 amino acid precursor protein that is primarily expressed in white adipose tissues. The mature protein (named "leptin," from the Greek word "lepto," meaning "thin"), consisting of the C-terminal 146 amino acids of the precursor, is secreted into the circulation from adipocytes. Human and mouse leptin genes share approximately 85% homology at the amino acid level.<sup>19</sup> Both genes consist of 3 exons separated by approximately 10.6 kb and approximately 2.3 kb introns, spanning approximately 20 kb. The leptin mRNA is approximately 4.5 kb in length, with approximately 55-bp 5' untranslated region (UTR) and approximately 3.9-kb 3' UTR. The protein coding sequence is contained in the second and the third exons.<sup>20-22</sup>

Despite the absence of primary sequence similarity, the tertiary structure of leptin is similar to that of members of the cytokine family, consisting of 4 antiparallel  $\alpha$  helices connected by 2 long crossover links and 1 short loop. The helical structure is highly conserved among the long-chain helical cytokine family members. The relatively loose, coil-structured crossover links and loops may provide flexibility for receptor binding-induced conformational changes, as is the case for hGH/hGHR.<sup>23-25</sup>

## REGULATION OF LEPTIN GENE EXPRESSION

The promoter region of the human and rodent leptin genes contains a TATA box-like sequence and a functional C/EBP (CCAAT/enhancer-binding protein) alpha binding site within the 200-bp region upstream of the transcriptional start site. The C/EBP site is likely responsible for the expression of leptin in mature adipocytes since C/EBP itself is a differentiation-induced transcription factor.<sup>26,27</sup> Another nuclear transcriptional factor, peroxisome proliferator-activated receptor (PPAR)-gamma, which is involved in adipocyte differentiation, downregulates leptin expression by apparent antagonism of C/EBP transactivation of leptin expression.<sup>28</sup> The anti-diabetic agent, thiazolidinedione, is a high-affinity ligand of PPAR-gamma and inhibits leptin expression in adipose tissue.<sup>29</sup>

Expression of leptin mRNA is increased by glucocorticoids<sup>30-32</sup> and insulin,<sup>33-35</sup> and decreased by  $\beta$ -adrenergic agonists and cyclic adenosine monophosphate (cAMP).<sup>30,36</sup> Leptin also appears to regulate its own expression. A rat mutation in the

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leptin receptor gene (*Lepr<sup>fa</sup>*) results in a gene dose-dependent increase of leptin mRNA expression in adipose tissue in 10-day-old rat pups segregating for *Lepr<sup>fa</sup>*. The effect of *Lepr<sup>fa</sup>* on leptin gene expression is independent of fat mass and insulin concentration in these rats.<sup>37</sup> This apparent autoregulation of leptin expression may be important in mediating the pulsatile release of leptin and restricting leptin overexpression because of the potent effects of leptin on energy homeostasis and sexual development.

Northern blot and reverse transcriptase polymerase chain reaction (RT-PCR) analyses have shown significantly different levels of leptin gene expression among different fat depots. For example, leptin mRNA expression is higher in subcutaneous than intra-abdominal fat depots in humans.<sup>38,39</sup> Interestingly, the amount of intra-abdominal fat conveys a significant risk factor for obesity-related medical complications, such as diabetes and coronary artery disease. Lower leptin expression in the intra-abdominal fat depots may contribute to their adverse effects, perhaps by effects on hepatic glucose homeostasis.

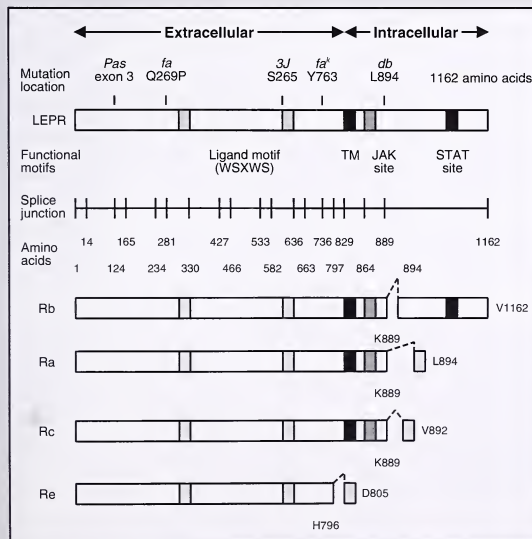
Preadipocytes, such as 3T3-F442A cells, do not express leptin.<sup>34</sup> Mature adipocytes, differentiated

from 3T3-F442A cells in culture, express very low levels of leptin mRNA (1% of that in epididymal adipose tissue).<sup>35</sup> However, mature adipocytes derived from subcutaneously implanted 3T3-F442A cells express leptin mRNA at levels about 15% of those in epididymal adipose tissue. These data suggest that factors required for high level expression of the leptin gene in vivo are lacking in the cell culture system.<sup>40</sup> Identification of these factors would shed important light on the in vivo regulation of leptin expression.

## MOLECULAR CLONING AND CHARACTERIZATION OF THE LEPTIN RECEPTOR

The leptin receptor gene was cloned by screening a cDNA expression library of choroid plexus using a leptin-alkaline phosphatase fusion protein as a probe. The receptor has a high affinity for leptin ( $K_d \approx 0.7 \times 10^{-9}$  M).<sup>41</sup> This gene was quickly demonstrated to be mutant in the *db* mouse and the *fa* rat.<sup>42-44</sup> The leptin receptor gene encodes a membrane protein that shares 24% sequence homology with gp130, a signal-transducing component of the

Figure 1



Schematic of functional domains, mutations, and splice variants of the mouse and rat leptin receptor (LEPR). The extracellular domain of the receptor is encoded by exons 1 through 15, the transmembrane domain by exon 16. The intracellular domain is encoded by exon 17 and either terminal exon 17', 18a, or 18b for the Rc, Ra, and Rb isoforms, respectively.<sup>43,45</sup> Exons 3 through 6 and 8 through 11 encode two potential leptin binding sites, containing the characteristic four conserved cysteine residues and the Trp-Ser-X-Trp-Ser motif (where X is a nonconserved amino acid). The second binding site appears to be important for leptin binding and signaling.<sup>120</sup> The transmembrane (TM) domain is 23 amino acids long. The Janus kinase (JAK) and STAT protein binding sites are encoded by exons 17 and 18b, respectively. The splice variants Rb, Ra, Rc, and Re have been described in at least two species. The Rd and Rf (not shown) have been identified in only one species: Rd in mouse, and Rf in rat.

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interleukin-6 receptor. The extracellular domain of the leptin receptor contains all of the characteristically conserved cysteine residues, the Trp-Ser-X-Trp-Ser motif, and other conserved residues characteristic of the cytokine receptor superfamily (Figure 1, page 19). The large extracellular domain of the receptor (approximately 865 amino acids) is followed by a single transmembrane domain (23 amino acids) and cytoplasmic domains of varying length (up to 273 amino acids) resulting from alternative splicing of the C-terminal exons.<sup>41,45</sup> Of the 6 alternative splice variants of LEPR described, 4 have been detected in at least 2 different species (Figure 1, page 19).<sup>45</sup> The longest isoform, "Rb" (1162 amino acids), is expressed predominantly in the hypothalamus, and at very low levels in many other tissues, including adipose tissue, skeletal muscle, pancreas, and liver. The "Rb" isoform of LEPR contains a Janus kinase (JAK) binding site (FWDDVPNP motif) and STAT binding site (YMPQ motif). JAK, a class of cytoplasmic tyrosine kinase, and STAT (signal transducer and activator of transcription, a family of latent cytoplasmic transcription factors) participate in signal transduction for members of the cytokine superfamily.<sup>46,47</sup> The "Rb" isoform of LEPR is the only isoform apparently not produced in the *db* mouse and is thereby shown to be essential for mediating leptin's effects on energy homeostasis.<sup>43,44</sup> A shorter isoform of LEPR, "Ra" (894 amino acids), is expressed at relatively higher levels in choroid plexus, kidney, and lung, and at lower levels in liver, muscle and adipose tissue.<sup>41,43</sup> By RT-PCR, mRNAs for the "Rc" isoform, and a putative soluble isoform "Re," also were detected in adipose tissue and heart.<sup>43</sup> The biologic functions of the shorter splice variants of LEPR are not clear, but may involve binding leptin molecules in the circulation and/or transport of leptin across cell membranes.

The leptin receptor gene includes 20 exons (18 coding), spanning about 70 kb.<sup>45,48</sup> Human and mouse leptin receptor genes share approximately 78% homology at the amino acid level.<sup>41</sup>

## MUTATIONS OF *Lepr* AND *Lepr* IN RODENTS

All known spontaneous mutations in the *Lepr* and *Lepr* genes in rodents have been recently characterized at the molecular level.<sup>19,43,44,49-55</sup> These results are summarized in Table 1 and Figure 1. Both the *ob* and *ob<sup>2J</sup>* mutations result in little or no detectable leptin protein in the circulation. The *db* and *fa* mutations result in functional disruption of 1 or more of the splice variants of the leptin receptor. The mutant animals are functionally leptin-resistant and have higher plasma leptin concentrations per unit of fat mass than lean

controls, presumably resulting in part from derangement of the apparent receptor-mediated autoregulation of leptin expression in adipose tissue.<sup>37</sup>

Studies of the *db* mutant confirmed the functional importance of the longest isoform ("Rb") of the receptor. The *db* mutation, which abolishes the "Rb" isoform of the receptor without affecting any of the other known splice variants, results in an obesity phenotype that is indistinguishable from that seen in null mutations, such as *db<sup>pas</sup>*, *db<sup>3J</sup>*, and *fa<sup>k</sup>*, which affect all known splice variants of *Lepr*.<sup>42,54,55</sup> The fact that the "Rb" isoform contains the complete JAK/STAT signaling motifs also is consistent with the apparent essentiality of this splice variant.

## MOLECULAR GENETICS OF *LEP* AND *LEPR* IN HUMANS

The human leptin gene (*LEP*) is located on chromosome 7q31.3,<sup>56</sup> the human leptin receptor gene (*LEPR*) maps to chromosome 1p31-p22.<sup>57</sup> A single guanine nucleotide deletion in codon 133 of *LEP* that leads to a frameshift was recently found in 2 obese cousins in a highly consanguineous family of Pakistani origin living in England. These children, an 8-year-old female (86 kg) and a 2-year-old male (29 kg), have very low plasma concentrations of a leptin molecule that is probably inactive (Table 1).<sup>58</sup> However, except for their extreme obesity, the gross phenotype of these children differs significantly from the *ob/ob* mouse in that they do not display apparent derangement of thermoregulation, overproduction of cortisol, or stunting in height (which may be mediated by the effects of excessive glucocorticoid in the mice). Hypothalamic hypogonadism would be expected, although these children are still prepubertal and the gonadal axis has not been formally assessed. A role for leptin in human energy homeostasis is confirmed by the obesity of these children.

A second mutation in the leptin gene (*LEP*), and the first mutation in the leptin receptor gene (*LEPR*) have been recently described in two families (Table 1).<sup>59,60</sup> The homozygous affected individuals in these families are hyperphagic and extremely obese. Hypogonadism and the absence of sexual maturation are common features in the adult patients. As predicted by the phenotype of *Lepr* mutant rodents, the humans homozygous for the *LEPR* mutation show evidence of autonomic nervous system dysfunction.

Significant coding sequence variants in *LEP* are extremely rare in humans.<sup>61-64</sup> However, extreme obesity in humans (BMI [body mass index-wt (kg)/ht (m<sup>2</sup>)] >42 from a US population; and BMI >35 from a French population) has been associated by genetic linkage analysis with the vicinity of the leptin

gene.<sup>65,66</sup> Some obesity-related traits, such as skin-fold thickness and serum proinsulin concentration in Mexican-Americans, also have been associated with the genetic region of the leptin gene.<sup>67</sup> Since significant variations in the coding sequence of the leptin gene have been reported only in the Pakistani family described above, these linkages could be due to variations in the noncoding region of *LEP* or could be due to another obesity gene in this region.<sup>59-62</sup>

Several nonconserved amino acid substitutions in the coding exons of the *LEPR* gene also have been identified in humans.<sup>68-71</sup> Sequence variations in the noncoding region of *LEPR* have been linked to acute insulin response to intravenous glucose tolerance testing (IVGTT) in Pima Indians, who have a very high prevalence of obesity and noninsulin-dependent diabetes mellitus (NIDDM).<sup>48,71</sup> Linkage be-

tween a marker in the leptin receptor region and BMI, body composition, and plasma insulin concentration also were observed in the Quebec Family Study.<sup>72</sup> Thus, these less drastic variations in coding sequences of *LEP* or *LEPR*, with attendant subtle alterations in leptin signaling, or variations of the promoter region that affect the regulation of *LEP* and *LEPR* gene expression may predispose individuals who carry these allelic variations to obesity.

## BIOLOGIC EFFECTS OF LEPTIN IN RODENTS

Intraperitoneal or intracerebroventricular (ICV) administration of recombinant leptin to *ob/ob* mice reverses virtually all the characteristics of the phenotype. Leptin decreases food intake, increases energy expenditure, including thermoregulatory

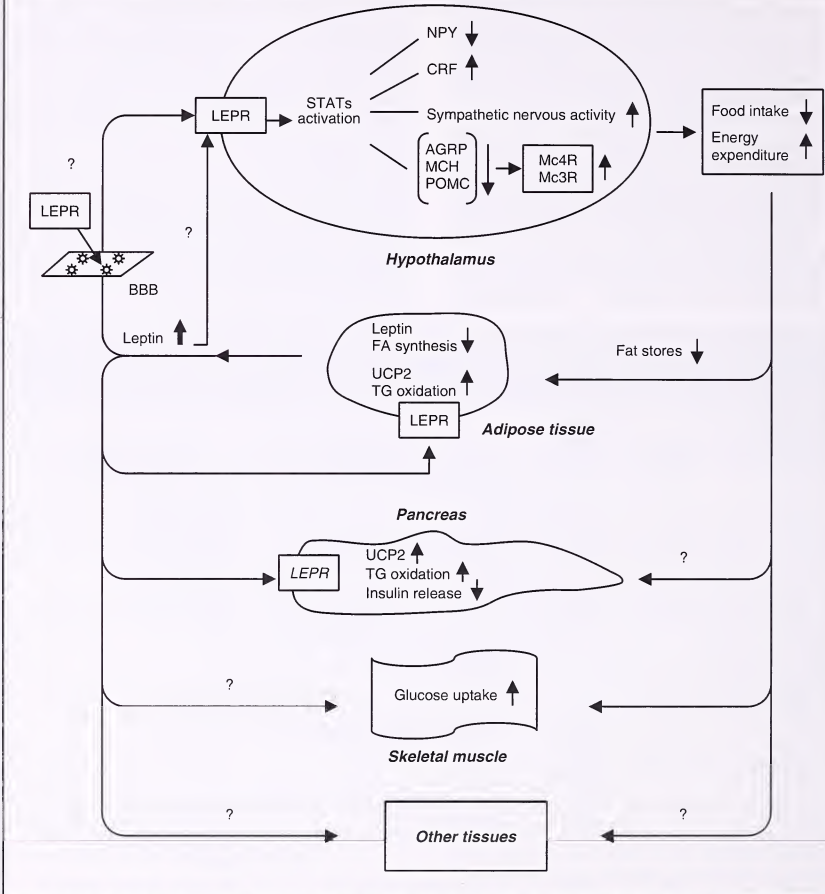
Table 1  
Spontaneous Mutations in the Leptin and Leptin Receptor Genes in Rodents and Humans

| Mutations or Gene Symbol | Species | Mutant Molecule | Nature of Mutation  | Result of Mutation  |
|--------------------------|---------|-----------------|---|---|
| <i>ob</i>                | mouse   | leptin          | C→T mutation in the last exon (Arg105Stop)  | No detectable leptin protein, but high level of mutant leptin mRNA <sup>19</sup>                            |
| <i>ob</i> <sup>2,3</sup> | mouse   | leptin          | Transposon insertion in the first intron; abnormal splicing; no leptin mRNA   | No detectable leptin mRNA and protein <sup>49</sup>   |
| <i>db</i>                | mouse   | leptin receptor | G→T mutation in the 3'UTR of "Ra" creates a splice donor site; insertion of first 106 bp of the last "Ra" exon into "Rb" mRNA | No "Rb" isoform; all other splice variants are normal <sup>143,44</sup>                                     |
| <i>db</i> <sup>3,1</sup> | mouse   | leptin receptor | 17-bp deletion in exon 11; frameshift (start at Ser625)   | No leptin receptor mRNA <sup>54</sup>   |
| <i>db</i> <sup>pas</sup> | mouse   | leptin receptor | Duplication of exons 3-6; frameshift  | No leptin receptor mRNA <sup>55</sup>   |
| <i>fa</i>                | rat     | leptin receptor | A→C mutation in exon 5 (Gln269Pro)  | Reduced surface expression of the mutant leptin receptor; no effect on affinity for leptin <sup>50,51</sup> |
| <i>fa</i> <sup>k</sup>   | rat     | leptin receptor | T→A mutation in exon 14 (Try763Stop)  | No leptin receptor mRNA <sup>52,53</sup>  |
| <i>LEP</i>               | human   | leptin          | A single guanine nucleotide deletion at codon 133; frameshift   | Little or no detectable leptin protein <sup>58</sup>  |
| <i>LEP</i>               | human   | leptin          | C→T mutation in the last exon (Arg105Trp)   | Mutant protein produced but not secreted <sup>59</sup>  |
| <i>LEPR</i>              | human   | leptin receptor | G→A mutation in the splice donor site of exon 16  | Truncation of leptin receptor after exon 15 with an extra Glu residue at the C-terminus <sup>60</sup>       |

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Figure 2



Schematic of the current view of the role(s) of leptin in energy homeostasis. Leptin is released by adipose tissue and circulates in the plasma. The concentration of leptin in plasma is very closely correlated with the amount of body fat. Plasma and/or CSF leptin molecules bind to a receptor (LEPR) in the hypothalamus, which triggers (via JAK/STAT) a series of changes of gene expression of several neuropeptides, including downregulation of NPY and upregulation of CRF and AGRP, and increases of sympathetic nervous activity. These changes result in decreased food intake and increased energy expenditure, and eventually lead to weight loss. Leptin also acts directly on peripheral tissues, regulating uncoupling proteins (UCPs) and leptin gene expression, fatty acid (FA) synthesis, and triglyceride (TG) oxidation. The decline of circulating leptin associated with reduced fat mass and/or hypocaloric intake leads to the opposite effects, triggering increased food intake and conservation of energy, which favors storage of body fat.

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thermogenesis and physical activity, and causes rapid weight loss (mainly adipose tissue loss) in the *ob/ob* mouse.<sup>73-77</sup> The effects of leptin on energy metabolism are summarized in Figure 2. Administration of leptin also decreases blood glucose and insulin concentrations in *ob/ob* mice,<sup>78</sup> and acutely stimulates glucose turnover and glucose uptake by skeletal muscles and decreases liver glycogen content in lean mice.<sup>79</sup> As predicted, administration of leptin has no effect in *db/db* mice.<sup>74,75</sup>

Leptin apparently affects food intake and energy expenditure via direct actions on peripheral tissues as well as effects mediated by the central nervous system (CNS). Leptin induces depletion of triglyceride in adipose tissue and pancreas by increasing intracellular fatty acid oxidation and gene expression of the enzymes involved in fatty acid oxidation.<sup>80,81</sup> Leptin also increases the expression of uncoupling protein-2 (UCP2) in adipose tissue and pancreas, which may partly account for the increase in energy expenditure after leptin administration.<sup>81</sup> These effects of leptin on adipose tissue and pancreas are apparently mediated by the leptin receptor in these tissues since the same effects are observed in both intact animals and isolated tissues. The effects are not observed in tissues isolated from *db/db* mice, or in *fa/fa* rats, suggesting the "Rb" isoform of the receptor is responsible for mediating these effects.<sup>81</sup> Leptin apparently also inhibits basal and glucose-stimulated insulin secretion by direct effects on pancreatic beta cells.<sup>82,83</sup>

## CNS-MEDIATED EFFECTS OF LEPTIN

The CNS-mediated effects of leptin on food intake and energy expenditure are mediated by specific neurons in the hypothalamus. The "Rb" isoform of the leptin receptor is primarily expressed in the arcuate, ventromedial, dorsomedial, and lateral hypothalamic nuclei,<sup>84</sup> which have been previously implicated in energy homeostasis.<sup>85</sup> The efferent signals from these neurons, affecting food intake and energy expenditure, are then "communicated" to peripheral tissues via the neuroendocrine and autonomic nervous systems.

The expression levels of several hypothalamic neuropeptides are influenced by leptin administration, and these neuropeptides may in turn mediate a portion of leptin's effects on energy intake and expenditure. One of these neuropeptides is neuropeptide Y (NPY). Central administration of NPY potently stimulates food intake, decreases sympathetic nervous activity, and increases plasma insulin and corticosterone concentrations.<sup>86</sup> ICV administration of leptin inhibits the expression and

release of NPY in the arcuate region of the hypothalamus in *ob/ob* mice.<sup>87,88</sup> Knockout of the *Npy* gene partially reduces the obesity phenotype of *ob/ob* mice.<sup>89</sup> However, *Npy* knockout mice are still sensitive to the anorexic effects of leptin,<sup>90</sup> suggesting the existence of NPY-independent downstream effectors of leptin action and food intake. ICV administration of leptin also increases the expression of corticotropin-releasing factor, a potent inhibitor of food intake, by 30% in the paraventricular nucleus of *ob/ob* mice.<sup>88</sup> Other possible candidates for downstream mediators of leptin effects include melanin-concentrating hormone,<sup>91</sup> agouti-related protein,<sup>92,93</sup> and urocortin,<sup>94</sup> all of which have been implicated in the regulation of ingestive behavior and/or energy expenditure in animals.

The CNS-mediated effects of leptin also are reflected in its critical role in normal reproductive function. Since ovaries of *ob/ob* and *db/db* mice function normally in wild-type host after ovarian transplantation, the infertility of *ob/ob* mice is apparently due to the neuroendocrine defect in the gonadal axis and not to a cell-autonomous defect in the ovary.<sup>95,96</sup> Leptin administration restores the reproductive function in both female and male *ob/ob* mice<sup>97,98</sup> and induces vaginal opening in prepubertal mice, indicating that plasma leptin concentration may be a signal for the onset of sexual maturity and maintenance of fertility.<sup>99</sup>

Fasting results in a rapid decline in circulating leptin concentration, and this decline appears to mediate some of the changes in gonadal, adrenal, and thyroid neuroendocrine axes induced by fasting. Short-term restriction of food intake in mice results in lower serum triiodothyronine and thyroxine levels and increased corticotropin and cortisol levels, concomitant with the decline in plasma leptin concentration. Repletion of exogenous leptin during the short-term starvation restores circulating triiodothyronine and thyroxine levels, reduces circulating cortisol levels, and prevents the starvation-induced delay of ovulation in female mice.<sup>100</sup>

## SIGNAL TRANSDUCTION OF LEPTIN/LEPTIN RECEPTOR

The structural similarities between leptin and members of the class I cytokine family suggest a similar mode of ligand-receptor binding and signal transduction for leptin and the other members of the family, including interferons, interleukins, growth hormone, and insulin-like growth factor 1 (IGF-1).<sup>23,41,46</sup> Binding of a ligand to this class of receptor triggers assembly of receptor components or dimerization of receptor molecules, and activates JAK, which is

bound to the cytoplasmic domain of the receptor. The activated JAK then phosphorylates tyrosine residues on the cytoplasmic domain of the receptor, which activates binding sites for the Src homology 2 group of latent cytoplasmic proteins, STATs. The phosphorylated STATs form homodimers and/or heterodimers, translocate into the nucleus, and participate in transcription regulation by binding to a specific STAT response element in the target genes.<sup>47</sup> Several STAT proteins have been implicated in leptin signaling. STAT 3 was shown to be phosphorylated in the hypothalamus of *ob/ob* mice but not *db/db* mice after systemic leptin administration.<sup>101</sup> STATs 1, 3, 5B, and/or 6 were reported to be phosphorylated upon leptin stimulation in *LEPR*-transfected COS cells and GT1-7 (a hypothalamic cell line) cells.<sup>102-104</sup> All these STAT proteins also have been shown to be involved in the signal transduction of many other members of the cytokine family. Leptin increases the association of phosphatidylinositol 3 (PI3) kinase with insulin receptor substrate (IRS)-1 and -2,<sup>105,106</sup> which are involved in signal transduction of insulin, cytokines, and growth factors. How the specificity of leptin signaling is defined by the activation of these STAT proteins and the changes of intracellular signaling molecules such as PI3 kinase and IRSs has yet to be worked out.

In addition, the possibility that the "Ra" isoform (Figure 2, page 22), which has a JAK binding site but no STAT binding domain, in combination with other molecules also is involved in the signal transduction of leptin cannot be ruled out. Although the "Ra" isoform does not induce phosphorylation of STATs 1, 3, and 5B in transfected COS cells,<sup>103</sup> this isoform has been reported capable of inducing expression of immediate early genes *c-fos*, *c-jun*, and *jun-B*, in transfected COS cells after leptin stimulation.<sup>107</sup>

## PLASMA LEPTIN CONCENTRATIONS IN HUMANS

Plasma leptin concentrations are highly correlated with total fat mass in humans ( $r=0.95$ ).<sup>108</sup> The concentration in plasma ranges approximately from 2 to 20 ng/mL in normal weight individuals, and 10 to 300 ng/mL in obese individuals. A single regression line relating fat mass to circulating leptin concentration fits both obese and nonobese subjects of the same sex, suggesting that the plasma leptin level is similarly regulated in both obese and lean individuals. In humans, plasma leptin concentrations per unit fat mass are 2- to 3-fold higher in females than in males.<sup>108</sup> These differences may be due in part to the higher percentage of body fat in subcutaneous depots in females. This depot has higher leptin

mRNA expression than intra-abdominal fat depots, especially in females.<sup>38,39</sup> In addition, the differences of ambient gonadal steroids also may play a role in the sexual dimorphism of leptin expression in humans. Premenopausal women have higher plasma leptin concentrations than postmenopausal women. In premenopausal women, serum leptin levels are higher in the luteal phase than in the follicular phase,<sup>108,109</sup> suggesting that estrogen and/or progesterone may increase leptin gene expression. On the other hand, testosterone replacement normalizes elevated serum leptin concentrations in hypogonadal males,<sup>110</sup> suggesting that androgens may suppress leptin expression.

## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

## LEPTIN RESISTANCE

The higher plasma leptin concentrations in obese humans indicate that leptin deficiency is not a common cause of obesity in humans. Thus, it has been suggested that the obese may be leptin-resistant. The mechanism of such apparent leptin resistance is not fully understood, but might be due to: (1) A defect in leptin signal transduction and the function of downstream signal mediators. As in the *db/db* mouse and *fa/fa* rat, mutations in the leptin receptor will obviously impair leptin signal transduction and cause leptin resistance. A null mutation in the leptin receptor gene and some subtle variations in both coding and noncoding sequences of the *LEPR* gene have been identified in humans.<sup>48,69,72</sup> These mutations of *LEPR* or mutations of the downstream mediator genes may reduce the intensity of leptin signal. Thus, a higher leptin concentration (therefore higher fat mass) would be required in these individuals to elicit an intensity of leptin signal equal to that in normal weight individuals. As a result, these individuals would appear to be leptin-resistant and obese (ie, have a higher "set point" of body weight); (2) Limited transport of leptin from plasma to cerebrospinal fluid (CSF). Since many arcuate neurons project axons to the median eminence, which is exposed to blood circulation directly, it is not clear whether leptin must cross the blood-brain barrier to act upon the CNS. However, rodents are more sensitive to ICV than to peripheral administration of leptin.<sup>73</sup> In obese humans, CSF leptin concentrations are disproportionately low relative to plasma leptin concentrations, although absolute concentrations of leptin in CSF are slightly higher than those in normal weight subjects. The ratio of CSF [LEP]:plasma [LEP] decreases as plasma leptin concentration increases, suggesting that there is a saturable transport of leptin from plasma into CSF. The limited transport of leptin from plasma to CSF could be one of the factors contributing to apparent leptin resistance in human obesity.<sup>111,112</sup>

## MOLECULAR PHYSIOLOGY OF LEPTIN IN HUMANS

Plasma leptin concentrations in humans show a diurnal rhythm, with the lowest concentrations occurring around noon to mid-afternoon and the highest concentrations occurring around midnight to early morning.<sup>113</sup> Plasma leptin concentrations also show ultradian oscillations, with a frequency of approximately 43.8 minutes. The frequency of pulsatility of plasma leptin concentration is similar between obese and nonobese individuals. The higher plasma leptin concentration in the obese is due solely to

increased leptin pulse height.<sup>114</sup> The humoral or neural signals that regulate the oscillations of the plasma leptin concentration are not clear at present. Autoregulation of leptin gene expression in adipose tissue by autocrine effects of leptin itself is one possibility.<sup>37</sup> The fluctuations of plasma leptin concentration are inversely related to those of adrenocorticotrophic hormone (ACTH) and cortisol. This finding, and reports that leptin inhibits corticotropin-releasing factor expression in the hypothalamus and substantially blunts the stress- and fasting-induced rise in ACTH and corticosterone<sup>100,114,115</sup> suggests that leptin suppresses the hypothalamic-pituitary-adrenal axis. Therefore, leptin-mediated alterations in ACTH and cortisol secretion or action are possible mechanisms by which a peripheral signal of nutritional status (leptin) could regulate CNS production of neuropeptides that modulate endocrine function and behavior.<sup>114</sup>

Leptin apparently circulates in the plasma in both free and bound forms. Multiple leptin-binding proteins have been detected by gel filtration and sucrose gradient centrifugation. The ratio of free to bound leptin is lower in obese (21.4%) than in lean (46.5%) subjects, although the absolute concentration of free leptin is higher in the obese. The molecular weights of these putative leptin-binding proteins range from 80 to 240 kd.<sup>116,117</sup> Only about 10% of bound <sup>125</sup>I-leptin in human plasma is immunoprecipitable with leptin receptor antibodies, suggesting that other proteins besides a soluble form of the leptin receptor molecule also bind leptin in the circulation.<sup>117</sup>

Short-term fasting substantially reduces the plasma concentration of free leptin and decreases the ratio of free leptin to bound leptin. In humans, 24-hour fasting lowers the free plasma leptin from 19.6 ng/mL to 1.3 ng/mL in lean subjects and 28.3 ng/mL to 14.7 ng/mL in obese subjects.<sup>117</sup> Maintenance of a hypocaloric diet (800 calories/d) reduces total plasma leptin concentration to approximately 50% of its concentration relative to weight-stable subjects with the same fat mass.<sup>118,119</sup> However, overfeeding does not increase leptin expression beyond that expected for the increase of fat mass.<sup>120</sup>

These effects of short-term fasting and overfeeding on plasma leptin concentrations, together with the biologic effects of leptin on food intake and energy expenditure, are most consistent with a physiologic model in which leptin has evolved primarily to keep body fat mass (energy reserves) at or above a set point. Caloric restriction threatens this energy reserve, triggering an immediate decrease in plasma leptin concentration, which in turn triggers a series of neuroendocrine changes designed to



increase food intake and decrease energy expenditure. Moderate increases in fat mass, with attendant increases in circulating leptin, probably have relatively small effects on this system of energy homeostasis. Larger increases, resulting from greater increases in fat mass or the exogenous administration of leptin, result in decreases in food intake and increases in energy expenditure.

This model of leptin physiology suggests that exogenous leptin may be useful in helping the formerly obese to maintain a reduced body weight by providing a leptin "signal" equal to that which preceded weight reduction.<sup>121</sup>

CONCLUSIONS

The primary physiologic role of leptin appears to be as a regulator of energy homeostasis by providing a signal to the CNS regarding the size of energy (fat) stores. This signal mediates changes in behavior and metabolism that tend to maintain body fat at a level determined by genetic, developmental, and environmental factors. The defense against lowered fat mass is much stronger than that against increased body fat. Evolutionary and experimental arguments support this conclusion. The major effects of leptin on energy metabolism are schematized in Figure 2 (on page 22).

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# Insulin, IGF-2 and Type 1 Diabetes Mellitus: Recently Implicated Genetic Loci

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Recently, great strides have been made in elucidating the genetic components of insulin-dependent diabetes mellitus (IDDM), one of the best examples of a multifactorial disease with both environmental and polygenic etiologies. This article focuses on one of the more recently implicated and best investigated loci to date, *IDDM2*. While its effects are no doubt less than that of the major histocompatibility complex, human lymphocyte antigen (HLA), *IDDM2* maps to chromosome 11p15.5, where at least 2 candidate genes are found, those for insulin (*INS*) and for insulin-like growth factor 2 (*IGF2*).

Family clustering and a high concordance rate in monozygotic twins indicate that genetic transmission of susceptibility is responsible for about half of the risk for the development of type 1 diabetes. The importance of the HLA locus, located on chromosome 6p21 (*IDDM1*), first came to light in the 1970s following association and linkage studies in affected and nonaffected siblings, and appears to account for 42% of the genetic component.<sup>1</sup> Type 1 diabetes is primarily a sporadic disease (90%); population-based (case-control) studies provided the early HLA association data. Family studies in which there were more than 1 affected child confirmed the association of specific HLA haplotypes (alleles) with type 1 diabetes (ie, the demonstration of linkage disequilibrium) and revealed preferential sharing of certain HLA haplotypes among affected sibs. The underlying biology of this linkage is not yet completely understood. It appears that the class II HLA molecules affect the immune response because of their highly polymorphic sequence variations, resulting in differences in the peptide-binding groove used in antigen presentation.<sup>2</sup>

That a locus involved in cellular immune recognition was involved in an autoimmune disease came as no surprise. However, 2 key questions remained: What other genes could play a role and how do we go about identifying these genes? Whereas reverse genetics (determination of the chromosomal location

and identification of new genes in spite of ignorance of the disease mechanism) has resulted in spectacular successes in identifying genes responsible for single-gene (mendelian) diseases, the application of this method to common polygenic phenotypes involves difficulties whose magnitude is hard to even estimate.<sup>3</sup> To date, no *novel* gene has been identified and cloned on the basis of its linkage to a complex (multifactorial) disease phenotype. Linkage can only narrow the locus to within several centimorgans (cM). In human genetic maps, 1 cM roughly corresponds to 1 million bp of DNA (1 Mb) and contains, on average, about 20 genes. Furthermore, positional cloning of mendelian disorder genes relies on the occasional patient with a large deletion/insertion or chromosomal rearrangement to further narrow the disease locus; this is not an option in complex disorders like diabetes, in which disease susceptibility is encoded not by gene-inactivating mutations but by subtle DNA sequence variants common in the general population.

Global searches of the whole genome, using hundreds of equally spaced microsatellite markers to detect linkage to specific chromosome locations, help to orient our search for disease-related candidate genes based on our knowledge of their participation in particular biochemical or cellular pathways.<sup>4,5</sup> While these studies are fraught with the statistical hazards of multiple comparisons and other methodologic controversies, they may help guide our quest for candidate genes. A disease is assigned to a chromosomal locus only after linkage has been formally demonstrated, replicated, and confirmed in at least 3 different datasets. The robustness of the linkage must be continually verified in additional datasets—preferably ones comprised of genetically homogeneous populations—and these regions need to be further saturated with markers to further define the loci. There are then association-based tests that take advantage of linkage disequilibrium (that is, the preferential association of specific marker polymorphisms with the disease) to narrow the locus to specific gene(s).

The presence of genes whose products are functionally related to the phenotype (candidate genes) in the region identified by linkage analysis can greatly accelerate this process. If no such genes exist, or if they are tried and ruled out, RNA sequences transcribed from the genetically defined interval (positional candidates) can be identified using transcript

maps of the human genome, such as the prototype recently published.<sup>6</sup> After the genes are fully cloned, polymorphisms in and around them can be identified and examined for association with a specific disease, eg, for alleles that are more frequent in diabetic than in nondiabetic subjects. However, the sequence hypervariability seen at *IDDM1*—the class II HLA locus—is the evolutionary result of adaptation to a wide variety of pathogens and is unlikely to be found in other functionally significant proteins. In most cases, subtle coding or even noncoding variants have to be examined for biologic significance. Table 1 presents a summary of loci detected by genome-wide scans for type 1 diabetes susceptibility. Linkage disequilibrium studies for *IDDM2* narrowed the locus to a variable number of tandem repeat (VNTR) polymorphisms immediately upstream of the gene for insulin; this was facilitated by an intense and systematic search for markers flanking the *INS* gene on chromosome 11p15.5. The importance of this region is well known because of its involvement in diseases other than type 1 diabetes (many tumors, including Wilms' tumors and adrenocortical tumors, and Beckwith-Wiedemann syndrome; see previous reviews in *GGH* 1994;10(1):1-4,6-10. Unfortunately, the current human genome map, in general, does not afford this high degree of marker density.<sup>7,9</sup>

## THE *IDDM2* LOCUS

Researchers suspected that insulin (acting as an antigen triggering autoimmune destruction of the pancreatic beta cell) may be the product of a candidate gene for type 1 diabetes, and linkage of this disease to a locus mapping to chromosome 11p15.5 (*IDDM2*) was established a decade ago (Figure 1). More recently, thorough association studies narrowed the locus to a polymorphism 356 bp upstream of the insulin gene (*INS*) promoter consisting of a VNTR of a 14-bp consensus sequence. A large number of alleles can be distinguished by size and by different variants of this consensus repeat unit. Most alleles in whites contain either 30 to 45 repeats (class I) or, less frequently, more than 150 repeats (class III). Intermediate (class II) alleles are rare.

Approximately 40% to 45% of whites have at least 1 class III allele, compared with <20% in diabetic patients. This suggests that class III (long) alleles are protective. This protective effect is observed in I/III heterozygotes and is, therefore, dominant. The relative risk for development of type 1 diabetes is increased 3- to 4-fold when a subject is homozygous for I/I (I/I versus I/III or III/III), and this specific polymorphism is estimated to account for 10% to 15% of the familial clustering of diabetes.<sup>10</sup>

Table 1  
Type 1 Diabetes Susceptibility Loci in Humans\*

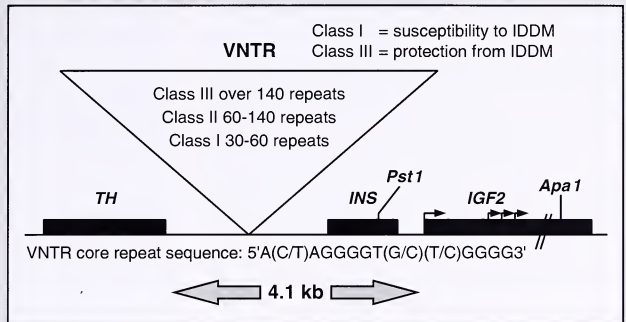
| Locus                              | Chromosomal Location | Detected in Davies Genome-Wide Scan? <sup>5</sup> | Detected in Other Datasets? <sup>†</sup> |
|------------------------------------|----------------------|---|--|
| <i>IDDM1</i> (HLA)                 | 6p21                 | Yes   | Yes                                      |
| <i>IDDM2</i> ( <i>INS</i> 5' VNTR) | 11p15.5              | Yes   | Yes                                      |
| <i>IDDM3</i>                       | 15q26                | Yes   | Yes                                      |
| <i>IDDM4</i>                       | 11q13                | Yes   | Yes                                      |
| <i>IDDM5</i>                       | 6q25                 | Yes   | Yes                                      |
| <i>IDDM6</i>                       | 18q21                | Yes   | Yes                                      |
| <i>IDDM7</i>                       | 2q31                 | No  | Yes                                      |
| <i>IDDM8</i>                       | 6q25-q27             | Yes   | Yes                                      |
| <i>IDDM9</i>                       | 3q21-q25             | No  | Cited in Todd <sup>4</sup>               |
| <i>IDDM10</i>                      | 10p11.2-q11.2        | Yes   | Yes                                      |
| <i>IDDM11</i>                      | 14q24.3-q31          | No  | Yes                                      |
| <i>IDDM12</i> ( <i>CTLA-4</i> )    | 2q33                 | No  | Yes                                      |
| <i>IDDM13</i>                      | 2q34                 | No  | Yes                                      |
| <i>IDDM15</i> (distinct from HLA)  | 6p21                 | No  | Yes                                      |
| Not assigned ( <i>GCK</i> )        | 7p                   | No  | Yes                                      |
| Not assigned                       | Xq                   | No  | Cited in Todd <sup>4</sup>               |

The *IDDM* nomenclature is officially assigned to a locus after linkage has been formally demonstrated, replicated, and confirmed in at least 3 independent datasets.  
<sup>†</sup> References to specific loci can be obtained by consulting *Online Mendelian Inheritance in Man* (<http://gdbwww.gdb.org/omim>).

Adapted with permission from Todd JA. *Proc Natl Acad Sci USA* 1995; 92:8560-8565.

Figure 1  
The *IDDM2* Locus

The *IDDM2* locus, located on human chromosome 11p15.5, has been mapped to a variable number of tandem repeat sequences lying 3' to the tyrosine hydroxylase gene (*TH*) and 5' to the insulin gene (*INS*) and its close (less than 2 kb) neighbor, the gene for insulin-like growth factor 2 (*IGF2*), whose 4 promoters are indicated by the arrows. Useful RFLP exon polymorphisms to study allele-specific transcription are noted for *INS* (*Pst1*) and *IGF2* (*Apa1*). Note that the *IGF2* gene is expressed from the paternal gene copy in most tissues, whereas the other imprinted genes at the 11p15.5 locus (not shown) are expressed by the maternal gene copy (these include centromeric to *TH*: *p57KIP2* and *ASCL2*; telomeric to *IGF2*: *H19*).



## FROM GENETICS TO GENE FUNCTION

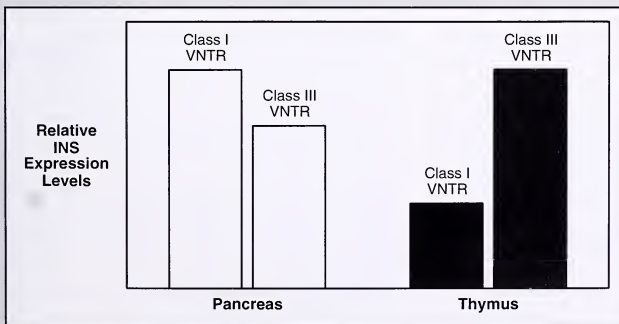
What biologic mechanism might account for this effect of the VNTR on diabetes susceptibility? Since the *INS* VNTR does not encode a protein sequence, it must exert transcriptional effects on nearby genes. Transcriptional effects of VNTRs elsewhere in the genome have been found, eg, those reported for *HRAS*<sup>11</sup> (Harvey rat sarcoma virus proto-oncogene), a membrane-associated small guanine triphosphate (GTP)-binding protein believed to participate in the development and/or maintenance of certain malignancies. Although *INS* may be the principle target of its 5' VNTR, this VNTR also

could affect transcription of *IGF2*, the gene encoding insulin-like growth factor 2, whose promoters are 5 to 24 kb downstream (Figure 1), a distance compatible with enhancer effects. Therefore, elucidation of the *IDDM2* mechanism requires a systematic search for allelic effects on transcription levels of genes that are in close proximity to the VNTR in tissues of known importance in diabetes.

## *INS* AS A CANDIDATE GENE FOR *IDDM2*

Recent studies have demonstrated that the VNTR does indeed exhibit tissue-specific allelic effects on *INS* transcription in vivo and in vitro (Figure 2).<sup>12-17</sup>

Figure 2



Schematic summarizing VNTR effects on *INS* mRNA levels in fetal tissue, genotyped for allele class. Data for fetal pancreas are adapted from Vafiadis et al.<sup>15</sup>; those for thymus are adapted from Vafiadis et al.<sup>16</sup>



Since the pancreas is the major tissue (and initially thought to be the only tissue) in which physiologically significant levels of insulin are produced, it was an important tissue to examine. To distinguish between mRNA from each gene copy in heterozygous samples of human fetal pancreas, a transcribed *Pst*I polymorphism within the 3' untranslated region of *INS* was used. By using this technique, pancreatic *INS* mRNA levels were found to be 15% to 20% lower from chromosomes bearing the class III VNTR than from those bearing the class I VNTR, easily genotyped by polymerase chain reaction (PCR).<sup>15</sup> Similar findings have been reported by others in adult pancreas.<sup>12</sup> Although statistically significant, this marginal loss of function is unlikely to account for the protective effect of class III VNTR, whose dominant nature suggests gain of function.

Mouse thymus expresses insulin<sup>18</sup> and many otherwise tissue-specific proteins (such as peptide hormones and exocrine pancreatic enzymes), presumably for the purpose of immune tolerance development. Human thymus also expresses insulin as mRNA and protein, albeit at levels  $10^3$  to  $10^4$  times lower than those in the pancreas. These observations prompted a search for *INS* VNTR allele-specific effects on thymic *INS* mRNA levels in 12 samples of human fetal thymus that were heterozygous at the VNTR (I/III) and informative for the transcribed *Pst*I polymorphism. Unlike pancreas, the thymic insulin mRNA value from VNTR class III haplotypes was 2- to 3-fold higher than from class I in 10 of the 12

samples ( $P < 0.007$ ).<sup>16</sup> Since antigen effects on thymocyte selection are dose-dependent,<sup>19</sup> this increased *INS* expression from VNTR class III chromosomes (ie, gain of function) might enhance thymic tolerance to insulin, thus explaining the dominant protective effect of this polymorphism. This is in favor of insulin being the *IDDM2* gene, but does not rule out the possibility that *IGF2* is involved, either in addition to *INS* or in place of *INS*.

### IGF2 AS A CANDIDATE GENE FOR IDDM2

The reasons to consider the product of *IGF2* in the pathogenesis of type 1 diabetes are summarized in Table 2. It is well appreciated that the IGF axis is important in lymphopoiesis and immune function.<sup>20,21</sup> IGF-2 is synthesized in and has biologic effects on tissues that are important in the pathophysiology of diabetes, such as pancreas, thymus, and lymphocytes. It is a particularly important fetal mitogen that has been shown to stimulate proliferation and/or prevent apoptosis. It has been hypothesized that IGF-2 may either function as a tolerogenic autoantigen in the thymus<sup>22</sup> or promote survival of self-reactive lymphocytes.<sup>20</sup> Involvement of IGF-2 in the pathogenesis of type 1 diabetes could explain parental imprinting effects reported in the genetics of *IDDM2*. Imprinting refers to differential transcriptional behavior (typically silencing) of a gene copy on the basis of the sex of the parent from whom it was inherited,<sup>23</sup> such as has been demonstrated for *IGF2* in some

Table 2  
Arguments Supporting a Role for *IGF2* in Type 1 Diabetes Pathophysiology

#### Physiologic Arguments\*

1. IGF-2 and type I IGF-1 receptor (mitogenic receptor) expressed in human fetal pancreas, thymus, and leukocytes
2. Type 2 IGF-2 receptor (involved in IGF-2 clearance) highly expressed in fetal thymus; suggests modulating IGF-2 levels may be important
3. IGF-2 transgenic animals show changes in lymphoid tissue, including clonal expansion of thymocytes, mainly mature CD4+ cells
4. IGF-2 known to promote cell survival (anti-apoptosis factor)

#### Genetic Arguments†

1. *INS* and *IGF2* mapped to within 2 kb of each other, a distance compatible with shared enhancer effects
2. Type 1 diabetes susceptibility in offspring of diabetic parents is related to paternal transmission (in some populations), suggesting imprinting effects
3. *INS* is not imprinted in human fetal pancreas; *INS* also is biallelically expressed in the majority of fetal thymi
4. *IGF2* is imprinted and expressed from the paternal allele in fetal pancreas and thymus
5. Placental *IGF2* mRNA levels are correlated with VNTR class
6. The 5' *INS* VNTR has transcriptional effects on *IGF2* (artificial constructs)

\*See Polychronakos et al<sup>20</sup> for literature review

†References given in text.

tissues in the human fetus and in the human placenta.<sup>24</sup> In contrast, *INS* is not imprinted in human fetal or adult pancreas,<sup>12,20,25</sup> despite the imprinted expression of the mouse insulin genes, *Ins1* and *Ins2*, in yolk sack, where only the paternal copies are transcribed.<sup>26</sup>

Linkage<sup>27</sup> or association at *IDDM2* has been reported in alleles of paternal<sup>28-30</sup> or maternal<sup>12,25</sup> origin, but other studies have found no parent-of-origin effect.<sup>31</sup> In addition to the intriguing mechanistic questions they raise, these parent-of-origin effects may provide clues as to the genes and biologic mechanisms involved at the *IDDM2* locus. Paternal bias would favor *IGF2* because of its exclusive paternal expression; an explanation for the maternal bias is less obvious, unless one postulates longer-range VNTR effects on imprinted, maternally expressed genes at the 11p15.5 locus.

We have recently demonstrated that higher steady-state *IGF2* mRNA levels are associated with paternal class I VNTR alleles in normal human placenta, a tissue in which *IGF2* is exclusively expressed from the paternal allele. Furthermore, by using a construct in which a class I or class III VNTR is placed upstream of an important fetal *IGF2* promoter (P3), greater reporter gene activity is observed with the class I VNTR allele. The physiologic significance of this could relate to the anti-apoptotic effect of IGF-2 on self-reactive T cells during fetal life, whereby the class I VNTR alleles, via increased *IGF2* transcription, favor the development of diabetes because of survival of self-reactive lymphocytes.<sup>32</sup>

It should be stressed that dividing VNTR alleles into classes affords only a broad categorization, as each class contains many distinct alleles of varying sizes. It is possible that expression of *IGF2* and/or *INS* associated with particular VNTR alleles also is affected differently by parental imprinting, known to be a tissue-specific, developmentally-specific, and polymorphic phenomenon.<sup>23</sup> In the case of *INS*, the higher thymic transcript levels from the class III haplotype seen in 10/12 specimens is present independently of the parental origin of the class III. However, imprinting is likely involved in the complete silencing of the class III allele in the remaining 2/12 thymi.<sup>16</sup> Pugliese et al<sup>17</sup> also found monoallelic *INS* expression in 3/10 thymi. In all 5 cases, it was the class III haplotype that was silenced. Therefore, it is possible that imprinting of thymic *INS* in this minority of individuals requires the presence of specific class III alleles. A precedent for such haplotype-restricted imprinting has been described: the polymorphic silencing<sup>33</sup> of the paternal copy of *IGF2R* (the IGF-2 receptor gene) is controlled by a sequence variant in cis.<sup>34</sup> Imprinted *IGF2* expres-

sion might be modulated by a similar phenomenon in leukocytes, where there is variable "relaxation" of imprinting, thereby allowing transcription from the maternal copy to a variable extent in different individuals.<sup>15,20</sup> Dependence of relaxation on specific VNTR alleles could explain the maternal effect, if *IGF2* were involved in the *IDDM2* effect instead of (or in addition to) *INS*.

## CONCLUSIONS AND FUTURE DIRECTIONS

Genetic evidence and functional considerations point to *IGF2* and/or *INS* as the *IDDM2* gene(s). Our knowledge of how *IDDM1* and *IDDM2* contribute to the diabetes phenotype continues to increase and is far in advance of our understanding of the many additional loci recently implicated. Furthermore, while the specific genes involved at these other loci are unknown at present, it is of interest that several genes coding for products that interact with IGF-2 can be found within some of them, such as those coding for the type 1 (15q26) and type 2 (6q27) IGF receptors and the IGF-binding proteins (2q34, 7p13). Is this mere chance, or does it explain some of the epistatic genetic interactions, whereby these loci are not independent but may be acting on the same or overlapping pathways involved in the pathophysiology of diabetes? With the speed at which technologic advances have accelerated our understanding of diabetes in the past 5 years, one can hope to see these questions answered in the not too distant future.

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## Congenital Leptin Deficiency Is Associated With Severe Early-Onset Obesity in Humans

The investigators report that 2 consanguineous, first-cousin offspring with hyperphagia and morbid obesity beginning in infancy had a homozygous abnormality of the *OB* gene encoding leptin, the appetite-regulating protein secreted by the fat cell. Each child had a homozygous deletion of 1 guanine nucleotide in codon 133 leading to a frameshift mutation and 14 altered amino acids. This truncated leptin protein could be synthesized but not secreted from Chinese hamster ovary (CHO) cells transfected with the mutant gene, and both children had extremely low serum immunoreactive leptin levels. They were hyperinsulinemic and normocortisolemic. The parents of both children and 1 sibling were heterozygous for this mutation; their serum leptin levels were normal, as was their body fat content.

Montague CT, et al. *Nature* 1997;387:903-908.

**Editor's comment:** These observations directly demonstrate for the first time the importance of leptin in appetite regulation in humans. All the obese subjects studied previously have not had an abnormality of genes encoding leptin or its receptor. The data also reveal that the heterozygote with 1 abnormal *OB* is normal. The phenotype of these children with hyperphagia, obesity, and hyperinsulinemia was quite similar to that of the

*ob/ob* mouse. It differed from the animal model in that the linear growth of the children was normal (75th percentile for chronologic age; no bone age data given), and they were not hypercortisolemic.

Jackson et al described a patient with childhood-onset obesity with dual mutations in the gene encoding prohormone convertase 1 (*PC1*), an endopeptidase necessary for prohormone processing. The patient was a compound heterozygote. One *PC1* gene contained a Gly483Arg mutation that caused trapping of the gene product within the endoplasmic reticulum. The other *PC1* gene had an A→C transversion in the donor splice site of exon 5, leading to deletion of this exon and a frameshift that resulted in a premature stop codon and truncated *PC1* protein. The investigators hypothesized that loss of *PC1* activity led to impaired processing of many protein prohormones, including neuropeptides involved in appetite regulation such as  $\alpha$ -melanocyte-stimulating hormone and glucagon-like peptide 1.

We truly are on the threshold of understanding the relationship between leptin, genetics, and obesity.

Allen W. Root, MD

Jackson RS, et al. *Nat Genet* 1997;16:303-306.

## Relationship Between Serum Leptin Concentration and Fetal Growth

Two recent articles in the *Journal of Clinical Endocrinology and Metabolism* concerned the relationship between the concentration of serum leptin and fetal growth.

Harigaya et al elucidated the role of leptin in the fetus. Blood samples from 116 infants were analyzed within 6 hours after birth. There was no difference in the concentration of leptin found in umbilical cord sera and infants' sera obtained within that 6-hour period. Ninety-one of these infants were term; 44 were classified as AGA (birth weight appropriate for gestational age); 28 were LGA (birth weight large for gestational age); and 19 were SGA (birth weight small for gestational age). Twenty-five were preterm. Infants with dysmorphic features, intrauterine infections, organic disorders, or chromosomal anomalies were excluded. Blood samples were compared with 28 umbilical cord samples taken at birth from the term group and 25 samples from healthy adults. Serum concentration of leptin and insulin levels were determined by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), respectively. Follow-up samples were taken from 48 of the 116 infants between 2 and 7 days of life. Leptin levels in term AGA infants were significantly lower than those of normal adults. The serum leptin concentration in LGA infants was significantly higher ( $12.8 \pm 10.2$  ng/mL) and those in SGA infants ( $1.6 \pm 1.1$  ng/mL) was significantly lower than in AGA

infants ( $P < 0.01$ ). The follow-up leptin concentration in 48 term infants in the LGA and AGA groups dramatically decreased within 48 hours of delivery; the leptin concentration did not change in the SGA group. A positive correlation was found between leptin concentrations within 6 hours of life and birth weights ( $r = 0.59$ ,  $P < 0.01$ ). Leptin levels within 6 hours of life positively correlated with gestational age. The authors concluded that serum levels of leptin correlate with the fetal body weight gain.

Koistinen and colleagues looked at leptin concentrations in cord blood to see if there was a correlation with intrauterine growth. To determine how fetal growth compares with leptin levels at birth, 50 full-term infants were studied (28 = AGA; 9 = LGA; and 13 = SGA). Blood samples to measure leptin and insulin levels were taken from umbilical cord at birth. Amniotic fluid samples were obtained by amniocentesis from 10 mothers within 1 to 8 days before delivery and from 20 mothers at the time of Cesarean section. Umbilical leptin levels were higher in LGA infants ( $35.7 \pm 8.0$   $\mu$ g/L;  $P < 0.005$ ) but lower in the SGA infants ( $3.3 \pm 0.05$   $\mu$ g/L;  $P < 0.001$ ) than in AGA infants ( $14.5 \pm 2.8$   $\mu$ g/L;  $P < 0.005$ ). Cord leptin levels correlated with birth weights, cord insulin concentrations, placental weight, and amniotic fluid leptin concentrations. Leptin concentrations in amniotic fluid were higher in LGA infants



than in AGA infants ( $4.8 \pm 0.7 \mu\text{g/L}$  vs  $3.1 \pm 0.5 \mu\text{g/L}$ ;  $P < 0.03$ ). The authors concluded that the strong relation between body weight and leptin concentration at term suggests that fatty mass is a major determinant of leptin secretion in utero.

Harigaya A, et al. *J Clin Endocrinol Metab* 1997;82:3281-3284.  
Koistinen HA, et al. *J Clin Endocrinol Metab* 1997;82:3328-3330.

**Editor's comment:** Although the physiologic role of leptin levels in utero is not completely understood, these 2 papers report new data on leptin levels at birth, their correlation with fat mass, and their postnatal decline during the first week of life.

The strong positive correlations found between serum leptin level and body weight gain in utero underscore the importance of this peptide as a marker of fetal growth. Thus, leptin could

be useful as a predictive factor of fetal outcome, although further studies need to be done to ascertain this fact. Insulin and leptin levels do not correlate significantly in Harigaya's study, suggesting different mechanisms of fetal growth modulation by these 2 growth factors in utero.

Of interest is that both groups used the same assay but did not get the same results for AGA infants. In Koistinen's paper, the figure of  $14.5 \pm 2.8 \mu\text{g/L}$  was given, but in Harigaya's paper the value was  $4.4 \pm 3.0 \mu\text{g/L}$ . The reason for this discrepancy is not apparent.

These papers supplement the lead article on leptin by Zhang and Leibel in this issue of GGH as Zhang and Leibel did not have the opportunity to present data on intrauterine growth and leptin levels.

Fima Lifshitz, MD

## Growth, Genetics, and Cancer

There is an undeniable relationship between cancer, growth, and genetics. Paraphrasing Eric R. Fearon, cancer is a genetic disease that arises from the accumulation of mutations that promote selection of clones of cells that display increasingly aggressive growth characteristics. Much of what is known about cancer genetics has come from studying hereditary cancer syndromes. Even though they collectively represent only about 1% of cancers, they have provided much insight into the pathogenetic mechanisms that give rise to cancer.

Fearon has recently examined 22 different hereditary cancer syndromes from a gene product functional perspective. Moreover, he has done this in the context of key cellular processes, such as cell proliferation, differentiation, apoptosis, and maintenance of genomic integrity. Thus, he organizes the syndromes into several functional categories. For example, several of the proteins are transmembrane receptors (proteins encoded by *MET*, *PTCH*, *RET*). Others are cytoplasmic regulatory or structural proteins (proteins encoded by *NF1*, *PTEN*, *APC*, *NF2*), transcription factors or regulators (proteins encoded by *p53*, *WT1*, *RB1*, *VHL*), or cell cycle regulators (proteins encoded by *CDK4*, *p16*). Finally, many proteins are involved in repair of DNA damage (proteins encoded by *MSH2*, *MLH1*, *PMS2*, *ATM*, *BRCA1*, *BRCA2*, *FACC*, *FACA*, *XPA*, *XPB*, *XPD*, *BLM*).

Several interesting observations come from this analysis. For instance, when genetic heterogeneity has been found, ie, hereditary nonpolyposis colorectal cancer, inherited melanoma, and familial breast cancer, all of the implicated genes function in a conserved pathway. For example, *MSH2*, *MLH1*, and *PMS2* in patients with hereditary nonpolyposis colorectal cancer adversely affect DNA mismatch recognition and repair.

One of the puzzling observations is that cancers are limited to certain tissues in most syndromes, yet the genes are widely expressed. It is suggested that many of the implicated genes

function in interesting or overlapping pathways that branch and converge differently in different cell types. Another explanation is that genes simply may have different functions in different cell types. Fearon emphasizes that other factors, such as other genes, diet, environment, and lifestyle, substantially affect the expression of cancer in mutation carriers.

Fearon ER. *Science* 1997;278:1043-1050.

**Editor's comment:** This excellent review puts a different slant on hereditary cancer syndromes. It not only organizes information from 10 years of literature concerning cancer syndromes but also presents the material in a functional context that allows one to create a big picture of how the syndromes relate to one another and to normal biologic processes.

William A. Horton, MD

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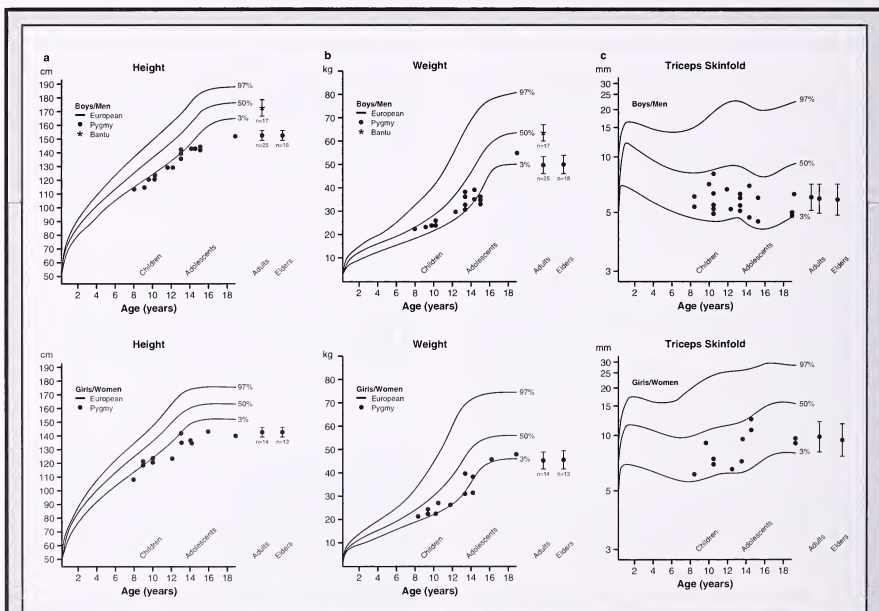
## Dissociation of Systemic GH-IGF-1 Axis From a Genetic Basis for Short Stature in African Pygmies

The investigators tested the hypothesis that the primary cause of short stature in African Pygmies resides in low levels of insulin-like growth factor 1 (IGF-1) and evaluated whether any observed alterations in their systemic IGF-1 status could be associated with malnutrition and/or altered immune status. Extensive serum assays and auxologic measurements were done for purposes of evaluating the hormonal and immune status versus the phenotypes of children, adolescents, and young and old adults. None had overt clinical or biochemical signs of malnutrition. Bantus living in the same equatorial rain forest of eastern Cameroon were used as controls.

Pygmies did not differ from Europeans or Bantus in mean serum IGF-1 or IGF-binding protein 3 (IGFBP-3) levels. How-

ever, in both African groups, IgG, IgM, IgE, C-reactive protein, and ceruloplasmin were above normal for Europeans, but the Pygmies had much higher IgG and IgM levels than the Bantus. Low IGF-1 levels were inversely associated with serum levels of IgG and IgM.

The authors concluded that in growing and adult African Pygmies without evidence of clinical or biochemical signs of nutritional deficiency, serum IGF-1 and IGFBP-3 are essentially normal. They believe that these studies disprove the previous hypothesis that there is a defect in IGF-1 production or growth hormone insensitivity, and that the short stature of African Pygmies is unrelated to a genetically determined lesion that becomes unmasked at puberty to suppress the surge in circulating IGF-1 levels. They suggest that much of the



Height (panel a), weight (panel b), and triceps skinfold thickness (panel c) plotted against age for African Pygmies are shown with a reference European (British) population (Tanner et al, 1966 a,b; Tanner & Whitehouse, 1975). Data for boys/men and for girls/women are shown on top and bottom part of the figure, respectively. For each anthropometric measurement, individual values are shown for children and adolescents, while mean and SD are shown for adults and elders; in addition the mean and SD values for the Bantu men are indicated using an asterisk symbol. Note that the heights of all Pygmies are below or close to the 3rd percentile of the European standard.

Reprinted with permission from Dulloo AG, et al. Dissociation of systemic GH-IGF-I axis from a genetic basis for short stature in African Pygmies. *Eur J Clin Nutr* 1996;50(6):371-380.

growth retardation of Pygmies may be due to excessive exposure to infections with resultant elevation of immunoglobulins, in spite of absent gross nutritional deficiency secondary to infection.

Dulloo AG, et al. *Eur J Clin Nutr* 1996;50(6):371-380.

**Editor's comment:** This study is very nicely executed, and extensive data are presented to raise much skepticism about the

previous hypothesis that an increase in IGF-1, which is normally associated with the adolescent growth spurt, does not occur in Pygmies. The data not only negate the former hypotheses but also reveal a new biochemical alteration (elevated immunoglobulins) that, in my opinion, may or may not be associated with growth retardation. This article was kindly brought to my attention by Dr. Guy Van Vliet.

Robert M. Blizzard, MD

## Nonadipose Tissue Production of Leptin: Leptin as a Novel Placenta-Derived Hormone in Humans

The authors report that leptin, the appetite-regulating polypeptide hormone secreted by adipocytes, also is synthesized and secreted by human placental chorionic villi and amnion cells. They demonstrated that plasma leptin concentrations were higher in pregnant women than in nonpregnant women of comparable body mass index (BMI), a finding made by other investigators. Whereas in nonpregnant women plasma leptin concentrations and BMI were directly related, this relationship was not demonstrable in pregnant subjects. Leptin values

declined rapidly after birth. Umbilical vein leptin concentrations were slightly higher than umbilical artery values. Leptin was detectable in amniotic fluid. Plasma leptin values were elevated in patients with hydatidiform moles and choriocarcinoma and fell rapidly with surgical removal or chemotherapeutic ablation of the tumor.

Expression of *ob*, the leptin gene, was demonstrated in placental chorionic villi and amnion. Immunoreactive leptin was present in trophoblasts of first trimester chorionic villi and syncytiotrophoblasts and amnion cells in the third trimester. The investigators suggest that placental leptin may be of physiologic importance in modulating the metabolic relationship between mother and fetus. Plasma leptin values may serve as a marker of tumors of placental origin.

Masuzaki H, et al. *Nat Med* 1997;3:1029-1033.

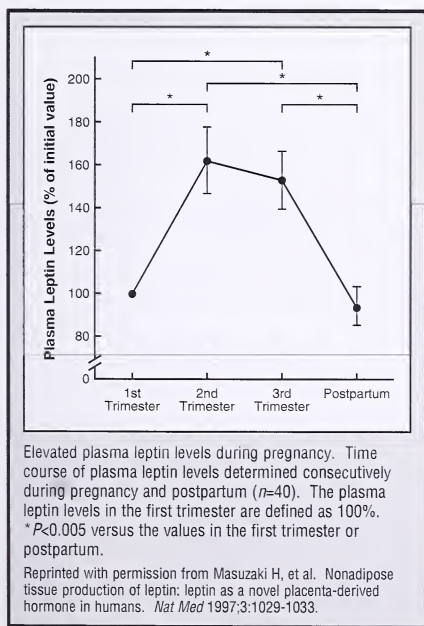
**Editor's comment:** This paper reports that leptin may be secreted by nonadipose placental cells, namely, placental trophoblasts and syncytiotrophoblasts, and secreted into amniotic fluid and the maternal circulation. Thus, leptin is another of the many protein hormones that are secreted not only by their primary tissue but also by the placenta. Placental leptin may modulate metabolic homeostasis in the pregnant woman and fetus. One wonders if, in the pregnancy complicated by placental insufficiency and intrauterine growth retardation, leptin deficiency may permanently alter hypothalamic appetite regulatory mechanisms that influence the postnatal growth of that individual.

Chehab points out that the elevated leptin concentrations in pregnant women imply an element of leptin resistance because appetite is not suppressed by pregnancy. Although these investigators did not note correlation between BMI and serum leptin concentrations in pregnant women, Hartmann et al reported that the 2 were highly correlated.

Allen W. Root, MD

Chehab FF. *Nat Med* 1997;3:952-953.

Hartmann BW, et al. *N Engl J Med* 1997;337:863.



## A Dominant-Negative Mutation of the Growth Hormone Receptor Causes Familial Short Stature

Ayling and colleagues report the identification of a new dominant-negative mutation in the growth hormone receptor (GHR) in a mother and daughter with short stature. This mutation (876-1 G→C transversion affecting the 3' splice acceptor site preceding exon 9) was not detected in maternal grandparents. The mutation results in a premature stop codon or a truncated GHR. The GHR belongs to a cytokine family of receptors that depend on JAK tyrosine kinases for activation. It is predicted that a truncated receptor would be incapable of association with JAK and, therefore, would have a dominant-negative effect on the GHR.

The clinical significance of this mutation relates to the phenotypic differences of the individuals from those with the Laron syndrome, in which midfacial hypoplasia, blue sclera, limited elbow extension, hypoglycemia, truncal adiposity, etc are often observed. In the latter, the mutations of the GHR have been primarily in the extracellular domain. In the 2 patients reported here, the GHBP, the extracellular portion of the GH binding receptor, was normal. The authors suggest that this new dominant GHR mutation should be looked for in children with familial short stature who have normal

GHBP (as opposed to low GHBP, which triggers the search for GHD genetic abnormalities currently), a group of children who were previously felt to have no known endocrine cause of their short stature.

Ayling RM, et al. *Nat Genet* 1997;16:13-14.

**Editor's comment:** This is an exciting contribution to the rapidly growing fund of information regarding the molecular causes of growth failure in children. It is not uncommon for a pediatric endocrinologist to be faced with extremely short children for whom no endocrinopathy can be identified. The work by Ayling and colleagues describes an additional genetic mutation that could present as growth hormone insensitivity syndrome. We look forward to studies of other families in hopes of determining the clinical magnitude of this new finding. The implications for potential treatment with insulin-like growth factor (IGF-1) are obvious, although the availability of IGF-1 as a therapeutic agent seems far distant in the future.

William L. Clarke, MD

## Androgen Insensitivity With Mental Retardation: A Contiguous Gene Syndrome?

A contiguous gene syndrome is the combination of clinical features resulting from a microdeletion of chromosomal DNA involving 2 or more contiguous gene loci. The location for the androgen receptor gene is Xq11-q12. Davies et al have identified a syndrome involving mental retardation and androgen insensitivity at Xq11.2-q12 between DXS1 and DXS905. Two affected individuals are reported with complete androgen insensitivity. This suggests that a gene for nonspecific mental retardation lies very close to the androgen receptor gene. They analyzed 4 patients with androgen insensitivity, 2 of whom also had mental retardation. Deletion analysis of the 2 individuals with mental retardation showed that the deletion extended past the androgen receptor gene in both directions, whereas in the individuals without mental retardation, the deletion was limited to the androgen receptor gene itself.

Androgens (testosterone and dihydroepiandrosterone) are steroid hormones secreted by the adrenal cortex that promote male sexual differentiation. The androgenic effect is mediated by the intracellular androgen receptors. Certain androgens bind to the androgen receptor and cause masculinization of the developing male fetus. A defect in the androgen receptor gene results in androgen insensitivity, which is a disorder of male sexual differentiation. This occurs when the target tissues fail to respond to the male sex hormones (androgens),

ie, the receptors are insensitive or resistant because of a defect in the androgen receptors (mutations). Androgen insensitivity syndrome can be complete or partial. In complete

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androgen insensitivity, the individuals have a normal male karyotype (46,XY), testes, and external genitalia like those of females (female phenotype). In partial androgen insensitivity syndrome, the receptor affinity is decreased for the ligand. The phenotype is extremely variable.

Davies HR, et al. *J Med Genet* 1997;34:158-160.

**Editor's comment:** Deletion analysis of the androgen receptor gene has improved our understanding of the causes of androgen insensitivity. More than 150 point mutations have been reported so far. The phenotype is extremely variable, probably reflecting the heterogeneity of the androgen receptor gene mutations. One cannot predict the phenotype based on these mutations because the same mutation may be associated with

a different phenotype, suggesting that other modifying genes play a role in androgen response. Mutations in the androgen receptor gene have been described in a number of clinical situations, including male infertility, prostate cancer, breast cancer, and Kennedy's disease. It is of particular interest that patients with androgen insensitivity and mental retardation have large deletions. There are many genes for mental retardation on the X chromosome; however, these 2 patients suggest at least 1 mental retardation gene is very close to the androgen receptor. Mental retardation seen in the other clinical situations involving the androgen receptor gene also may suggest contiguous gene deletions.

Judith G. Hall, MD

## Growth Pattern During the First 36 Months of Life in Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency)

Gasparini et al followed 17 female and 7 male infants with congenital adrenal hyperplasia due to 21-hydroxylase deficiency from diagnosis until 36 months of age. All were initially treated with cortisone acetate (25 mg/m<sup>2</sup>/d in 3 divided doses given q8h) and 9 $\alpha$ -fluorohydrocortisone (0.05 mg q12h). Every 3 months for the first 12 months and every 6 months thereafter, the height was recorded as length for chronologic age (CA) and ponderal growth as percentage of ideal body weight (IBW). Annual bone ages (BA) were obtained. At diagnosis, males tended to be less compromised in linear growth than females (standard deviation score for less [SDS-L]  $-0.5 \pm 0.7$  vs  $-1.1 \pm 1.1$ ) but were significantly more compromised in weight ( $76.3 \pm 16.7\%$  IBW vs  $91.7 \pm 8.0\%$ ,  $P < 0.05$ ). By 3 months of age, the females' percentage of IBW remained unchanged; that of males normalized and remained similar to females through the next 36 months of observation. SDS-L at 3 months in females increased to  $0.41 \pm 0.88$  ( $P < 0.005$ ) and remained constant thereafter. Males, on the other hand, showed a significant and progressive decrease in SDS-L at 6 months ( $-1.41 \pm 0.96$ ;  $P < 0.05$ ), but from that point on showed a progressive increase, reaching and maintaining normal values by 18 to 24 months of age. No differences were noted between males and females with regard to 17-hydroxyprogesterone (17-OHP) levels or the BA:CA ratio, which approximated 1. 17-OHP levels were distributed over a wide range, and in all patients a correlation was found between SDS-L for target height and the SDS-L at 2 years and 3 years. In particular, the SDS-Ls of both males and females at 3 years were comparable to that of the midparental height of their parents.

The authors point out that the traditional treatment of 21-hydroxylase deficiency has included cortisone acetate, 25 mg/m<sup>2</sup>/d. However, the decrease observed in linear growth

in the males was interpreted as possible overtreatment; thus, the cortisone acetate dose was decreased despite elevated levels of 17-OHP. Twelve of the patients were followed longitudinally until 7 years of age. Those individuals maintained a BA:CA ratio between  $0.83 \pm 0.19$  and  $1.01 \pm 0.29$  despite cortisone acetate doses between  $15.9 \pm 6.0$  mg/m<sup>2</sup>/d and  $20.0 \pm 8.0$  mg/m<sup>2</sup>/d. These patients reached a height that correlated with their predicted adult height despite inadequately suppressed 17-OHP, at least for the first 7 years of life.

Gasparini N, et al. *Horm Res* 1997;47:17-22.

**Editor's comment:** This is an important and interesting paper. It should provide encouragement to those who utilize cortisone acetate replacement therapy at doses of  $<25$  mg/m<sup>2</sup>/d in attempts to maintain normal linear growth in their patients despite elevated 17-OHP levels. The reader is referred to a recent review (*J Clin Endocrinol Metab* 1996;81:3180-3191) on the use of adrenalectomy as treatment for congenital adrenal hyperplasia and for further discussion of a variety of

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different treatment options available for patients with this disorder. Unfortunately, in the United States, the major supplier of injectable cortisone acetate has discontinued its production, and pediatric endocrinologists will be forced to become familiar with other glucocorticoid agents or other forms of treatment.

Gasparini et al did not report on any adverse clinical events during times of physiologic stress that may have occurred in patients receiving <25 mg/m<sup>2</sup>/d. Such information would be important to have prior to making recommendations regarding their proposed therapeutic regimen.

William L. Clarke, MD

**2nd Editor's comment:** The reader also is referred to an article by Kerrigan et al (*J Clin Endocrinol Metab* 1993;76:1505-1510), reporting that the production rate of hydrocortisone is less than that calculated by Migeon's group (approximately 6.1 mg/m<sup>2</sup>/d vs approximately 12 mg/m<sup>2</sup>/d). It is not surprising, therefore, that a dose less than twice the production rate (25 mg/m<sup>2</sup>/d), which is the dose previously accepted by endocrinologists as being a suppressive dose in congenital adrenal hyperplasia, may be more than necessary to adequately treat the disorder.

Robert M. Blizzard, MD

## Opitz Syndrome Gene Found

Opitz syndrome, which was originally described as G and BBB syndromes, is characterized by midline defects, including hypertelorism, hypospadias, lip/palate/laryngotracheal clefts, and imperforate anus. Clinically indistinguishable forms have been genetically mapped to the X (Xp22) and 22 (22q11.2) chromosomes. A consortium of several groups (see references) headed by Andrea Ballabio in Milan has now found the gene that harbors the mutations responsible for the X-linked form.

After first constructing a physical map of the Xp22 breakpoint region, the investigators next identified expressed sequences from which they were able to assemble a consensus cDNA sequence of 3,452 bp. The predicted protein product is 667 amino acids, which was named MID1. Expression of the *MID1* gene was then studied. Transcripts were found in virtually all normal fetal tissues examined, especially kidney, brain, lung, and placenta, and in the heart and brain of adults. No transcripts were detected in samples from an affected male. Studies in the mouse revealed that *mid1* is expressed ubiquitously in early embryos, with the highest levels found in the first and second branchial arches.

The sequence of predicted protein places it in the so-called B-box family of zinc-finger proteins. These proteins contain a "RING-finger" and 2 "B-box" domains, which are thought to mediate protein-protein interactions. Genes belonging to this family encode transcriptional regulators.

Mutation analyses were carried out in patients from 22 independent families; mutations were found in 4 families. The gene was disrupted by the pericentric inversion in the family used for mapping. The other mutations were an in-frame 3-bp deletion, a frameshift that produces a premature stop codon, and a tandem duplication of 24 bp. All are predicted to disrupt the function of the protein.

The authors conclude that the *MID1* encodes a protein whose function is important for development of midline structures.

Green EA, et al. *Science* 1997;278:615-630.  
Henikoff S, et al. *Science* 1997;278:609-614.  
Quaderi NA, et al. *Nature Genet* 1997;17:285-291.  
Tatusov RL, et al. *Science* 1997;278:631-637.

**Editor's comment:** Genes relevant to early human development are being discovered at a rapid pace, primarily from positional cloning of "birth defect syndrome" genes. This is very exciting, but it is difficult to keep track of the different genes and mutations. The situation is analogous to the chondrodysplasia situation in the early 1990s, when there were well over 100 distinct disorders, multiple ways of classifying them, and considerable debate over grouping versus separating conditions with subtly different clinical features. Fortunately, molecular genetics helped to sort out the situation by revealing that a large portion of these disorders fell into a relatively small number of "chondrodysplasia families" that shared common genetic origins.

The situation is more complicated when the entire spectrum of birth defects is considered. However, attempts are now being made to "organize" genes into families, which will likely lead to a reconsideration of how these syndromes are classified and managed nosologically. This move to better organize genetic information into a family context was very evident at the 1997 American Society of Human Genetics meetings in Baltimore, as well as in the 1997 Genomics issue of *Science*. These efforts should eventually help keep clinicians from becoming lost in the maze of molecular genetic information.

William A. Horton, MD

## In The Next Issue

### Growth Hormone Replacement in Adults

Peter Sönksen, MD,  
and J Weissberger, MD

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## ***GROWTH, Genetics, & Hormones***

**Volume 14, Number 2**

**Post-Program Self-Assessment/CME Verification**

**Answer Sheet**

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Answer each of the questions or statements contained in the Post-Program Self-Assessment/Course Evaluation. There may be more than one correct answer. Participants are encouraged to refer to the related article for evaluation of their responses. To receive Category 1 credit, complete the self-assessment exam/course evaluation and record your responses on the answer sheet. Enclose a check for \$20 made payable to the University of Virginia or complete credit card information. Mail the answer sheet and fee to:

*GGH*

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A certificate will be mailed to you. This enduring material was approved for credit June 1998. Eligibility for credit expires June 2001.

## Evidence From Turner's Syndrome of an Imprinted X-Linked Locus Affecting Cognitive Function

The investigators studied 80 Turner syndrome (TS) patients with a single X chromosome. In 55 patients, the X was maternally derived (45,X<sup>m</sup>) and in 25, paternally derived (45,X<sup>p</sup>). Members of the 45,X<sup>p</sup> group were significantly better adjusted, with superior verbal and higher order executive function skills, which mediate social interactions.

The investigators conclude that the observations suggest there is a genetic locus for social cognition that is imprinted by the X<sup>p</sup> in TS patients and not by the X<sup>m</sup>. Neuropsychological and molecular investigations of 8 females with only partial deletions of the short arm of the X chromosome indicated that the putative imprinted locus escapes X-inactivation, and probably lies on Xq or close to the centromere on Xp. If this locus is expressed only from the X chromosome of paternal origin, the existence of this locus could explain why 46,XY males, who always have a maternally derived X, are more vulnerable to developmental disorders of language and social cognition, such as autism, than are 46,XX females.

The techniques used in differentiating the behavioral differences of 45,X<sup>p</sup> from 45,X<sup>m</sup> are listed in the Table. The 2 groups, as compared with normal 46,XY and 46,XX, are presented in the Figure.

Skuse DH, et al. *Nature* 1997;387:705-708.

### Scale for Measuring Social Cognition

Complete the following section by circling 0 if the statement is not at all true of your child, 1 if it is quite or sometimes true of your child, and 2 if it is very often true of your child:

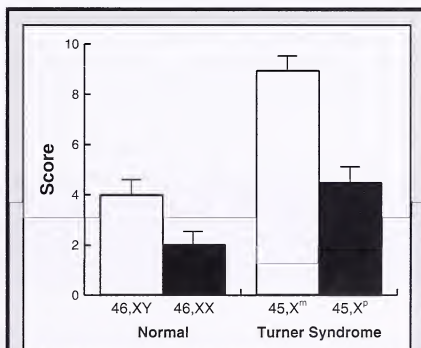
- |   |   |   |   |
|---|---|---|---|
| 0 | 1 | 2 | • lacks an awareness of other people's feelings                                 |
| 0 | 1 | 2 | • does not realize when others are upset or angry                               |
| 0 | 1 | 2 | • is oblivious to the effect of his/her behavior on other members of the family |
| 0 | 1 | 2 | • behavior often disrupts normal family life                                    |
| 0 | 1 | 2 | • is very demanding of people's time  |
| 0 | 1 | 2 | • is difficult to reason with when upset  |
| 0 | 1 | 2 | • does not seem to understand social skills, eg, interrupts conversation        |
| 0 | 1 | 2 | • does not pick up on body language   |
| 0 | 1 | 2 | • is unaware of acceptable social behavior                                      |
| 0 | 1 | 2 | • unknowingly offends people with behavior                                      |
| 0 | 1 | 2 | • does not respond to commands  |
| 0 | 1 | 2 | • has difficulty following commands unless they are carefully worded            |

Internal consistency for set of 12 questions: Standardized item alpha 0.94

**Editor's comment:** The hypothesis presented and the evaluation and testing of the hypothesis are unique and reflect the contributions of an important subgroup of investigators—specifically, the neuropsychoneuroendocrinologists. Collaboration of neuropsychoneuroendocrinologists with pediatric endocrinologists, geneticists, psychiatrists, and others is significantly contributing to therapeutic considerations with which endocrinologists and geneticists deal.

The hypothesis of these investigators is not totally proven as there are other possible explanations for the findings, but the possible correctness of the hypothesis as demonstrated by the investigators will stimulate further and related studies that may expand our knowledge of the relationships between imprinting, growth, intellect, and social or cognitive function. Watch for further studies and developments.

Robert M. Blizzard, MD



Subscale scores (mean + SE) of questionnaire on social-cognitive impairment (see Table). Higher scores indicate poorer social cognitive skills. The 45,X<sup>m</sup> Turner syndrome females score higher than 45,X<sup>p</sup> females and both normal groups ( $P < 0.0001$ ). Normal males score higher than normal females ( $P < 0.001$ ); the effect size of this difference is 0.58, implying that the upper 50% of females score higher than approximately 72% of males. The ratios of mean social-dysfunction scores male:female and 45,X<sup>m</sup>:45,X<sup>p</sup> are very similar (2.2:1 and 2.1:1, respectively). The overall higher scores for the Turner syndrome subjects, compared with normal females, may reflect the contribution made by visuospatial abilities to social cognition. These abilities are impaired equally in both monosomic groups. No information regarding parental origin of the normal X chromosome was made available to parents, their consultants, or members of the research team gathering these or other data.



**GROWTH, Genetics, & Hormones Volume 14, Number 2**  
**Post-Program Self-Assessment/CME Verification**

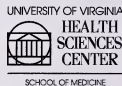
**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on page 38 of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The following characteristics occur in both *ob/ob* and *db/db* mice:
  - a. obesity
  - b. hypothalamic infertility
  - c. impaired thermoregulatory thermogenesis
  - d. large heads
2. Which of the following increase(s) expression of leptin mRNA?
  - a. Thyroxine
  - b. Cortisol
  - c. Growth hormone
  - d. Insulin
3. Leptin is virtually absent in the circulation of the \_\_\_\_\_.
  - a. *ob/ob* mouse
  - b. *db/db* mouse
  - c. *fa/fa* rat
4. The two reported children with very low serum leptin concentrations differ from mice with leptin deficiency in the following respect(s):
  - a. mild versus gross obesity
  - b. normal versus high cortisol levels
  - c. normal linear growth versus short linear growth
5. Central administration of neuropeptide Y \_\_\_\_\_.
  - a. stimulates food intake
  - b. stimulates sympathetic nervous activity
  - c. stimulate plasma insulin
6. Familial clustering and a high concordance rate in monozygotic twins indicate that genetic transmission of susceptibility is responsible for about \_\_\_\_% of the risk for the development of insulin-dependent diabetes mellitus.
  - a. 33%
  - b. 50%
  - c. 67%
  - d. 75%
7. Reverse genetics is the determination of the chromosomal location and identification of new genes in spite of ignorance of the disease mechanism.
  - a. True
  - b. False
8. The application of reverse genetics to common polygenic phenotypes is \_\_\_\_\_.
  - a. easy
  - b. moderately easy
  - c. moderately difficult
  - d. difficult
9. \_\_\_\_\_ loci have been detected by genome-wide scans for susceptibility for insulin-dependent diabetes mellitus.
  - a. Four
  - b. Eight
  - c. Thirteen
  - d. Sixteen
10. Genetic evidence and functional considerations point to \_\_\_\_\_ as the *IDDM2* gene(s).
  - a. *INS*
  - b. *IGF2*
  - c. *IGF1*
  - d. *NPY*

**CME Accreditation  
Statement**

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The University of Virginia School of Medicine designates this educational activity



for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Answer Key: 1. a,b,c 2. b,d 3. a 4. b,c 5. a,c 6. a 7. a 8. d 9. d 10. a,b

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Deal, Polychronakos, Zhang, and Leibel report no conflicts. Drs. Lifshitz, Clarke, Horton, Hall, Rosenfeld, and Slyper report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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## Growth Hormone Deficiency in Adults

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### INTRODUCTION

Growth hormone deficiency (GHD) in adulthood (AGHD) has recently received much attention, particularly in Europe. There has been a dramatic upsurge of awareness of the role and importance of AGHD since a landmark epidemiologic study from Sweden showed an excess mortality in AGHD<sup>1</sup> and the results of the first double-blind, placebo-controlled trials of growth hormone (GH) replacement in AGHD showed material benefit.<sup>2,3</sup> Previously, it was widely assumed that GH had no physiologic relevance once linear growth ceased, and the limited availability of pituitary-derived human GH meant there was insufficient material to explore its effects in adults. The introduction of recombinant human GH (rhGH) in the mid-1980s and its subsequent use in clinical trials has forced a major reappraisal of the importance of maintaining an adequate presence of GH during adult life.

In the last 9 years, many European centers have reported their experience with rhGH in AGHD, mainly using formal placebo-controlled clinical trials. Many meetings have been held to discuss the results of these trials and their implications for routine patient care. Most of the patients entered

into these trials had other pituitary hormone deficiencies, for which they received stable conventional hormone replacement in appropriate doses. Despite differences in study design, patient selection, and rhGH used, the findings have been remarkably consistent and unexpectedly positive. Collectively, they indicate that the majority of AGHD patients, whether the GHD is of childhood onset (COGHD) or acquired onset in adult (AOGHD) life, are compromised both physically and psychologically and can derive substantial, sometimes dramatic, benefit from GH replacement.

Many of the benefits that GH replacement brings are now well documented as a result of numerous double-blind, placebo-controlled trials.<sup>4</sup> Description of the new syndrome of AGHD has resulted,<sup>5</sup> the main features of which are presented in Table 1 (page 42) and are discussed in more detail below.

### BODY COMPOSITION

Apart from stimulating longitudinal bone growth, GH is strongly anabolic and lipolytic and has a powerful antinatriuretic action. GHD children (CGHD) are not only short but also have abnormal body composition with excessive fat, reduced lean tissue, and contracted extracellular fluid (ECF). It is now clear that these abnormalities of body

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## Letter to the Editor:

Recently, we evaluated a 40-year-old woman with McCune-Albright syndrome in whom breast cancer was diagnosed at age 38. At age 2 months, menarche and premature thelarche were diagnosed. Growth stopped at age 9. No therapy produced cessation of menses. The association of premature menarche and later development of breast cancer suggests a potential relationship between these 2 conditions. This patient was exposed to substantial estradiol from 3 months to 9 years of age. We postulate that this may have been sufficient to cause her breast cancer.

Known risk factors for breast cancer exist, including early menarche, late menopause, late pregnancy, prolonged use of hormone replacement therapy, obesity, and elevated plasma estradiol. The majority of these are associated with prolonged or increased exposure of breast tissue to estrogen. Early menarche and late menopause are obvious causes of increased duration of estrogen exposure. Obesity is associated with an increase in aromatase activity in adipose tissue and increased peripheral production of estrone and estradiol. Estrogen replacement during the menopause also increases exposure.

The most common hypothesis regarding estrogen-induced carcinogenesis is that estrogens bind to estrogen receptors; stimulate the transcription of estrogens, which enhance cellular proliferation; and increase the rate of proliferation. Increased estrogen exposure would be expected to cause an increased number of genetic mutations in breast tissue. The rate of proliferation also would decrease time available for DNA repair of new mutations.

An alternate hypothesis has been suggested. Estradiol can be metabolized to 4-OH-estradiol, a catechol estrogen, and then to 3,4-quinone. This compound can bind covalently to guanine, activate glycosidase, and result in depuration of DNA. Upon replication of cells with removal of guanine, the preferential substitution is thymidine during the replication process. This results in G → T point mutations.

A reasonable further hypothesis is that the genomic effect of estradiol to increase proliferation and the genotoxic effects of metabolites act in an additive or synergistic fashion to cause cancer. This concept was recently reviewed at a conference at the Westlands Center in Chantilly, Virginia, dedicated to discussion of the carcinogenic effects of estradiol.

We would like to determine if other patients with McCune-Albright syndrome also have experienced early onset of breast cancer. If a sufficient number of cases can be identified, the association would become more than anecdotal. We encourage physicians caring for adult McCune-Albright patients to respond to this letter by reporting other cases.

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Table 1  
**Clinical Features Suggesting  
Growth Hormone Deficiency in Adults**

### Past history:

- Known pituitary pathology/treatment
- Full conventional hormone replacement

### Associated with symptoms (sometimes only on direct questioning), including:

- Impaired psychologic well-being
  - Poor general health
  - Reduced vitality and energy
  - Impaired emotional reaction
  - Depressed mood
  - Impaired self-control
  - Anxiety
  - Increased social isolation
- Increased abdominal adiposity
- Reduced strength and exercise capacity

### Together with such signs as:

- Mixed truncal/generalized obesity
- Increased waist:hip ratio
- Thin, dry skin; cool peripheries; poor venous access
- Mild/moderate reduction in muscle strength
- Moderate reduction in exercise performance
- Psychologic state characterized by low, labile mood

### Supplemented by test results:

- Stimulated growth hormone level <3 ng/mL
- Low or low-normal serum IGF-1
- Unfavorable serum lipid profile
- Low glomerular filtration rate and renal plasma flow
- Reduced lean body mass/increased fat mass
- Reduced basal metabolic rate
- Reduced bone density

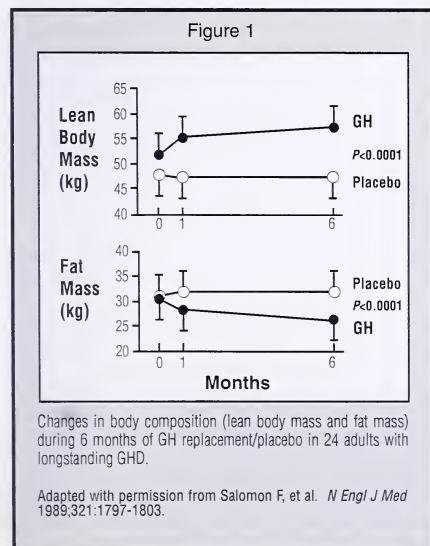
composition also are present in AGHD<sup>3,6</sup> and are reversible with GH replacement.<sup>2,3,7,8</sup>

## Fat Mass

In a cohort of 24 severely GHD adults that we first studied in 1987, mean fat mass (derived from the measurement of total body potassium) was 37.9% of body weight, exceeding that predicted from age, sex, height, and weight by an average of 7%.<sup>3</sup> This was very similar to the experience with a much larger cohort of 101 GHD Swedish adults who had a mean excess of body fat (determined from combined measurements of total body water and total body potassium) of approximately 6 kg.<sup>6</sup> As is the case in CGHD, the excess fat in AGHD tends to accumulate predominantly in the abdominal region and particularly in the intra-

abdominal organs.<sup>8</sup> A reduction in fat mass with GH replacement in adults has been a uniform finding.<sup>2,3,8</sup> In our patients, a mean reduction of 5.7 kg (18%) of body fat was observed after 6 months (Figure 1), with the greatest change occurring in the abdominal region, as shown by changes in skinfold thickness and waist:hip ratio. The selective effect of rhGH on abdominal fat in AGHD has been elegantly demonstrated by computed tomography (CT) scanning, with abdominal fat (both intra-abdominal and subcutaneous) found to decrease by an average of 30% after 6 months of treatment, compared with a 13% reduction in more peripheral sites.<sup>8</sup> These changes all occurred with no alterations in dietary intake. With calorie restriction, however, remarkable losses of fat have occurred with simultaneous increases in lean body mass (LBM). This is well demonstrated by the case of Mrs. E.L. (illustrated in Figure 2).

*Mrs. E.L. developed pituitary apoplexy in 1953. Following this, she was given a course of deep X-ray therapy to prevent any tumor recurrence and given levothyroxine 0.15 mg, cortisone acetate 37.5 mg/d, DDAVP 0.1 mL/hs, and estrogen/progesterone. She remained on this regimen until 12 months before she was sent to St. Thomas', when her estrogen/progesterone preparation was withdrawn.*



**Figure 2**

**Patient E.L.:**

- GH commenced December 1990
- Maintenance dose: 0.04 - 0.05 U/kg/day
- IGF-1: 2.9 → 32.0 nmol/L

**Patient E.L. — Body Composition**

|                       | Dec. '90 | Dec. '91 |
|-----------------------|----------|----------|
| Weight (kg)           | 104.7    | 78.0     |
| Lean Body Mass (kg)   | 40.1     | 47.2     |
| Fat Mass (kg)         | 64.6     | 30.8     |
| Sum of Skinfolts (mm) | 22.0     | 144.0    |

Case study of Mrs. E.L., who suffered from hypopituitarism secondary to pituitary apoplexy in 1953. She was wheelchair-bound and unable to lose weight until she started GH replacement in December 1990.

*Over the preceding 5 years she had become increasingly weak. When seen at St. Thomas', she was virtually wheelchair-bound. Her weight had increased due to her inactivity and had resisted her continuous dietary attempts to control it. She was depressed, saw little future in life, and had begun to drink more alcohol than was good for her as a form of escape.*

*Following the addition of rhGH to her drug regimen, she lost weight dramatically (on the same calorie-restricted diet on which she had previously gained weight), while simultaneously substantially increasing her LBM. Her mobility increased dramatically, as did her quality of life. A subsequent long-overdue knee replacement went smoothly and improved her mobility further. She withstood additional corticosteroid and immunosuppressive therapy required for bullous pemphigoid, without significant loss of quality of life or mobility. She remains mobile and active, although her weight has crept up a little over the years.*

**Lean Body Mass**

In our original study, GHD adults had values for LBM derived from total body potassium measurements that were 7% to 8% lower than values predicted from the age, sex, height, and weight of the subjects. After 6 months of GH replacement, LBM was normalized, increasing on average by 10% to 11% (5.5 kg), with most of this increase occurring in the first month of treatment (Figure 1). Other investigators, using several



different methods for determining LBM, have also demonstrated increases of 7% to 10% over a 6-month period of rhGH treatment. Open studies conducted over longer periods of rhGH replacement extending beyond 5 years suggest that the restoration of LBM is largely complete within the first 6 to 12 months, with less but still important increases occurring thereafter. This is in contrast to the usual loss in LBM with increasing age.

Skeletal muscle, which comprises approximately 50% of LBM, is substantially reduced in AGHD. The distribution of muscle and fat in cross-sectional mid-thigh CT scans from 22 adults with COGHD was 63% and 37%, respectively, compared with the distribution in healthy age-matched controls of 85% muscle and 15% fat.<sup>2</sup> We made similar observations in 20 patients with AOGHD, the mean cross-sectional area on CT of the dominant quadriceps muscle (expressed per kilogram body weight) being 15.5% lower than that in controls matched for age, sex, and activity. Following 6 months of rhGH treatment in AGHD, significant increases in cross-sectional muscle areas on CT of 5% to 8% have been observed in the thigh<sup>2,9</sup> and other regions.<sup>8</sup>

The deficit in LBM in untreated AGHD represents not only a loss of cell mass and tissue protein but also a substantial contraction of ECF. Estimates of extracellular water in 101 AGHD patients (based on the measurement of total body potassium and total body water) were on average 15% lower than predicted values.<sup>6</sup> After 6 months of rhGH treatment in 10 AGHD patients, there was a mean increase in LBM of 4.6 kg, of which as much as 3.0 kg could be attributed to increases in ECF.<sup>8</sup> These observations are in keeping with the well-described antinatriuretic

action of GH<sup>10,11</sup> which appears to involve relatively minor activation of the renin-angiotensin-aldosterone axis<sup>12-14</sup> and a probably more important direct effect of rhGH and/or insulin-like growth factor 1 (IGF-1) on renal tubular sodium reabsorption.<sup>15-17</sup>

### Bone Mass

The importance of GH for skeletal growth and maturation in childhood is well established, but much less is known about its role in maintaining bone mass during adult life. Several studies have demonstrated reduced cortical and trabecular bone mass in AGHD of COGHD. In such cases, the osteopenia may be the result of a failure to achieve normal adult height and a deficient accretion of bone during childhood and adolescence rather than an accelerated loss of bone during adulthood. One study involving only patients with AOGHD, however, also demonstrated diminished cortical and trabecular bone mass.<sup>17</sup> Osteopenia was most marked in patients who had acquired GHD in early adulthood, again presumably because of a failure of accretion of sufficient bone in the years during which peak bone mass is normally attained. However, bone mass also was significantly reduced in those patients who had developed AOGHD after the age of 30 years, suggesting that GHD does indeed lead to a loss of bone after peak bone mass has been attained. This remains a slightly controversial topic. The balance of evidence indicates that GH is essential for achieving normal peak bone mass and that GHD with onset after peak bone mass has been achieved has a relatively minor, but still significant, effect on increasing the rate of bone loss. The clinical significance of the collective findings has not been firmly established, but there are at least 2 studies showing an increase in osteoporotic fractures in adults with GHD.<sup>18,19</sup>

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We now know that GH replacement in adults stimulates the processes of bone remodeling, resulting in changes in various serum and urinary markers of bone turnover. In the early stages this may result in a decrease in bone density as remodeling is activated.<sup>20,21</sup> Only after about 6 months does bone density begin to increase with longer-term rhGH treatment.<sup>21,22</sup> Longer-term data over 5 or more years indicate that there is a steady gradual accumulation of bone; this continues in a linear fashion so long as the rhGH continues to be administered. After rhGH is stopped, the accretion of bone continues for at least 6 months.

## Skin

The fine facial wrinkling characteristic of hypopituitary patients may be related to GHD. Skin thickness and total skin collagen are reduced in hypopituitary patients, despite conventional replacement therapy; the converse is true in acromegaly.<sup>23</sup> An increase in skin thickness was demonstrated following rhGH treatment in normal elderly males selected on the basis of low IGF-1 levels.<sup>24</sup>

In most people's experience, there is typically a dramatic change in facial appearance with GH replacement. This might be partly explained not only by changes in skin texture and thickness but also by a loss of excess subcutaneous fat combined with an increase in tissue hydration.

Hypopituitary adults are usually described as having dry skin, whereas excessive sweating is a typical feature of acromegaly. Eccrine sweat glands have been shown to possess GH receptors.<sup>25</sup> Sweat secretion, measured in response to pilocarpine iontophoresis, was significantly lower in AGHD patients than in age- and sex-matched control subjects; during rhGH treatment, there was a significant increase in the sweat secretion rate that was perceived by the patients.<sup>2</sup> GHD adults have been shown to have an impaired ability to dissipate heat by sweating following heat stress or exercise,<sup>26</sup> and this may be a contributory factor to their reduced exercise capacity.

## PHYSICAL PERFORMANCE

### Muscle Strength

In our original study, isometric quadriceps force in AGHD was significantly reduced, on average by 26%, compared with matched controls.<sup>27</sup> Most of the deficit could be explained by the reduction in muscle cross-sectional area. However, after correction for this, an additional small deficit was still present in the AGHD patients, possibly explained by a lack of training.

The effects of relatively short-term (up to 6 months) rhGH treatment on muscle strength in AGHD were evaluated in several studies. A significant increase in limb girdle strength was found in our patients; however, quadriceps isometric force did not increase significantly in any of the studies despite clear increases in thigh muscle cross-sectional area. Only with more prolonged rhGH treatment of at least 12 months has a significant increase in quadriceps force been demonstrated; after 3 years, a near normalization

of muscle strength can be expected.<sup>28</sup> Measuring muscle strength is not easy, and the failure to demonstrate an effect in many cases is because the variance of the method is too great to be able to demonstrate an effect in the small number of patients included.

### Exercise Capacity

Maximal exercise performance in AGHD has been assessed by cycle ergometry. Before treatment, values for maximum oxygen uptake were significantly reduced, being on average 72% to 82% of those predicted for age, sex, and height.<sup>7,29</sup> Following rhGH treatment for up to 6 months, maximum oxygen uptake increased significantly, reaching a mean of 97% of predicted in our patients. Parallel increases in power output and work capacity also have been observed.<sup>30</sup> Submaximal exercise performance representing most closely the exercise taken in normal life, measured as anaerobic threshold, increased significantly with rhGH treatment, suggesting that physical activities of daily living would be accomplished with less metabolic stress and thus with less subjective sensation of effort. In contrast to the relatively rapid normalization of exercise capacity we observed in patients with AOGHD, data from patients with COGHD suggest that it may take up to 3 years to attain maximum benefit.<sup>31</sup> Possible explanations for the enhanced exercise performance with rhGH treatment include the increase in skeletal muscle mass, increased ECF, improved cardiac function (see below), improved heat dissipation through increased sweating capacity, and as yet undetermined alterations in fuel utilization during exercise.

## CARDIAC FUNCTION

Echocardiography in our original study revealed a small but significant increase in resting left ventricular (LV) end-diastolic volume (mean, 2%) and stroke volume (mean, 6%) with rhGH treatment, most likely resulting from an increase in plasma volume (preload) secondary to the antinatriuretic effect of GH.<sup>13</sup> There also was, however, a significant increase in LV wall mass, which occurred in the absence of any change in mean arterial pressure. The mean increase in LV wall mass of 5% was comparable with the 5% to 10% increase in thigh muscle and LBM in the same patients, suggesting an anabolic action of GH on both skeletal and cardiac muscle. In adults with COGHD, cardiac mass was reduced and cardiac function was impaired,<sup>32,33</sup> and both were normalized after 6 months of rhGH treatment, returning to baseline 6

months after treatment stopped.<sup>32</sup> A sustained increase in cardiac output has been observed in AGHD after 3 years of rhGH treatment.<sup>34</sup>

Two remarkable case reports of cardiac cachexia in GHD adults that responded dramatically to GH replacement further emphasize the importance of GH in attaining and maintaining optimal cardiac function.<sup>34-36</sup>

## RENAL FUNCTION

Glomerular filtration rate and renal plasma flow were significantly lower in AGHD compared with age-matched healthy individuals.<sup>2</sup> GH replacement resulted in a normalization of both variables and in our patients induced a rapid and marked increase in creatinine clearance and a fall in blood urea.<sup>3</sup> It is not known to what extent these findings reflect changes in plasma volume, cardiac output, kidney size, or other intrarenal effects.

## METABOLISM

### Energy Expenditure

The anabolic action of GH is associated with an increase in energy expenditure. Basal metabolic rate (BMR) increased by an average of 22% in our patients after 1 month of rhGH treatment and was still 16% higher than baseline after 6 months.<sup>2</sup> The increase in BMR at 6 months could be largely explained by the increase in LBM (known to be the major physiologic determinant of BMR), whereas the mean BMR at 1 month was still significantly raised even when expressed per kilogram of LBM. GH increases the peripheral conversion of thyroxine ( $T_4$ ) to triiodothyronine ( $T_3$ ),<sup>2,31</sup> and rhGH treatment in AGHD results in a rise in circulating  $T_3$  levels both in patients on  $T_4$  replacement and those not needing it. This may help to explain the initial increase in BMR. Stimulation of previously underactive metabolic processes, particularly protein synthesis<sup>20,37,38</sup> and fat oxidation,<sup>38-41</sup> also may contribute to the calorogenic effect of GH. From a practical therapeutic aspect, free  $T_3$  levels should be monitored when GH replacement is started, and reduction in  $T_4$  replacement dose may be needed to prevent iatrogenic hyperthyroidism. Plasma thyrotropin and free or total  $T_4$  are of little or no value in monitoring thyroid status in GHD patients on GH replacement.

### Carbohydrate Metabolism

The increase in fat mass and its central distribution in AGHD is associated with insulin resistance,

reflected in a strong correlation between fat mass and fasting insulin and C-peptide levels in these patients.<sup>42</sup> This contrasts with the increased insulin sensitivity seen in children with GHD.

Following rhGH treatment in AGHD for 1 to 3 months, modest increases have been observed in fasting and postprandial blood glucose concentrations (within the normal range) as well as in fasting serum insulin and C-peptide concentrations.<sup>3,20</sup> This is in keeping with a further increase in insulin resistance, which was confirmed recently using a glucose clamp technique.<sup>43</sup> Glycosylated hemoglobin concentrations, however, have shown no significant changes after up to 3 years of rhGH treatment.<sup>3,8,28</sup> It also is reassuring that glucose, insulin, and C-peptide concentrations and insulin sensitivity assessed by glucose clamp all tend to return towards baseline values by 6 months of rhGH treatment, suggesting an over riding beneficial effect of shedding fat and increasing LBM on insulin action. It will be of interest to see whether insulin sensitivity is eventually normalized with long-term treatment.

### Protein Metabolism

Whole-body protein synthesis (assessed by the continuous infusion of  $C^{13}$ -leucine) was found to be lower in GHD than in matched controls,<sup>37</sup> as were leucine flux, leucine oxidation, and the incorporation of leucine into protein.

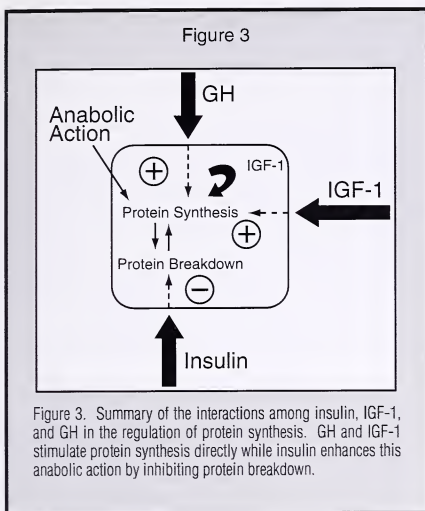
In a double-blind, placebo-controlled study involving 18 patients and using a continuous infusion of  $C^{13}$ -leucine, stimulation of protein synthesis was demonstrated after 2 months of rhGH treatment. This remained significant even when results were corrected for the increase in LBM.<sup>38</sup> However, when  $C^{13}$ -leucine turnover was examined after a longer period (6 months) of treatment, no increase in leucine flux or protein synthesis was observed,<sup>37</sup> possibly because a new steady state had been achieved.

GH, IGF-1, and insulin act synergistically to promote anabolism during rhGH treatment. GH and IGF-1 stimulate protein synthesis and the accompanying rise in circulating insulin inhibits proteolysis (Figure 3).<sup>44,45</sup>

### Lipoprotein Metabolism

In AGHD, mild increases in total and low-density lipoprotein (LDL) cholesterol levels have been observed in up to 50% of patients compared with age-, weight-, and sex-matched controls, in keeping with earlier findings in GHD children.<sup>46,47</sup> GHD adults also tend to have low high-density lipoprotein (HDL) cholesterol levels and high





triglyceride levels. Following rhGH treatment in AGHD, several studies have demonstrated a significant fall in total and LDL cholesterol concentrations, accompanied by a modest rise in HDL cholesterol and no change in triglyceride levels.<sup>20,47-49</sup> However, data on lipoprotein(a), an independent risk factor for the development of atherosclerosis and myocardial infarction, have been less consistent with either a rise<sup>48</sup> or no change<sup>49</sup> being observed. This may be due to differences in assays, patient selection, or duration of rhGH treatment.

Alterations to the lipid profile during rhGH treatment could occur through direct actions of GH and/or IGF-1 on lipoprotein synthesis or clearance. An indirect effect also is possible via the rise in circulating  $T_3$  levels or the reduction in central adiposity accompanied by an amelioration of the insulin-resistant state.

The clinical relevance of the unfavorable lipid profile of AGHD and its improvement with rhGH treatment is apparent when considered in light of a large retrospective epidemiologic study from Sweden. Overall mortality in 333 hypopituitary patients was found to be almost 2-fold higher than that in an age- and sex-matched normal population, despite adequate thyroid, adrenal, and sex hormone replacement.<sup>1</sup> The increase in overall mortality was largely accounted for by an increase in cardiovascular deaths. It was suggested that GHD,

which could be assumed to be present in most of the hypopituitary patients, was a factor in the increased cardiovascular mortality. This proposed association has been strengthened by the demonstration of an increased prevalence of atherosclerosis in hypopituitary adults on full conventional hormone replacement.<sup>50</sup> Very long-term observations will be required to determine if GH replacement in AGHD results in a regression of atherosclerosis and ultimately in a reduction in cardiovascular mortality.

## PSYCHOLOGIC WELL-BEING

Detailed assessment of psychologic well-being was performed in AGHD in our initial study, using 3 well-validated but independent generic questionnaires.<sup>51</sup> Responses prior to treatment were compared with those from normal subjects matched for age, gender, ethnic origin, socioeconomic class, and area of residence. The GHD patients perceived themselves as having a poorer quality of life than the normal subjects. Particular areas of concern to the patients were low energy, emotional lability, low mood, and social isolation. They also regarded themselves as having a poorer level of general health, less self-control, less vitality, and more anxiety. Indeed, over one third of the patients scored in the range consistent with a psychiatric disturbance requiring therapy.

It is likely that these findings reflect the GHD state per se and are not simply attributable to the presence of a chronic disorder. In a recent comparison of AGHD with age- and sex-matched diabetic patients using standardized psychiatric rating and diagnostic measures, significantly more of the GHD group were identified as definite psychiatric cases (46% vs 24%), with the most frequent diagnosis being major depression.<sup>52</sup> Moreover, in our original placebo-controlled study, treatment of GHD for 6 months resulted in a substantial improvement in psychologic well-being, with many patients verbally reporting a feeling of increased energy and well-being within a few weeks. The responses to the questionnaires confirmed the clinical impression, with statistically significant improvements in perceived physical health and quality of life, especially in the areas of energy and mood.<sup>51</sup>

It is likely that the increases in LBM and skeletal muscle mass contribute to the sense of improved well-being with rhGH treatment, particularly in the long-term. Other possible mechanisms include improved tissue hydration and perfusion, the



increase in circulating  $T_3$ , and direct effects of GH and/or IGF-1 within the central nervous system. There is evidence of a direct central effect, with the demonstration of abnormal sleep patterns in young GH-deficient adults that improve after short-term rhGH treatment.<sup>53</sup> It has been shown that GH replacement penetrates the blood-brain barrier, stimulating a rise in the cerebrospinal fluid  $\beta$ -endorphin concentration with a simultaneous fall in concentrations of homovanillic acid and vasoactive intestinal polypeptide concentrations.<sup>54</sup>

## SIDE EFFECTS

The target dose of rhGH chosen for the initial trials in AGHD was 0.06 to 0.07 IU/kg/d (0.02 to 0.023 mg/kg/d), which is similar to that routinely used in CGHD. Side effects forced early dose reductions in a significant proportion of the patients, and this, together with the finding of elevated circulating IGF-1 levels in some cases, suggested that this dose is supraphysiologic for most adults. Subsequent trials have employed lower rhGH doses. With experience, the average replacement dose has fallen to less than 0.02 IU/kg/d (0.006 mg/kg/d).

Clinical evidence of sodium and water retention following rhGH treatment in AGHD is the most common side effect, reflecting the hormone's potent antinatriuretic action. Weight gain, swelling of the ankles, a sensation of tightness in the hands, or symptoms of carpal tunnel compression frequently occur within days or weeks. These symptoms are often transient or resolve rapidly with dose reduction. In many cases, they can be explained purely through normalization of tissue and extracellular hydration. Blood pressure has not changed significantly with rhGH treatment. Arthralgias involving small or large joints also occur in some patients soon after commencing treatment, but usually there is no evidence of effusion or inflammation and X-ray films show no abnormality. These changes also settle spontaneously with time but occasionally necessitate a dose reduction. They are possibly due to swelling of articular cartilage and reactivation of cartilage growth, since patients not uncommonly liken them to the growing pains of adolescence. With lower starting doses and more gradual titration, the incidence of side effects should continue to decrease.

The main limiting factor to widespread use of rhGH in hormone replacement is now cost. In the climate of restricting health-care costs that exists in most

countries, it is not easy to convince funding agencies that GH replacement is worth the high cost.

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## Orexins and Orexin Receptors: A Family of Hypothalamic Neuropeptides and G Protein-Coupled Receptors That Regulate Feeding Behavior

Two neural proteins with appetite-stimulating properties, their receptors, and respective genes have been identified and characterized in humans, rats, and mice. They are termed orexins (after the Greek word orexis, which means appetite). Orexin-A is a 33 amino acid peptide (molecular weight, 3.56 kD); orexin-B is a 28 amino acid peptide (molecular weight, 2.94 kD); they are derived from a common gene located on human chromosome 17q21 by alternative splicing. Two orexin receptors also have been found;  $hOXR_1$  (425 amino acids) and  $hOXR_2$  (444 amino acids), and have structures typical of 7 transmembrane G protein-coupled receptors. They are somewhat homologous to receptors for neuropeptide Y and thyrotropin-releasing hormone.

In the rat brain, orexin is located in the lateral and posterior hypothalamic regions (the "feeding center"), but not in the ventromedial, arcuate, or paraventricular nuclei (the "satiety center"). *Prepro-orexin* mRNA levels were upregulated upon fasting, suggesting a physiologic role for the peptides as mediators in the central feedback mechanism that regulates feeding behavior. Administration of orexin-A or -B into the lateral ventricle of male rats stimulated an immediate increase in food consumption.

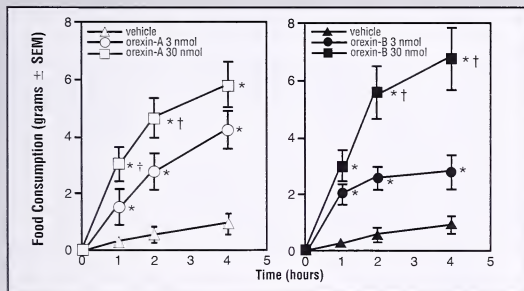
Sakurai T, et al. *Cell* 1998;92:573-585.

**Editor's comment:** This interesting finding adds another player to the many that regulate appetite and energy metabolism in humans and animals. Exploration of its interaction with leptin will be of great importance. It is possible that the orexin system may contribute to disorders associated with either hyperphagia or anorexia in humans. The possibility that polymorphic variants of orexin-A or orexin-B and their receptors determine appetite in normal individuals and in patients with feeding disorders will undoubtedly be explored, as will the therapeutic potential of receptor agonists in patients with wasting disorders and of antagonists in obesity.

The Editorial Board calls to your attention that an excellent review of leptin physiology by Zhang and Leibel appeared in *GGH* (1998;14[2]:17-26), which you may wish to read as a foundation for understanding the many articles that are appearing and will appear in the literature concerning appetite and obesity very soon.

Allen W. Root, MD

### Stimulation of Food Consumption by Intracerebroventricular Injection of Orexin-A and -B in Freely-Fed Rats



Designated amounts of synthetic human orexin-A (left) or -B (right) were administered in a 5  $\mu$ L bolus through a catheter placed in the left lateral ventricle in early light phase. Cumulative food consumption was plotted over the period of 4 hours after injection. Asterisks (\*) indicate significant difference from vehicle controls ( $P < 0.05$ ;  $n = 8-10$ ; ANOVA followed by Student-Newman-Keuls test). Crosses (†) designate significant difference between 3 nmol and 30 nmol injections ( $P < 0.05$ ;  $n = 8-10$ ; ANOVA followed by Student-Newman-Keuls test). Similar results were obtained in at least 4 independent sets of experiments. The same vehicle control curve was replotted in both panels.

Reprinted with permission from Sakurai T, et al. *Cell* 1998;92:573-585.

### Further Reports of Leptin Abnormalities in Humans

Montague et al (*Nature* 1997;387:903-908) described the only case reported until now of leptin deficiency. No cases of leptin receptor gene (*LEPR*) deficiency have been described. The 2 articles reviewed here describe the clinical and hormonal consequences of a genetic defect in either the secretion of leptin, the adipocyte hormone that regulates appetite and energy metabolism, or the synthesis

of its receptor. Phenotypically, patients with leptin deficiency or insensitivity are morbidly obese and, as adults, hypogonadotropic.

Strobel et al describe a Turkish family in which 3 extremely obese, hyperphagic members had a homozygous C  $\rightarrow$  T substitution in codon 105 (exon 3) of the *OB* (leptin) gene,

leading to replacement of arginine by tryptophan. Body mass index values were markedly elevated (46.9, 55.8, and 32.5) at 34, 22, and 6 years of age, respectively; all had inappropriately low serum leptin levels. Family members who were heterozygous for this mutation were phenotypically normal with normal serum leptin concentrations. The older affected subjects were clinically hypogonadotropic (primary amenorrhea in the 34-year-old female; little virilization in the 22-year-old male). Basal serum concentrations of testosterone were low, but the response to human chorionic gonadotrophin was normal; serum level of luteinizing hormone (LH) was low in relation to the low testosterone. Follicle-stimulating hormone (FSH) was normal, as were the secretory responses of FSH, LH, and testosterone to gonadotropin-releasing hormone. The 22-year-old patient had low sympathetic tone (abnormal cold pressor response and orthostatic hypotension). In transfection studies (COS-1 cells), it was demonstrated that this mutation did not inhibit synthesis of leptin but impaired its processing through the secretory pathway.

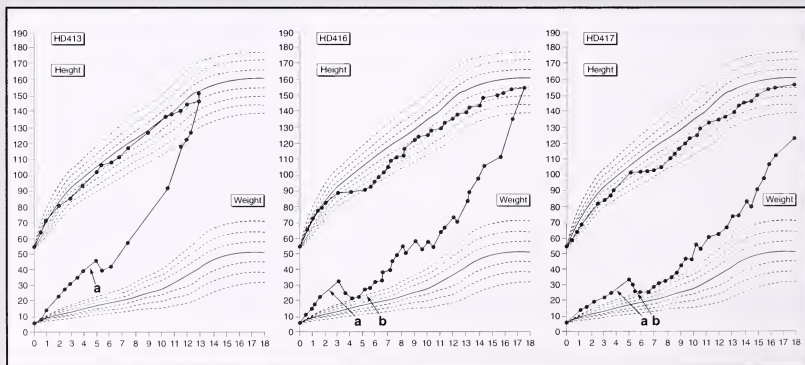
Clement et al reported 3 obese female siblings of Kabilian ancestry who had serum concentrations of leptin that were 4-fold higher than the upper limit of normal. They had a homozygous mutation (G→A) in the splice donor site of exon 16, leading to skipping of exon 16 and the synthesis of a leptin receptor (*OB-R*) lacking the transmembrane and intracellular domains of the wild-type *OB-R*. Since this

mutation led to secretion of the extracellular domain of the *OB-R* that could dimerize and bind leptin, serum leptin-binding activity was quite high in the homozygous and heterozygous subjects, accounting in part for their elevated serum leptin values. The homozygous mutants had aggressive food-seeking behavior and became obese within the first months of life, similar to subjects with the Prader-Willi syndrome. Growth faltered at approximately 5 to 6 years of age, despite the obesity (Figure). They were found to have subnormal provoked and spontaneous growth hormone (GH) secretion and inappropriately low levels of insulin-like growth factor-1 (IGF-1) and IGF-binding protein 3 (IGFBP-3). During administration of GH, growth rate improved and IGF-1 and IGFBP-3 values rose. The older subjects had primary amenorrhea in the presence of low LH and FSH levels; slightly low free thyroxine values; and perhaps slightly prolonged thyrotropin secretory responses to thyrotropin-releasing hormone, suggesting partial deficiency of this tripeptide. Despite attempts at food restriction, weight gain progressed inexorably; 1 subject died at 19 years of age at a weight of 133 kg; the 2 surviving siblings weighed 159 kg at 13 years and 166 kg at 19 years, respectively.

Strobel A, et al. *Nature Genet* 1998;18:213-215.

Clement K, et al. *Nature* 1998;392:398-401.

**Editor's comment:** These articles confirm the essential role of leptin in the regulation of appetite and weight in



Height and weight curves for the 3 affected sisters from birth to adult age. The letter **a** indicates a period of food and food-intake restriction. The restrictive diet of 500 kcal/d resulted in weight loss and in a dramatic decrease in growth velocity that persisted even after food-intake increase and weight regain. The letter **b** indicates the introduction of treatment with levothyroxine and the start of treatment with exogenous growth hormone. The x-axis indicates age in years; the y-axis indicates height in centimeters or weight in kilograms.

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humans and its likely importance in pubertal development. The influence of leptin on sympathetic tone is of interest, although the mechanism by which it exerts this effect is unknown at present. The finding of suboptimal GH secretion in the leptin-resistant subjects is unexpected, as patients with leptin deficiency reported to date have had normal linear growth patterns. The criteria employed for the diagnosis of GH deficiency in the leptin-resistant subjects seem reasonable, although the decline in growth rate appeared to coincide initially with a period of food restriction and weight loss. If the leptin receptor influences GH secretion in humans, it may be through regulation of hypothalamic GH-releasing hormone synthesis or secretion.

Allen W. Root, MD

**2nd Editor's comment:** The investigators of both papers are commended for performing very important studies.

While the data in the second paper pertaining to obesity, sexual infantilism, hyperinsulinemia, and leptin levels are interpreted correctly, I am reluctant to interpret the auxologic and/or biochemical data as being convincing evidence of GH deficiency. The growth curves are not the growth curves of GH-deficient children. The growth of the 3 affected children was never below the 3rd percentile. Two unaffected children (No. 412 and No. 419) were essentially of comparable heights at essentially the same ages as No. 413 and No. 417, who were affected; the latter 2 had not had sex steroids to enhance their growth. The low GH levels are compatible with those often seen in obese children. The IGF-1 levels are marginally low, but no sex steroids were present to stimulate GH production and, consequently, generation of IGF-1. In my opinion, further observations on similar patients are needed for the argument of GH insufficiency to be convincing.

Robert M. Blizzard, MD

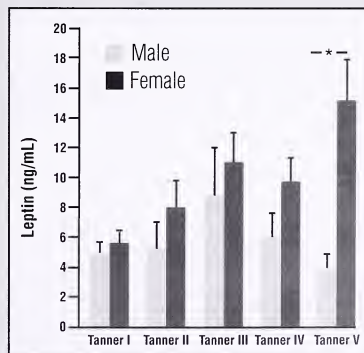
## Leptin Plasma Levels in Healthy Spanish Children and Adolescents, Children With Obesity, and Adolescents With Anorexia Nervosa and Bulimia Nervosa

In order to determine normal circulating levels of leptin throughout adolescence as well as in children with eating disorders (obesity, anorexia nervosa, and bulimia nervosa), 100 normal children were prospectively included in this study. They were divided into 5 different groups, corresponding to each of the 5 Tanner stages. These children had height and growth velocities between  $\pm 1$  SD and body mass index (BMI) within  $\pm 2$  SD. Their bone ages were similar to their chronologic ages. These normal subjects were compared with 14 prepubertal obese children with BMI  $> 2$  SD. Fasting blood samples were taken in the morning. Leptin levels were measured at 6 and 12 months when 25% and 50% of BMI-SDS reduction was achieved by the obese group, respectively.

Eleven Tanner stage V females with anorexia nervosa also were included in the study. Leptin levels in these anorexic patients were measured after weight gains of 8% to 10% above their original weight. Bound and free leptin levels also were determined in 3 anorexic and 3 bulimic patients.

Significant changes in leptin level were observed throughout puberty at different Tanner stages. Normal males exhibited only 1 peak level at Tanner stage III, while normal females revealed 2 peaks at Tanner stages III and V. Linear correlation between leptin and BMI-SDS was found in lean normal subjects. Mean leptin levels for obese prepubertal children were significantly elevated compared with age- and sex-matched controls. Normal leptin levels were reached after 1 year on a low-calorie diet and weight loss of at least 50% of the initial BMI-SDS. No direct correlation was found between BMI-SDS and leptin levels in the obese group.

Patients with anorexia nervosa exhibited lower plasma leptin levels compared with age- and sex-matched controls. Those patients with bulimia have higher leptin levels than patients with anorexia nervosa and do



Mean circulating leptin plasma levels ( $\pm$  SEM) in healthy Spanish males and females at different stages of sexual maturation. A significant effect of both sex and Tanner stage was found on leptin levels (2-way ANOVA;  $P < 0.0001$ ). Circulating leptin levels are significantly sexually dimorphic only at Tanner stage V(\*).

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not differ significantly from controls. Total and free leptin levels were higher in bulimic patients than in anorexic patients, but no differences were found in levels of the bound form.

Argente J, et al. *J Pediatr* 1997;131:833-838.

**Editor's comment:** This paper reports new leptin level data throughout normal developmental stages and its correlation with body weight in 2 different pathological states, as well as after a phase of weight recovery in obese and anorexic patients. The data presented in this article confirm previous findings during adulthood reported by Ferron et al (*Clin Endocrinol [Oxford]* 1997;46:289).

The authors also demonstrated differences between different leptin fraction levels in those patients with anorexia and bulimia; however, these values were not compared with those of normal subjects. Previous

reports in adult populations already have described the biokinetics of the different leptin fractions in normal and obese subjects. Thus, it would have been interesting to see in this particular study not only how the leptin profiles change throughout the normal development stages but also the kinetics of different leptin fractions in normal subjects throughout childhood. Although determination of leptin values may be helpful to assess adipose tissue stores, it still is not clear what their clinical role is in the diagnosis or prognosis of severe eating disorders.

Zhang et al recently contributed an outstanding lead article concerning leptin physiology in GGH (1998;14[2]:17-26), which readers will find most enlightening.

Fima Lifshitz, MD

*J Clin Invest* 1998;98:1277-1282.

*Diabetes* 1996;45:1638-1643.

## The APECED Gene and Its Products and Polyglandular Autoimmune Disease I

Autoimmune polyglandular syndrome type 1, also termed autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a monogenic, autosomal recessive disorder associated primarily with autoimmune hypoadrenalism, hypoparathyroidism, and chronic mucocutaneous candidiasis; it also is associated to a lesser extent with ectodermal dystrophies (vitiligo, alopecia), pernicious anemia, hepatitis, hypogonadism, hypothyroidism, and diabetes mellitus.

By linkage analysis, this disorder has been mapped to chromosome 21q22.3. Two groups of investigators have now isolated and characterized the gene that is mutated in and responsible for APECED.

Since it is likely that the product of this gene is a regulator of transcription of genes associated with immune function, it has been named *AIRE* (autoimmune regulator). It contains 2,027 bp with an open reading frame of 1,763 bp encoding 14 exons; the main transcription product (*AIRE-1*) has 545 amino acids without a transmembrane or signal peptide, but with a nuclear targeting signal, 2 cysteine-rich zinc-finger DNA-binding domains (amino acid 299-340 and 434-475), a proline-rich region, and 3 LXXLL sequences—domains associated with DNA and protein interaction. Two alternatively spliced products also have been found composed of a separate first exon and exons 8-14: *AIRE-2* (348 amino acids) and *AIRE-3* (254 amino acids). *AIRE-1* is expressed in the thymus, adrenal cortex, and pancreas as well as in the spleen, lymph node, bone marrow, fetal liver, and testis.

Homozygous and compound heterozygous mutations in *AIRE* have been found in Finnish, Swiss, Dutch, and German families with APECED, including 5 mutations resulting in frameshifts and truncated proteins and loss of the zinc-finger domains. No mutations in *AIRE-2* or *AIRE-3* have been found to date in patients with APECED.

Nagamine K, et al. *Nature Genet* 1997;17:393-398.  
Finnish-German APECED Consortium. *Nature Genet* 1997;17:399-403.

**Editor's comment:** Autoimmune polyglandular syndrome type 2 (primarily autoimmune hypoadrenalism, diabetes mellitus, and thyroid disease) and other polyglandular syndromes such as pernicious anemia and autoimmune thyroid disease are clearly linked to the HLA system (chromosome 6p) and presumably result from an aberration in immune surveillance related to antigen presentation or recognition. Since APECED is not linked to HLA, the protein product of *AIRE* (which has many characteristics of a regulator of gene transcription) introduces another potential pathway of immune regulation. As the genes regulated by *AIRE* are identified and the biologic functions of the products of these genes determined, our understanding of the mechanisms of immunity and disorders thereof will increase. Parenthetically, it is interesting to note that the Down syndrome critical region also is assigned to chromosome 21q22.3. It is possible that the propensity for autoimmune thyroid disease characteristic of the Down syndrome patient may be related to *AIRE*.

Allen W. Root, MD

## Efficacy and Safety of Growth Hormone Treatment in Children With Prior Craniopharyngioma: An Analysis of the Pharmacia and Upjohn International Growth Database (IGDS) From 1988 to 1996

This article presents data regarding the use of human growth hormone (hGH) in children with craniopharyngioma. Extensive data (collected from 1988 to 1996) were extracted from the Pharmacia and Upjohn International Growth Database. The database showed that 488 patients had a prior history of craniopharyngioma (280 boys, 208 girls). The modality of treatment of craniopharyngioma was known in 451 cases: 251 were treated with surgery alone; 144 had surgery plus irradiation; 12 received only irradiation; and 44 had received no surgery or radiation. hGH treatment was begun at a median time of 1.56 years (mean,  $2.23 \pm 1.88$  years) after tumor diagnosis and was given in a mean dose of  $0.49 \pm 0.15$  IU/kg/wk (0.15 mg) in 3 to 7 injections. Of the group, 40.4% were treated with hGH alone, but others received hydrocortisone and other replacement hormones.

Three hundred ninety-four children completed 1 year of hGH treatment; 152 who were prepubertal at the start of treatment completed 5 years of hGH treatment. The median height SDS increment was 0.9 after 5 years. The gain in height SDS was not influenced by tumor recurrence. Bone age increased 4.5 years in 5 years. Seventy-eight males and 53 females who completed hGH treatment to ultimate height were at a median height SDS of  $-0.7$ ; 58.8% were above  $-1$  SD in relation to target height. Mean height velocity during the final year of hGH treatment was 4.3 cm/y. Adverse effects included tumor recurrence, with 63 recurrences in 54 patients (11%) after a median of 3.7 years after the initial diagnosis; the longest interval between initial diagnosis and tumor recurrence was 10.3 years.

The authors point out that the response of children with treated craniopharyngioma to exogenous GH was similar to that seen in idiopathic growth hormone deficiency. Growth over 5 years was not influenced by the recurrence of tumor. They also state that they were unaware in every case of the factors involved in the decision to discontinue hGH, but that final height had not been achieved in many of these individuals at that time. Finally, they point out that the recurrence rate of 11% is greater than the rate of 6% to 7% reported in the National Cooperative Growth Study (NCGS) sponsored by Genentech Inc.

Price D, et al. *Hormone Res* 1998;49:91-97.

**Editor's comment:** *These are important data and help answer the question: "When does one begin GH therapy in children with treated craniopharyngioma?" The individuals reported in this study began their treatment at a mean of 2.3 years after tumor diagnosis. What remains unclear is why the decision was made to begin therapy at that time.*

*The authors are correct in pointing out that their recurrence rate is greater than that from the NCGS in the United States for craniopharyngioma (6.4%). The conclusions from NCGS and the current report suggest that exogenous GH does not increase the risk for tumor recurrence.*

William L. Clarke, MD

*For a complete review of the diagnosis and management of craniopharyngioma, see GGH 1994;10(3):6-10.*

## Metabolic Effects of Long-Term Growth Hormone Treatment in Prepubertal Children With Chronic Renal Failure After Kidney Transplantation

Patients included in this report on metabolic data for the German Study Group for Growth Hormone Treatment in Chronic Renal Failure (CRF) had a height SDS of  $\leq -2.0$  and/or a height velocity  $< 25$ th percentile, a glomerular filtration rate (GFR) of  $< 60$  mL/min/1.73 m<sup>2</sup> in conservatively treated patients, and a GFR  $> 20$  mL/min/1.73 m<sup>2</sup> in patients after renal transplantations (RT). Fifty-three children were prepubertal at the start of recombinant human growth hormone (rhGH) therapy and remained prepubertal throughout the observation period. Twenty-nine of the patients were on conservative treatment for CRF, 14 patients were on dialysis, and 10 other patients had functioning renal allografts. All were on immunosuppressant therapy with cyclosporine, azathioprine, and methylprednisolone.

Twelve healthy prepubertal children being evaluated for idiopathic short stature formed the control group. None had rhGH deficiency but had received rhGH therapy. The CRF patients received rhGH at a dose of 28 to 30 IU/m<sup>2</sup>/d (0.31 to 0.33 mg/kg/d). Control subjects received rhGH 24 IU/m<sup>2</sup>/d (0.26 mg/kg/d). Biochemical examinations included HbA<sub>1c</sub>, GFR, and a standard oral glucose tolerance test (OGTT), including insulin values.

Prior to administration of rhGH, HbA<sub>1c</sub> and glucose responses during the OGTT were significantly elevated in all patient groups compared with controls. Fasting and integrated glucose concentrations were significantly higher in dialyzed patients than in those treated conservatively or those with RT. As anticipated in RT patients, the fasting 2-hour postprandial glucose was

positively correlated with the daily corticosteroid doses. Fasting serum insulin levels were elevated in the renal failure patients, with the highest levels being in the posttransplant group.

Fasting and OGTT glucose responses did not change throughout the observation period. However, fasting and stimulated insulin levels were 2-fold increased compared with baseline after the first year of rhGH therapy in the dialysis and RT patients, as well as in the controls. Insulin levels in the conservatively treated group became significantly elevated only after the second treatment year. Four patients, 2 on conservative treatment, 1 on dialysis, and 1 RT recipient, developed transient impairment of oral glucose tolerance as defined by the National Diabetes Group of the National Institutes of Health.

In conclusion, the authors observed a selective increase in fasting and glucose-stimulated insulin secretion without a change in glucose tolerance in patients with CRF after RT, but also in short normal children in response to rhGH therapy. This phenomenon was exaggerated in patients on dialysis and after RT, and persisted for up to 5 years of rhGH treatment. Although the absence of increased glucose intolerance during long-term rhGH treatment is

reassuring with respect to the diabetogenic potential of rhGH, the persisting hyperinsulinemia, combined with the dyslipidemia associated with CRF, raises concerns that rhGH therapy may contribute to the long-term risk for premature atherosclerosis in patients with childhood-onset CRF.

Haffner D, et al. *Pediatrics* 1998;43:209-215.

**Editor's comment:** *This interesting study demonstrates that the effects of rhGH on glucose-stimulated insulin secretion are not different for children with CRF and those with idiopathic short stature. The authors point out that associated hyperinsulinemia may be of particular concern in children with uremia and other factors, including dyslipidemia, which may contribute to atherosclerosis. These data are compatible with those reported on US patients and summarized in GGH (1996;12[4]:49-53) by Dr. Richard Fine. He points out in his review that there have been no clinical consequences associated with the hyperinsulinemia, as corroborated by the German Study Group. However, the long-term effect of such treatment remains to be shown.*

William L. Clarke, MD

## Familial Hyperinsulinism Caused by an Activating Glucokinase Mutation

Hyperinsulinism (HI) is relatively common. It is the most common cause of hypoglycemia. Affected individuals are at risk for seizures and permanent brain damage. Glaser et al describe a family with HI associated with a mutation in the glucokinase gene. Glucokinase is an enzyme with low affinity for glucose that controls the rate-limiting step of beta-cell glucose metabolism.

A mutation of the glucokinase gene has been detected in a 31-year-old white male, his 2 children, his sister, and his father. The mutation was sought in the proband after he became unconscious with low plasma glucose (38 mg/dL)

and elevated insulin levels. Counter regulatory hormone responses were normal, and so were pancreatic CT and MRI findings. The proband's 2 children had hypoglycemic seizures and also were diagnosed with HI. Urinary amino acids and urinary and plasma carnitines were normal, as was a pancreatic ultrasound. The proband's sister was diagnosed as having hypoglycemia at the age of 15 years; she had low fasting blood glucose and high plasma insulin levels, and later developed multiple sclerosis. Oral glucose tolerance tests in the proband and his sister showed hypoglycemia 3 hours after taking glucose. During hypoglycemia, their plasma insulin and C-peptide concentrations were elevated. In the proband and his sister, exogenous insulin administration resulted in a decrease in plasma glucose and plasma C-peptide concentrations. The proband's sister's children were normal. The proband's father had symptoms of hypoglycemia, which were controlled by diet. At the age of 48 years, the father developed insulin-dependent diabetes mellitus. All the affected family members were treated with diazoxide 100 to 300 mg/d. This same mutation was not detected in 37 unrelated white families, including 6 with an apparently autosomal dominant form of hyperinsulinism.

The Val455Met mutation in the glucokinase gene results in an increased affinity of glucokinase for glucose, resulting in a higher rate of glycolysis and therefore a higher rate of insulin secretion. This represents a

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clinically and biochemically distinct autosomal dominant form of familial hyperinsulinism.

Glaser B, et al. *N Engl J Med* 1998;338:226-230.

**Editor's comment:** Identification of this mutation in the glucokinase protein is another step in understanding glucose homeostasis. It is clear that different mutations within the same gene give rise to different phenotypes requiring different therapies. The Val203Ala mutation within the same gene results in loss of function and gives rise to maturity-onset diabetes mellitus. That particular glucokinase mutation has been identified in about 50% of individuals with gestational diabetes. By contrast, this new type of mutation leads to hyperinsulinism and hypoglycemia. This contrast illustrates that the domain of mutations within a gene can lead to striking differences in phenotypes.

Judith G. Hall, MD

**2nd Editor's comment:** Most patients with familial HI have a defect in the sulfonyleurea protein resulting from a

*SUR gene mutation. An excellent article to review in conjunction with this article is by Permutt et al, entitled "FHI: An Inherited Disorder of Spontaneous Hypoglycemia in Neonates and Infants" (Diabetes Reviews 1996;4:347-353). Permutt et al provide the foundation to better understand the etiologies and variations of familial HI, previously called leucine-sensitive hypoglycemia and/or nesidioblastosis.*

Robert M. Blizzard, MD

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## Births After Intracytoplasmic Injection of Sperm Obtained by Testicular Extraction From Men With Nonmosaic Klinefelter's Syndrome

Klinefelter's syndrome results from the presence of an extra X chromosome (47,XXY) in males. It is a relatively common sex chromosomal abnormality, occurring in about 1 in 500 males. Some individuals with Klinefelter's syndrome are mosaics, ie, they have both 46,XY and 47,XXY cells. Individuals who are mosaic (46,XY/47,XXY) may have some degree of spermatogenesis and may be fertile, compared with nonmosaic Klinefelter men (47,XXY), who typically have azoospermia and infertility.

Palermo et al have reported 2 couples in which the nonmosaic Klinefelter's syndrome males had undergone testicular sperm extraction (followed by in vitro fertilization by intracytoplasmic injection of single sperm) and thereby were able to father healthy newborn infants.

In the case reports, the men were 32 years and 34 years old and their wives were 32 and 33 years old. Both women were healthy and normal, while both men had nonmosaic Klinefelter's syndrome (47,XXY). Both men had gynecoid habitus, gynecomastia, and bilateral atrophic testes. The first man had bilateral varicocele; the second man had a moderate-size left varicocele. Both men had high serum gonadotropin and low serum testosterone levels. Both men had only Sertoli cells on testicular biopsies. Three semen analyses of the first man showed normal volumes and fructose and a single abnormal nonmotile sperm in 1 semen specimen.

Analysis of the 3 semen samples from the second man revealed low volume, normal fructose, and no sperm.

Both women were given leuprolide (a gonadotropin-releasing hormone agonist) subcutaneously to inhibit gonadotropin secretion and then a combination of human menopausal gonadotropin and follicle-stimulating hormone intramuscularly. Oocytes (15 to 40) were retrieved by ultrasonographically guided transvaginal needle aspiration after intramuscular administration of chorionic gonadotropins.

Simultaneous testicular biopsies were performed in the men. Both men had received testolactone 3 months before having a testicular biopsy. After intracytoplasmic sperm injection and fertilization of oocytes, embryos (3 for each woman) were selected and transferred. Both couples refused preimplantation diagnosis.

Both women received daily intramuscular injections of 50 mg progesterone in oil until fetal heartbeats were confirmed by ultrasound. The ultrasound of the first woman at 32 days of embryo transfer revealed 2 asymmetric uterine sacs, only one of which had a fetal heartbeat. Ultrasound of the second woman showed 2 intrauterine sacs, both with fetal heartbeats. Amniocentesis at 20 weeks showed a fetal karyotype of 46,XY in the first pregnancy and fetal karyotype of 46,XX and 46,XY in the second pregnancy.



A healthy boy was born to the first couple; he had a birth weight of 2,778 g at 38.5 weeks gestation. Two healthy children, a 2,551-g boy and a 2,410-g girl, were born by cesarean section to the second couple.

Palermo G, et al. *N Engl J Med* 1998;338:588-590.

**Editor's comment:** Assisted reproductive technology has become increasingly important and has revolutionized the treatment of infertility. Preimplantation diagnosis is now possible in which DNA can be analyzed from a single blastomere. This allows selection of disease-free embryos for transfer to the uterus. In the pregnancies described above, the parents chose to take no risk of loss at that stage, but

*opted for more conventional prenatal diagnosis by amniocentesis. Intracytoplasmic sperm injection is a relatively new development in assisted technology and provides new hope for couples in whom in vitro fertilization has failed or when there is paucity of viable sperm. This technique is quite promising for nonmosaic Klinefelter's syndrome men, who may now be able to have their own biologic children through this new technology. Interestingly, the number of men recognized to be infertile has been increasing for more than a decade. Precise diagnosis of male infertility will help to provide options to couples.*

Judith G. Hall, MD

## Screening for Retinopathy in the Pediatric Patient With Type 1 Diabetes Mellitus

Diabetic retinopathy is the leading cause of blindness in the United States, in patients between the ages of 20 to 74 years. Individuals with type 1 diabetes mellitus are at a high risk for developing diabetic retinopathy. The American Academy of Pediatrics has recently published a statement regarding recommendations for ophthalmologic evaluation of asymptomatic children with type 1 diabetes mellitus.

The statement provides background about diabetic retinopathy and the rationale for the ophthalmologic examination for diabetic retinopathy. An examination schedule for diabetic retinopathy for asymptomatic individuals with type 1 diabetes mellitus also is suggested (Table).

The objective and goals of the statement are to (1) develop a program for assessing children with type 1 diabetes mellitus on a regular basis to prevent diabetic retinopathy as part of the diabetic management; (2) identify children who may be at risk for developing diabetic retinopathy; (3) refer patients appropriately for ophthalmologic examination; and (4) educate individuals with diabetes and their families regarding the benefits of good diabetic control. The members of the committee believe referral to an ophthalmologist for follow-up is essential because ophthalmologists are much better able to detect early retinopathy than primary care physicians. HMOs often are reluctant to refer patients to ophthalmologists for such exams, and this poor practice is unacceptable.

Sections of Endocrinology and Ophthalmology American Academy of Pediatrics. *Pediatrics* 1998;101:313-314.

**Editor's comment:** The American Academy of Pediatrics guidelines will be useful for pediatricians and pediatric endocrinologists taking care of children with type 1 diabetes mellitus. Good control of diabetes mellitus is a

*first step in preventing diabetic retinopathy. Prompt laser eye surgery can prevent further visual deterioration and delay the onset of blindness as a result of diabetic retinopathy.*

Judith G Hall, MD

### Suggested Ophthalmologic Examination Schedule for Asymptomatic Pediatric Patient With Type 1 Diabetes

#### INITIAL DISCUSSION

Within the first year after diagnosis, child and/or parents should receive counseling by a pediatrician or pediatric endocrinologist regarding the need for ophthalmologic examination and early intervention.

#### INITIAL EXAMINATION BY AN OPHTHALMOLOGIST\*

3 to 5 years after diagnosis if >9 years of age

#### FOLLOW-UP EXAMINATION†

Annually

#### DURING PREGNANCY

During first trimester, then every 3 months until delivery

\* Poor control or deterioration may indicate an earlier initial examination. An ophthalmologic examination also should be performed in poorly controlled patients before intensification of therapy.

† Abnormal findings will dictate more frequent follow-up examinations.

## Partial Hormone Resistance in Mice With Disruption of the Steroid Receptor Coactivator (SRC-1) Gene

The authors demonstrate that in mice inactivation of the steroid receptor coactivator (*Src-1*) leads to decreased growth of the gonads and sex hormone-dependent structures (uterus, prostate) but does not impair fertility. *Src-1* influences gene transcription by increasing histone acetyltransferase activity and other mechanisms, thus enhancing receptor-mediated nuclear gene transcription. The investigators inactivated *Src-1* by deleting ~9 kb of its genomic sequence (446 amino acids) from embryonic stems and then inserting these cells into blastocysts of a strain of C57 mice, thus generating chimeric founders. They then bred heterozygous and homozygous *Src-1*-deficient mutant animals. Heterozygous animals were normal. Homozygous *Src-1*-deficient animals were phenotypically normal, but responded subnormally to several steroid hormones. Uterine growth in response to estrogen was significantly attenuated when compared with normal animals, as was testosterone-induced prostate growth. Testicular size was decreased and breast development impaired in homozygous mutants. Serum concentrations of estradiol and testosterone were slightly elevated in the *Src-1*<sup>-/-</sup> animals. Surprisingly, fertility was normal. In part, the defect in *Src-1* expression was compensated for by increased synthesis of related steroid receptor coactivators such as *TIF2*. The writers concluded that *Src-1* is important for efficient steroid hormone action in vivo.

Xu J, et al. *Science* 1998;279:1922-1925.

**Editor's comment:** Nuclear estrogen receptor (ER)-associated proteins help mediate the transcription-activating effects of the estrogen-ER complex.<sup>1</sup> Flanking the DNA binding domain (DBD) of the ER are 2 independent transcription-activating domains (AF-1 on the amino terminal side of the DBD and AF-2 on the carboxyl terminal side of the DBD, overlapping but distinct from the hormone binding domain). Transcription activated through the AF-2 site is mediated by several coactivating proteins that bind to the AF-2 site after the change in conformation of the ER that accompanies its binding to ligand estrogen occurs.<sup>1</sup> One wonders how soon it will be until patients with partial insensitivity to steroid hormones due to inactivating mutations of steroid receptor coactivating proteins are identified clinically and genomically.

Allen W. Root, MD

Halachmi S, et al. *Science* 1994;264:1435-1438.

**2nd Editor's comment:** Could these ER-associated proteins or similar augmentors contribute to the variations in breast size among women of similar body size and/or adiposity?

Robert M. Blizzard, M.D.

## Of Fingers, Toes, and Penises\*

*Hox* genes encode DNA-binding transcription factors that regulate and coordinate the relative positioning of structures during embryologic development.<sup>1</sup> There are 4 *Hox* complexes in vertebrates. Experimental mutations in *Hox* genes alter the position of the regulated skeletal structures (limbs, digits) as well as their size. Kondo et al developed mice with compound mutations in different *Hox* genes: (1) the hypodactyly allele, which is a deletion of *Hoxa-13*; and (2) null alleles in *Hoxa-13* or *Hoxd-11*, -12, and -13. These compound mutant mice had total digital agenesis and agenesis of the genital eminence with absence of the penis in male animals and absence of the bladder and urethra in females. The authors concluded that *Hoxa* and *Hoxd* genes regulate development of the morphogenetic ends of the body—digits at the ends of limbs and genital structures at the end of the trunk.

Kondo T, et al. *Nature* 1997;390:29.

\*Capital letters (*HOX*, *HOXA*, *HOXD*) designate human genes; small letters (*hox*, *hoxa*, *hoxd*) represent animal genes.

**HOX genes.1** Mutations in *HOXd-13* are associated with synpolydactyly, an autosomal dominant disorder characterized by duplication of the 3rd and 4th fingers and syndactyly of the 3rd, 4th, and/or 5th toes. The mutation in *HOXd-13* (chromosome 2q31) involves expansion of a sequence of 15 consecutive alanine residues in the N-terminal region of the normal gene to one with 22 to 29 alanine residues and is thought to be associated with gain-of-function of this protein. A dominant-negative mutation (deletion of 20 amino acids necessary for DNA binding) in *HOXA-13* (chromosome 7p14-p15) has been associated with the hand-foot-genital syndrome, a disorder characterized by hypoplasia of the thumbs and great toes, clinodactyly of the 5th fingers, abnormalities of the carpal and tarsal bones, penile hypospadias in males, and abnormal urethras and ureters and malformed uteri in females. It is likely that mutations in 1 or more *HOX* genes may be found in patients with minor skeletal malformations (clinodactyly, brachydactyly, brachymetacarpia, brachymetatarsia).

Allen W. Root, MD

Innis JW. *Curr Opin Pediatr* 1997;9:617-622.

**Editor's comment:** These experimental findings shed light on the genetic mechanisms that are aberrant in clinical human malformation syndromes involving the

## Randomized Trial of Growth Hormone in Short Normal Girls

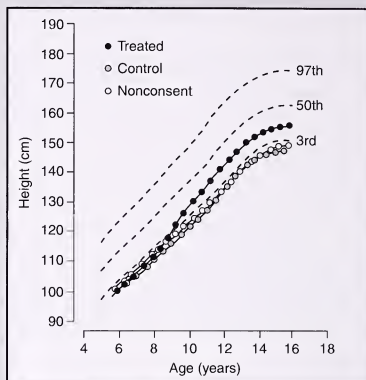
In a randomized study, the investigators administered recombinant human growth hormone (rhGH) (30 IU/m<sup>2</sup>/wk [0.33 mg/kg/wk]) to 7 normal short girls (height more than 2 SD below mean height for age) for an average of 6.2 years each. They compared the growth of these children to that of 6 girls who were randomized to a control, untreated group and 19 girls who did not consent to a randomized selection process.

The authors found (Figure) that at near-final height (chronologic age, 16 years) the rhGH-treated subjects were: (1) substantially taller (155.3 cm) than either untreated group (randomized, 147.8 cm; nonrandomized, 149.3 cm); (2) all within their target height range; and (3) 3.5 cm taller than their pretreatment predicted height (whereas the other groups were a mean of 5.5 cm below predicted height). Neither bone age nor puberty advanced more rapidly in rhGH-treated subjects than in the other groups. The increased stature in the rhGH-treated subjects was realized before the onset of adolescent maturation. The authors concluded that rhGH increased final height in normal short girls without affecting the timing or rate of progression of puberty.

McCaughey ES, et al. *Lancet* 1998;351:940-944.

**Editor's comment:** The majority of studies of the effectiveness of rhGH in increasing height in short normal subjects have been performed in males and have been disappointing.<sup>1</sup> McCaughey et al now report that rhGH can increase adult stature in females. Buchlis et al<sup>2</sup> also report that the adult height of short females receiving rhGH was 6.8 cm greater than that of (historical) control subjects, while adult stature of rhGH-treated males was only 3.0 cm greater than that of (untreated) control males. These observations in small groups of short females are tantalizing. Coupled with the observation that rhGH increases adult stature in patients with Turner syndrome,<sup>3</sup> one wonders if the genes on the Y chromosome that influence growth and program the taller stature of

### Mean Growth Patterns for Treated Girls and Controls



Reprinted with permission from McCaughey E, et al. *Lancet* 1998;351:940-944.

males somehow inhibit the growth-promoting effects of exogenous GH in this sex.<sup>4,5</sup>

Allen W. Root, MD

- Guyda HJ. *Trends Endocrinol Metab* 1994;5:334-340.  
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### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.



## Mutations in *PROP1* Cause Familial Combined Pituitary Hormone Deficiency

The investigators identified 4 families with combined pituitary hormone deficiency due to homozygous or compound heterozygous inactivating mutations of *PROP1*, the "prophet of *Pit-1*," a transcription factor necessary for expression of *POU1F1* (the human homologue of mouse *Pit1*). *POU1F1* is essential for differentiation of the somatotrope, lactotrope, and thyrotrope. Its deficiency results in hypoplasia of the pituitary gland and subnormal secretion of growth hormone (GH), prolactin (PRL), and thyrotropin. *PROP1* has 3 exons encoding a 226 amino acid paired-like homeodomain protein with DNA binding properties. Each of the 3 mutant *PROP1* genes resulted in decreased DNA binding of the product and, hence, to decreased transactivation of a reporter gene. Patients with mutations of *PROP1* lacked not only GH, PRL, and thyrotropin

secretion but also luteinizing hormone and follicle-stimulating hormone as well, and were sexually immature. Magnetic resonance imaging revealed hypoplastic pituitaries. The authors concluded that *PROP1* is important for differentiation of gonadotropes and, through expression of *POU1F1*, of somatotropes, lactotropes, and thyrotropes.

Wu W, et al. *Nature Genet* 1998;18:147-149.

**Editor's comment:** These elegant studies identify yet another gene necessary for differentiation of the anterior pituitary that now includes *Lhx3* (LIM homeodomain *Zn-binding transcription factor*), *POU1F1*, *GH-releasing factor*, and its receptor.

Allen W. Root, MD

## Frequency of Inherited Bleeding Disorders in Women With Menorrhagia

Menorrhagia is a common gynecologic problem and accounts for 12% of referrals to gynecologists. It involves abnormal uterine bleeding occurring at regular intervals that is excessive in amount and duration. Adolescent girls may have excessive uterine bleeding as they establish their menstrual periods; however, if it is persistent, then a gynecologic evaluation is warranted. There are many etiologies. However, if the pelvic examination is normal, then genetic bleeding disorders should be considered.

Kadir et al screened 150 women with menorrhagia in order to find out what proportion of women with menorrhagia have a genetically related bleeding disorder. Uterine blood loss was assessed by means of a pictorial blood assessment chart. The following were determined for each woman: full blood count, blood grouping, activated partial thromboplastin time, factor VIII activity, von Willebrand factor antigen activity, and factor XI levels.

The authors found that 26/150 (17%) women with menorrhagia who had a normal pelvic examination had a genetically related bleeding disorder: 15/26 had mild von Willebrand's disease; 3/26 had moderate to severe von Willebrand's disease; 4/26 had mild factor XI deficiency; 1/26 had mild von Willebrand's disease and factor XI deficiency; 1/26 had combined von Willebrand's disease, factor XI deficiency, and factor X deficiency; 1/26 was a carrier of hemophilia A; and 1/26 had platelet dysfunction. Overall, 13% had von Willebrand's disease and 4% had factor XI deficiency. Menorrhagia since menarche was noted in 11/123 women (8.9%) without a bleeding disorder, 13/20 women (65%) with von Willebrand's disease, and 4/6 (66.7%) with factor XI

deficiency. Women with von Willebrand's disease and factor XI deficiency had prolonged activated partial thromboplastin time. They found that individuals with von Willebrand's disease had a history of easy bruising, bleeding after tooth extraction, postpartum hemorrhage, and postoperative bleeding.

The authors suggest that clinicians treating individuals with menorrhagia should take a careful medical history and test for inherited bleeding disorders, especially von Willebrand's disease. More than 50% of the affected women would have been missed if screening had been done only on the basis of symptoms.

Kadir RA, et al. *Lancet* 1998;351:485-489.

**Editor's comment:** Kadir et al found that 1 in 6 women presenting with menorrhagia and a normal pelvic exam has a hereditary bleeding disorder. They point out that they may have a selected population. Nevertheless, the diagnosis of a hereditary bleeding disorder has important implications for prenatal diagnosis, genetic counseling, and future invasive procedures for the affected woman. Because von Willebrand's disease is so common (1.4% of the population), it seems likely that cases of menorrhagia that may be due to von Willebrand's disease are being missed. Although the incidence of bleeding disorders in adolescent females will be less than in adult women, some cases of bleeding disorders—particularly von Willebrand's disease—are being missed. Endocrinologists and gynecologists should be aware of this possibility and avoid it by taking a thorough history and screening for the disorder.

Judith G. Hall, MD



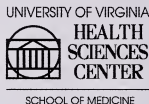
**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Which of the following are reduced in hypopituitarism in adults?
  - a. skin thickness
  - b. total skin collagen
  - c. LBM
  - d. BMI
2. The use of GH in AGHD may not lead to increased bone density for a year or more.
  - a. true
  - b. false
3. The use of hGH Rx has been shown to be psychologically beneficial in >90% of adults with GHD.
  - a. true
  - b. false
4. hGH is unknown to:
  - a. increase anabolism
  - b. increase energy expenditure
  - c. increase peripheral conversion of  $T_4$  to  $T_3$
  - d. increase fat oxidation
  - e. increase insulin sensitivity to CGHD
  - f. increase IGF-1
  - g. decrease GFR

**Answer Key:** 1. a, b, c 2. a. 3. b. 4. a, b, c, d, e, f

**CME Accreditation Statement**

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The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Sönksen and Weissberger report no conflicts. Drs. Lifshitz, Clarke, Horton, Hall, Rosenfeld, and Slyper report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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## Pediatric Endocrinopathies Related to Reduced Fetal Growth

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### INTRODUCTION

Over the past years, pediatric endocrinology has witnessed the fragile scaffolding of a novel concept based upon 4 solid cornerstones. The concept associates reduced prenatal growth to a series of postnatal endocrinopathies. It is currently unknown whether the latter are sequelae of the former or whether both are the result of a more fundamental defect that remains to be defined.

The *first cornerstone* for this concept—as for all medical paradigms—consists of a series of carefully documented clinical observations. The *second cornerstone* is the principle of the “critical window,” originally derived from undernutrition studies in early life (Figure 1, page 2)<sup>1,2</sup> and nowadays established not only within the cascades of genetic switches directing tissue differentiation but also within other developmental events that proceed rapidly and are therefore vulnerable. The *third cornerstone* is the recognition that some components of the endocrine system (such as the anterior pituitary, the gonads, and the inner zone of the adrenal cortex) are more active in early life than in childhood or adulthood, implying that modulation of pronounced prenatal activity might account for some of the postnatal variations that had remained unexplained. The *fourth cornerstone* was

provided by Barker and coworkers,<sup>3</sup> who compiled evidence consolidating the aforementioned principles as indeed applicable to endocrine-related disorders in adulthood and senescence.

Convincing evidence linking reduced prenatal growth to pediatric endocrinopathies is accumulating. This article summarizes available data on these relationships in regards to the emerging implications for clinical practice. In particular, we focus on some aspects of the somatotrophic axis, adrenarche and pubarche, sexual differentiation and gonadal function, lipid metabolism, and insulin sensitivity. Most of the discussed observations concern children with either no dysmorphic condition or with a Silver-Russell morphotype.

### SOMATOTROPIC AXIS

By definition, approximately 3% of human infants are born small for gestational age (SGA), or less than the 3rd percentile. The vast majority of these infants experience an early and rapid catch-up growth and reach normal stature by 2 years of age.<sup>4</sup> Approximately 10% maintain a height below  $-2$  SD, at least throughout childhood.<sup>5,6</sup> The mechanisms underlying the growth failure in this small percentage remain incompletely understood. In short SGA children there is an increased incidence of growth hormone deficiency (GHD), either in the classic form, which is detectable by conventional stimulation

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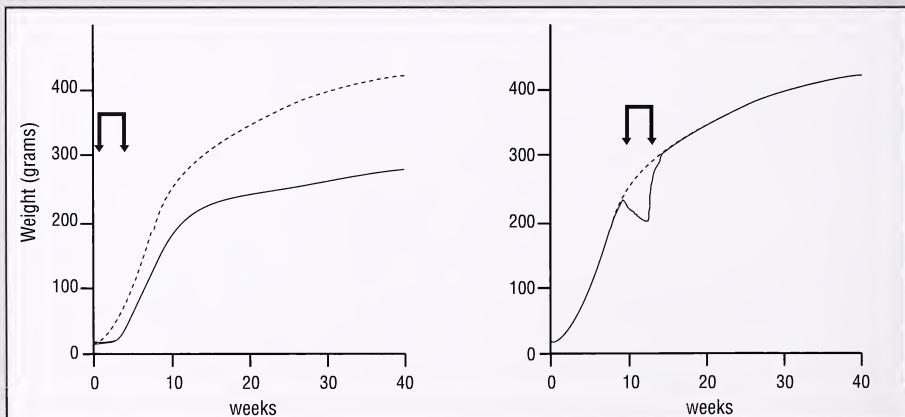
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tests, or in the more subtle neurosecretory form, which is detectable by growth hormone (GH) profile studies.<sup>7</sup> However, the majority of short SGA children appear to be neither GHD nor GH-resistant, as indicated by basal serum levels of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) that are within the normal range, and/or by normal IGF-1 and IGFBP-3 responses to exogenous GH, and/or by relatively low serum leptin concentrations.<sup>8,9</sup> Thus, available evidence points towards some form of IGF-1 resistance as the predominant factor responsible for the persistent growth failure in this group. The pathophysiology of this type of IGF-1 resistance remains to be clarified. It may have been induced by an adverse prenatal environment that programed the growth plates at a lower level of responsiveness within a critical window of time.<sup>10-12</sup> In addition, GHD in SGA children is commonly associated with IGF-1 resistance, which is thought to account for an average reduction of 20% in the growth-promoting efficacy of GH therapy when given to these children.<sup>12</sup> When a supraphysiologic dose of GH is administered (Figure 2), the growth response of short SGA children regardless of their secretory GH status readily matches that of other GHD children, indicating that the IGF-1 resistance towards growth can be overcome and that a normal height and weight can be obtained at least throughout childhood.<sup>12</sup> Therefore, it is anticipated that the indications and the doses for GH therapy in children will become increasingly interlinked with the emerging

principles of endocrine programming in early life. GHD children, if not born SGA, are candidates for continuous GH therapy using a physiologic dose and, if born SGA, using a higher dose.

For non-GHD SGA children, the 2 major determinants of the growth response to exogenous GH are (1) the dose of GH administered and (2) the age of the child. The higher the GH dose and the younger the child, the more pronounced the response.<sup>7,12</sup> Two treatment avenues are being explored (Figure 3). The more conventional strategy is to treat these SGA children as if they were GHD, ie, with a continuous GH regimen using a slightly supra-physiologic dose (0.03 mg/kg/d) throughout childhood. A more innovative strategy aims at restoring the altered responsiveness within the growth plate by administering a high dose (0.07 to 0.1 mg/kg/d) of GH treatment early in life and over only 2 to 3 years. This approach results in an early and rapid normalization of body size, thus mimicking, albeit at a later age, the spontaneous catch-up growth that failed to occur in these children. More importantly, there are now preliminary data up to 5 years after GH withdrawal indicating that early and high-dose GH treatment may have the capacity to reprogram the growth pattern at a higher level in these children (Figure 4, page 4).<sup>13</sup> If these findings are confirmed, they may result in the development of an early and brief treatment regimen with fewer injections and an improved cost:benefit ratio compared with the more conventional strategy.

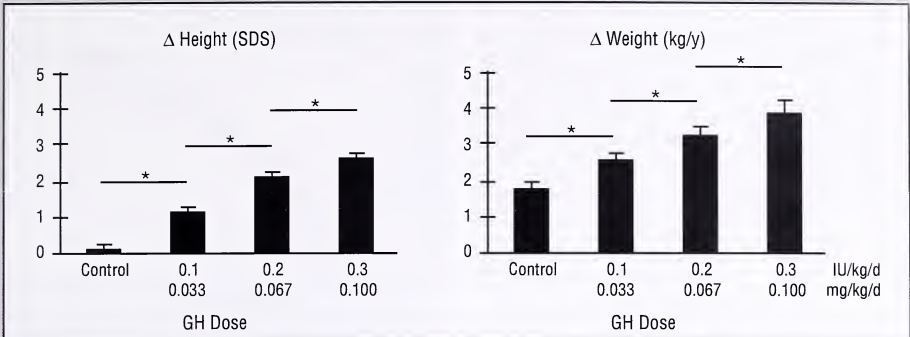
Figure 1



Evidence for "the critical window" phenomenon is demonstrated. Dotted lines indicate the average weight gain from birth in rats. Full lines represent the average weights of infant rats who were undernourished (noted by arrows) early in life or later (after 3 weeks). Permanent effects occur if undernutrition is present early, suggesting "a critical window of time" for a phenomenon to occur for subsequent adversity.

Adapted with permission from McCance RA, et al. *Proc R Soc Med (Lond)* 1962;156:326-337; Widdowson EM, et al. *Proc R Soc Med (Lond)* 1963;158:329-342.

Figure 2



GH-induced catch-up growth is demonstrated to be dose dependent. Epianalysis results of height and weight (means  $\pm$  SEM) from 3 independent randomized controlled studies in short prepubertal SGA children without GHD who were treated with 1 of 3 doses of GH over 2 years are presented. \*  $P < 0.01$ .

Adapted with permission from de Zegher F, et al. *Trends Endocrinol Metab* 1998;9:233-237.

Finally, we emphasize that to date, the different GH-treatment regimens applied to SGA children have a reassuring safety profile and do not lead to inappropriate acceleration of bone maturation or to disproportionate growth, eg, within the craniofacial complex.<sup>12,14</sup>

### PRONOUNCED ADRENARCHE AND PRECOCIOUS PUBARCHE

Almost 80% of the fetal adrenal consists of the so-called fetal adrenal zone, which is located in the inner area of the cortex and which virtually disappears in early infancy. It has been known for 25 years that

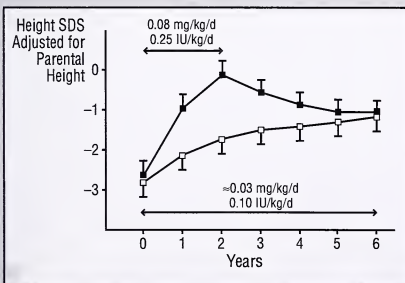
reduced fetal growth is associated with reduced size of the fetal adrenal zone and reduced concentrations of dehydroepiandrosterone sulfate (DHEAS) in fetal serum. "Adrenarche" refers to the endocrine process that results in rising serum concentrations of adrenal androgens produced by the zona reticularis within the inner part of the adrenal cortex. Adrenarche occurs normally between 6 and 8 years of age. "Pubarche" refers to the appearance of pubic hair. By definition, pubarche before the chronologic age of 8 years is considered to be precocious.

Two independent studies recently indicated that adrenarche is more pronounced in children born SGA as compared with appropriate-for-gestational age (AGA) controls, particularly if the SGA children had experienced spontaneous catch-up growth.<sup>15,16</sup> These biochemical findings are already becoming of clinical relevance as idiopathic precocious pubarche, a frequent clinical expression of early adrenarche, also has now been associated with reduced fetal growth (Figure 5, page 4).<sup>17</sup>

### MALE PSEUDOHERMAPHRODITISM AND SUBFERTILITY, OVARIAN HYPERANDROGENISM AND ANOVULATION

The embryonic gonads play a crucial role in sexual differentiation. Subsequently, during fetal and neonatal life the human gonads present a high profile of secretory activity. In turn, this phase is followed by a decade of low activity under neuroendocrine inhibition, and then by gonadal hormone production and pubertal development, which leads to a fully reproductive status.

Figure 3

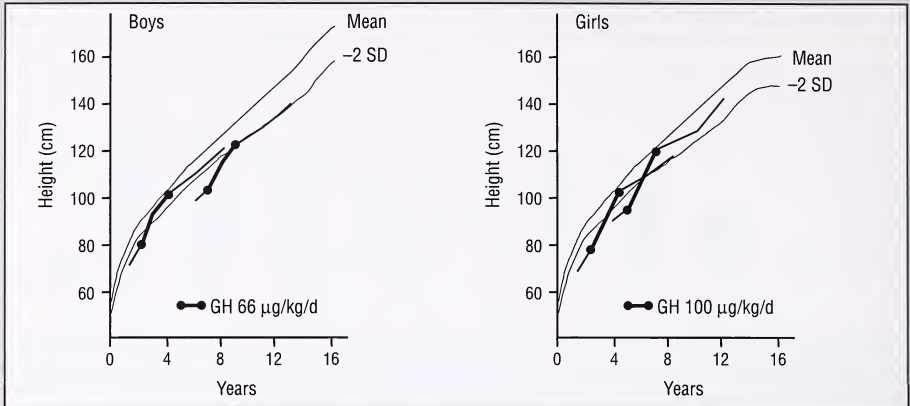


Two years of GH treatment at high dose ( $n = 13$ ) produces the same ultimate growth 6 years later as 6 years of treatment at a lower dose ( $n = 14$ ). The mean age at start of treatment was 4 years in these prepubertal SGA children.

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Figure 4

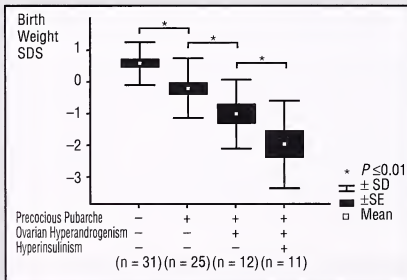


The growth curves of these 4 prepubertal children illustrate that marked catch-up growth during high-dose GH treatment for 2 years may be followed initially by catch-down growth over a few years, but subsequently by sufficient spontaneous growth to maintain height at a higher level than the original level.

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Androgen insensitivity, a form of male pseudohermaphroditism,<sup>18</sup> was found to result in a slightly reduced birth weight ( $-0.4$  SD on average).<sup>19</sup> Even more intriguing is the recent finding that unexplained forms of male pseudohermaphroditism appear to be associated with a markedly reduced birth weight ( $-2$  SD on average).<sup>20</sup>

Figure 5



The birth weights (SDS) of postmenarcheal control girls are compared with postmenarcheal girls having had precocious pubarche with or without ovarian hyperandrogenism (OH) and with or without hyperinsulinemia. The diagnosis of OH was based on the response of 17 hydroxyprogesterone to LHRH agonist. Hyperinsulinemia when demonstrated occurred during a standardized OGTT.

Adapted with permission from Ibanez L, et al. *J Clin Endocrinol Metab* 1998;83:3558-3562.

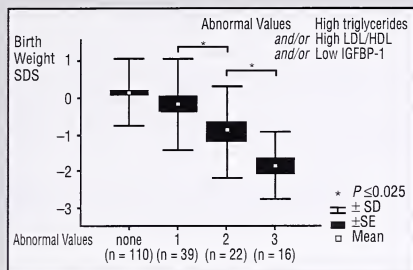
While reduced fetal growth has been associated with subsequent testicular dysfunction for half a century,<sup>21,22</sup> only recently has a specific link with male subfertility been recognized.<sup>23</sup> Reduced fetal growth is thought to be accompanied by an early decrease in the number and/or function of Sertoli cells, which, in turn, appear to be one of the key determinants of adult spermatogenesis.<sup>23</sup> Reduced fetal growth also has been associated with a reduced fraction of primordial follicles in fetal ovaries,<sup>24</sup> with ovarian hyperandrogenism in adolescent girls (Figure 5),<sup>17</sup> and with anovulation, particularly dating from late adolescence.<sup>25</sup> The timing of menarche and menopause, however, are quite independent of birth weight.<sup>26,27</sup>

## DYSLIPIDEMIA AND INSULIN RESISTANCE

A few years ago, Barker et al identified the relation between reduced fetal growth and syndrome X in senescent men.<sup>28</sup> One of the mechanisms by which prenatal undernutrition may induce postnatal insulin resistance is by altering—within a critical window of time—the relative rates of multiplication of cells destined to form the perivenous and the periportal zones within the hepatic lobule, such that a relative but permanent excess of glucokinase and lack of phosphoenolpyruvate carboxykinase expression ensue.<sup>29</sup>

Asymptomatic insulin resistance has now been documented in SGA children with and without spontaneous catch-up growth, and is often detectable before

Figure 6



Birthweight SD scores of girls (age range 5 to 20 years) with (from left to right) either none, one, two, or all three of the following abnormal serum concentrations: high triglycerides, high LDL/HDL ratio, low IGFBP-1.

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puberty.<sup>17,30</sup> In the sequence of pediatric endocrinopathies linked to reduced fetal growth, insulin resistance was found to be associated with the lowest birth weights (Figure 5). In accord with the latter findings, reduced fetal growth also has now been linked to low serum IGFBP-1 concentrations, which are thought to reflect hepatic hyperinsulinism in children and adolescents. Hypertriglyceridemia and an increased serum low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio often occur (Figure 6), indicating that children and adolescents with SGA already tend to display an atherogenic lipid profile.<sup>31</sup>

## CONCLUSION

There is increasing evidence that reduced prenatal growth is associated with a modulation in the function of multiple components within the postnatal endocrine system. A minority of children born SGA experience a persistent reduction of postnatal growth, which has been attributed to either classic or neurosecretory GHD and/or to some form of IGF-1 resistance. The short stature of most prepubertal SGA children can be normalized with a variety of GH treatment regimens; however, on average, a slightly higher than conventional dose seems to be required. Adrenarche appears to be amplified in SGA children, and idiopathic precocious pubarche has been associated with relatively low birth weights. Similar associations have been found for male pseudohermaphroditism and subfertility and for ovarian hyperandrogenism and anovulation. Finally, insulin resistance has been documented in both prepubertal and pubertal SGA children with and without spontaneous catch-up growth, and dyslipidemia also has been linked to reduced fetal growth. Thus, children born small appear to be at increased risk for experiencing endocrine and metabolic

dysfunction; in addition, the lower the birth weight, the more prone the child seems to be to developing a broad spectrum of endocrine and metabolic anomalies (Figures 5 and 6). The challenge now is to identify the molecular and cellular mechanisms underlying the aforementioned associations so that more than merely symptomatic treatments can be designed. A new chapter in pediatric endocrinology has begun.

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## Recognition of Children With Psychosocial Short Stature: A Spectrum of Presentation

The conclusion of Gohlke et al is that psychosocial short stature (PSS) should be considered in every patient with "idiopathic" growth hormone deficiency (GHD) or when growth hormone (GH) treatment is ineffective in a child with presumed GHD. Symptoms such as hyperphagia, abnormal eating habits, disturbed behavior, global developmental lag, enuresis, or encopresis should prompt consideration of PSS, especially when there is a possibility of associated sexual abuse, which is frequently present in patients with this symptom complex.

Forty of the 65 children studied were single family cases; however, 25 represented multiple cases within 12 families. In half of the families, the parents were divorced or separated. The educational and work background of the parents was usually in the lower and unskilled categories. Unemployment of fathers was significantly excessive.

Sex distribution was equal. A remarkable finding was that only 29% of full-term newborns had a birth weight >3,000 g. Twenty-one percent of all patients were premature (<37 weeks). Bone age was delayed by an average of 1.9 years. Body mass index was normal in all patients.

Fifty-four percent had eating problems, 42% behavioral problems, 26% encopresis, and 18% nocturnal enuresis; 12% urinated in inappropriate places or as an aggressive act. Many suffered physical abuse as well as psychological abuse. Bizarre eating habits, etc, were common in the physically abused. The authors noted that their data after assessment were very different from the data given in the physician referral letters; items such as hyperphagia, enuresis, and encopresis were not mentioned at all in referral letters but were often obtainable when inquiry was made.

Three case histories were presented that emphasized different important aspects of this syndrome. In case No.1, failure to respond to GH treatment as expected in a child with presumed GHD was present. GH insensitivity may be the presenting sign of PSS. In case No. 2, separation of the affected children with PSS from the abusing family did not result in catch-up growth. When this happens, an adverse environment should be looked for in the second home. In addition, some patients who have an adequate initial response to GH subsequently fail to grow at an

appropriate rate for a child with GHD under treatment; when this occurs, PSS should be suspected. The authors emphasize that not all PSS patients have classic findings of PSS. Case No. 3 demonstrated that abuse cannot always be proven, although several features of PSS may be present.

In the discussion, emphasis is made that a detailed psychological history should be taken for hyperphagia, polydipsia, and hoarding or scavenging food in all children suspected of having GHD.

Gohlke BC, et al. *J Pediatr Endocrinol Metab* 1998;11:509-517.

**Editor's comment:** *This syndrome is underdiagnosed in most pediatric clinics. These 65 children with PSS presented over a 7-year period to the pediatric endocrine clinic at Great Ormond Street. This syndrome is much more common than most pediatric endocrinologists believe, and the diagnosis often is not made because the physician does not think of the possibility. In retrospect, I myself have missed the diagnosis several times, although I am considered an authority in the field. Exemplary is my misdiagnosing GHD initially but finally diagnosing PSS in presumed GHD children who were not responding to GH satisfactorily.*

*This article is abstracted because of its importance and because clinicians dealing with short stature need to consider the syndrome, know its variations, and suspect its presence, particularly in children of low normal or low birth weight and in short children who come from disrupted homes.*

Robert M. Blizzard, MD

Previous publications relating to PSS appearing in *Growth, Genetics, & Hormones* are listed immediately below.

Abuse or Psychosocial Dwarfism: An Update. 1985;1(4):1-4.

Physiological GH Secretion During the Recovery From PSS. A Case Report. 1989;5(2):14.

Psychosocial Growth Failure: A Positive Response to GH and Placebo. 1992;8(4):13.

Letter to and Letter from the Editor. 1993;4(1):9.

A New Stress-Related Syndrome of Growth Failure and Hyperphagia in Children Associated With Reversibility of GH Insufficiency. 1997;13(1):9-11.

## A Prolactin-Releasing Peptide in the Brain

The investigators isolated a gene for a 7-transmembrane, G-protein-associated, orphan receptor, itself expressed primarily in the pituitary; they then transfected this receptor into Chinese hamster ovary cells and thereafter isolated 2 peptides from hypothalamic extracts that bound to this receptor and had specific prolactin-releasing activity. They identified the bovine, rat, and human genes coding for these peptides. The human gene encodes an 87 amino acid peptide with a 22 amino acid signal peptide, prolactin-releasing 31 amino acid (amino acids 23-53) and 20 amino acid (amino acids 34-53) peptides, and a carboxyl terminal 34 amino acid sequence. The carboxyl terminal glycine of both peptides must

be amidated for full bioactivity. The prolactin-releasing activity of the 31 amino peptide (PrRP31) was comparable to that of thyrotropin-releasing hormone. This report also demonstrated the importance of the arachidonic acid signal transduction pathway in prolactin secretion, as it was this signal that was employed to monitor the isolation and identification of these peptides.

Hinuma S, et al. *Nature* 1998;393:272-276.

**Editor's comment:** *A specific prolactin-releasing substance to complement the prolactin inhibitory activity of dopamine has*

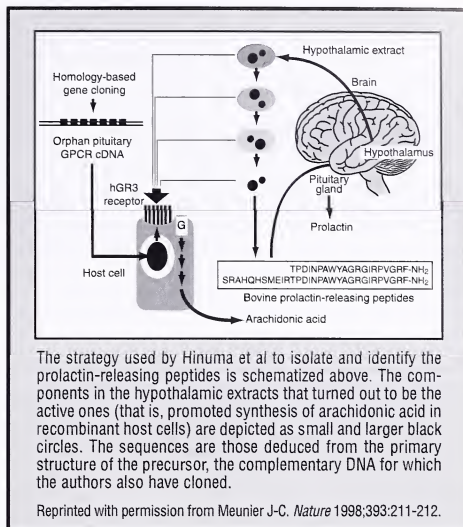


long been sought and now appears to have been isolated. The physiologic role of the prolactin-releasing peptides is unclear, and their diagnostic or therapeutic relevance remains to be assessed. The "reverse" process by which the prolactin-releasing peptides were found (ie, identification of an orphan receptor and then its ligands) indicates the revolution in biologic investigation in which we have the privilege of participating. Details concerning the lactotroph cell membrane receptor specific for the prolactin-releasing peptides are awaited. Meunier's comments and entire article (*Nature* 1998;393:211-212) are exceedingly worthwhile reading.

Allen W. Root, MD

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## GH Dependence and GH Withdrawal Syndrome in GH Treatment of Short Normal Children: Evidence From Growth and Cardiac Output

Lampit et al evaluated the efficacy of interrupted growth hormone (GH) therapy in prepubertal children with idiopathic short stature (ISS). Their protocol was to treat normal short children for a period of 3 years or until they reached the 25th percentile and then to discontinue therapy at a young age (no more than 9 years of age) and follow them until final height. The criteria for ISS were height  $< -2$  SD, growth rate more than  $-1$  SDS, bone age  $< 75\%$  of chronologic age, and serum GH concentration following arginine stimulation of  $> 10$   $\mu\text{g/L}$ . Thirty-four children were studied, 12 of whom served as a control group. In addition to measuring the children, Doppler echocardiographic evaluation was performed before recombinant human GH (rhGH) therapy, yearly for 2 years during therapy, and at 6 and 12 months after the cessation of therapy.

The children receiving rhGH were treated until their height reached the 25th percentile but for no longer than 3 years, even if they had not reached this percentile. Nineteen of the children completed 3 years of rhGH therapy (0.9  $\text{mg/m}^2$  daily). During the first year of treatment, the growth velocity accelerated as expected. After withdrawal of rhGH, growth decelerated in every child over a 6-month period to a velocity that was significantly lower than pretreatment values. The growth velocity recovered to pretreatment values by the fourth semiannual measurement. Height SDS also increased in the treatment group and then declined somewhat in the second year of therapy. The GH response to arginine was not significantly different after rhGH therapy. Insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) remained unchanged throughout the

study. However, systolic and diastolic parameters fell significantly during the initial 6 months of rhGH withdrawal and remained low for 12 months. Aortic cardiac output also fell significantly during the initial 6 months of rhGH withdrawal. No child had any symptoms referable to these cardiac changes.

The authors state that these data suggest that it may be feasible to interrupt rhGH prior to puberty in order to achieve an improved final height, but they will not know this for certain until the patients reach their final height. More interestingly, their report suggests that rhGH treatment is associated with the development of a physical adaptation to continuous high levels of GH. The rhGH withdrawal symptoms were not induced by alterations of serum GH or IGF-1.

Lampit M, et al. *Eur J Endocrinol* 1998;138:401-407.

**Editor's comment:** This is a fascinating report. It has become more and more apparent that adults with GH deficiency (GHD) have significant improvement in cardiac function when they are restarted and maintained on replacement therapy. It would appear that the administration of rhGH to children who do not manifest GHD induces a dependency on rhGH for cardiac function.

Indeed, when rhGH is interrupted there is a significant reduction in cardiac output. Although the authors state that there have been no clinical symptoms associated with the cardiac findings, the effect of either longer periods of rhGH administration or withdrawal



withdrawal has not been studied. It would be of interest to repeat these studies periodically over several years before concluding that there are no permanent changes associated with the

initiation and subsequent withdrawal of rhGH in normal short children.

William L. Clarke, MD

### Quality of Life of Young Adults With Idiopathic Short Stature: Effect of Growth Hormone Treatment

The authors assess the quality of life (QOL) and well-being of 89 fully grown, young adults with idiopathic short stature (ISS), some of whom had been treated with recombinant human growth hormone (rhGH) (N=24, 16 males; 0.2 to 0.3 mg/kg/wk for 3.8 to 8.1 years) and others who had not (N=65, 40 males). They also compared the data for these subjects with data for the average Dutch population of similar age. The mean adult height for all ISS children was  $-2.35$  SD. Except for a decrease in the number of rhGH-treated subjects with a partner, there was no difference between the rhGH-treated and nontreated subjects with ISS in educational attainment, state of general health, personality inventory, or psychosocial/employment difficulties encountered because of short stature. The adult heights of the 2 populations were similar. The rhGH-treated subjects achieved an adult stature that was 3.3 cm greater than the pretreatment predicted adult height (range,  $-9.9$  to  $+13.4$  cm); interestingly, the rhGH-treated individuals estimated their adult height to be 13 cm (range, 0 to 28 cm) greater than they would have reached without rhGH administration, a perception also shared by their parents. Although expressing a desire to be taller, when the rhGH-treated and nontreated ISS subjects were asked if they were willing to risk loss of longevity in order to achieve greater stature by taking a lifelong medication, or to risk loss of life by a height-increasing surgical procedure, only a minority (11% to 22%) indicated a willingness to do so. Most ISS subjects were satisfied with their heights. In comparison to the general

Dutch population, there were no meaningful differences in QOL of the ISS subjects, whether treated with rhGH or not (see Table). The significance of the lower frequency of a partner in the rhGH-treated ISS subjects was unclear but not considered significant as it did not differ from the general Dutch population. The authors concluded that "the QOL of rhGH-treated and untreated young adults with ISS was similar and equal to the general population."

Rekers-Momborg LTM, et al. *Acta Paediatr* 1998;87:865-870.

**Editor's comment:** These data indicate that: (1) subjects with ISS do not differ from taller peers in their QOL; (2) administration of rhGH does not meaningfully increase adult stature or improve the QOL of treated subjects; and (3) the perception of the effectiveness of rhGH in increasing height is vastly overestimated by the treated subjects and their families. In the experience of this physician, it is the concern of the parents rather than of the child who has been brought with ISS for pediatric endocrine consultation. It is essential that the pediatric endocrinologist confronted with the normal child with ISS fully inform the family about the limited expectations of therapy with rhGH on adult stature and future well-being, and emphasize the greater likelihood that the child's stature will have minimal impact on his function as an adult.

Allen W. Root, MD

### The Mean (SD) Values for Dimensions of the Dutch Restricted Version of the Minnesota Multiphasic Personality Inventory (NVM) for rhGH-Treated and Control Children With Idiopathic Short Stature (ISS)

| Dimension of the NVM   | Treated ISS<br>(n = 17) | Controls ISS<br>(n = 47) | Standard Population<br>(n = 809) |
|------------------------|-------------------------|--------------------------|----------------------------------|
| Negativism             | 17.9 (10.3)             | 15.7 (9.7)               | 14.7 (7.7)                       |
| Somatization           | 5.9 (6.6)               | 4.7 (4.4)                | 5.3 (5.3)                        |
| Shyness                | 8.6 (7.5)               | 9.6 (7.6)                | 8.0 (6.4)                        |
| Severe psychopathology | 3.1 (2.4)               | 2.7 (3.5)                | 2.7 (2.7)                        |
| Extroversion           | 20.4 (5.1)              | 19.7 (4.8)               | 17.1 (5.3)                       |

Reprinted with permission from Rekers-Momborg LTM, et al. *Acta Paediatr* 1998;87:865-870.

### Final Height After Combined Growth Hormone and Gonadotropin-Releasing Hormone Analogue Therapy in Short Healthy Children Entering Into Normally Timed Puberty

Controversy continues as to the feasibility of using gonadotropin-releasing hormone analogues (GnRHa) in combination with recombinant human growth hormone (rhGH)

to increase final height by delaying puberty and slowing bone maturation in short non-growth hormone-deficient (GHD) children. Lanes and Gunczler evaluated the effect of 2½ years of

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## ***GROWTH, Genetics, & Hormones***

Volume 15, Number 1

### **Post-Program Self-Assessment/CME Verification**

#### **Answer Sheet**

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combined rhGH and GnRHa therapy in 10 short children (7 girls, 3 boys) with a mean chronologic age of  $11.8 \pm 1.2$  years, mean bone age of  $11.2 \pm 0.9$  years, and mean height  $-2.4 \pm 0.4$  SD below the 50th percentile. These children had an initial mean predicted height of  $150.7 \pm 9.8$  cm (target height,  $160.7 \pm 5.3$  cm) and all were in Tanner stage-II puberty. The control group included 7 girls and 3 boys with a mean chronologic age of  $11.4 \pm 1.0$  years, mean bone age of  $11.0 \pm 0.8$  years, and mean height  $-2.3 \pm 0.4$  SD below the 50th percentile who were in Tanner stage-II puberty. They had a mean predicted height of  $151.8 \pm 10.1$  cm (target height,  $159.5 \pm 5.1$  cm). No subject had GHD as determined by responses to clonidine stimulation. The subjects in the study group received rhGH at a dose of  $0.1$  U/kg/d SC 6 days a week ( $0.2$  mg/kg/wk) and GnRH (leuprolide acetate) at a dose of  $0.3$  mg/kg IM q28d. Subjects were treated for  $30 \pm 5.2$  months.

As anticipated, combined therapy resulted in interruption of pubertal development. Growth velocity decreased from  $6.5 \pm 1.6$  cm/y to  $3.9 \pm 1.3$  cm/y during the second year of combined therapy, resulting in a height z-score reduction from  $-2.4 \pm 0.4$  to  $-2.6 \pm 0.7$  SD (see Figure). Bone age maturation declined as well, averaging  $0.5$  bone age year/year of treatment. Predicted final height improved slightly by 12 months, but at the end of treatment was similar to baseline. The predicted final height of the study population after 2 years of therapy was  $150 \pm 8.0$  cm while that of the control population was  $151.2 \pm 9.4$  cm. After the discontinuation of combined therapy, growth velocity did not improve but the bone age advanced more rapidly, averaging  $2.0 \pm 0.4$  cm/y of follow-up. The mean final height of the study group was  $151.7 \pm 2.4$  cm, not greater than the mean pretreatment predicted final height of  $150.7 \pm 9.8$ .

Lanes R, Gunczler P. *Clin Endocrinol* 1998;49:197-202.

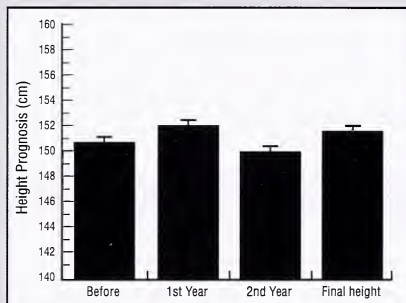
**Editor's comment:** Although this is a study of a relatively small number of subjects, the inclusion of a very well-defined control group strengthens the findings. In this particular study, it is very clear that the combined use of rhGH and GnRHa did not increase final adult height. Whether initiating therapy in children

in slightly earlier stages of puberty or administering larger doses of rhGH might have resulted in a better outcome remains to be seen. The authors point out that this is only the second study that they know of following children receiving combined therapy to final height. The other study was conducted by Balducci and colleagues. The findings by Balducci's group produced similar results as reported by Lanes and Gunczler. These findings are important and should caution pediatric endocrinologists to be conservative in suggesting that such therapy may be of benefit to non-GHD short children who are entering puberty. Previously, several studies using GnRH alone were equally none productive.

William L. Clarke, MD

Balducci L, et al. *J Clin Endocrinol Metab* 1998;80:3596-3600.

**Predicted Adult Height  
Before and During Treatment  
and Final Height of Patients**



Reprinted with permission from Lanes R, Gunczler P. *Clin Endocrinol*. 1998;49:197-202.

## Morbidity in Turner Syndrome

This is a report using data from the Danish Cytogenetic Central Register and the Danish National Registry of Patients to assess the morbidity of women with Turner syndrome (TS) during the 10 years from January 1, 1984, to December 31, 1993. This study includes all women living in Denmark during that period. Five hundred ninety-four women with TS were identified. The observed number of diagnoses among TS patients was compared with the expected number calculated from the incidence in the study base. Not surprisingly, the relative risk of having an endocrine diagnosis, particularly hypothyroidism and thyroiditis, was much greater in women with TS than in the general female population (see Table). Insulin-dependent and noninsulin-dependent diabetes also were increased. Congenital malformations were most consistent among patients with 45,X karyotype. Osteoporosis and fractures occurred more

frequently in women with TS, especially in the metacarpal bones and in the femoral neck. Fractures of the spine, ulna, and radius also were seen more frequently. The relative risk of cancer among women with TS does not seem to be elevated compared with the general population, except for cancer of the rectum and colon. In addition, women with TS had a significantly increased relative risk of heart disease, arteriosclerosis, hypertension, and vascular disease of the brain. Finally, the relative risk of cirrhosis of the liver was significantly elevated. As expected, the relative risk of endocrine diseases reached a maximum in the young age groups, while the relative risk of fractures reached a maximum in the older age group. The authors stress that women with TS have a risk profile similar to that of postmenopausal women.

Gravholt C, et al. *J Clin Epidemiol* 1998;51:147-158.



**Editor's comment:** This is a very large and carefully performed epidemiologic study. The finding that women with TS have a relative risk profile similar to that of post-menopausal women suggests ways in which pediatric and adult endocrinologists might intervene to significantly alter this morbidity. In Denmark, it is suggested that most women with TS receive lifelong sex steroid replacement

therapy. The specific age at which therapy is initiated remains somewhat controversial as pediatric endocrinologists attempt to maximize the height of these young girls. It is important, however, to stress to these young girls the importance of continuing therapy throughout adulthood as a possible protection against excess morbidity.

William L. Clarke, MD

### Relative Risk of Endocrine Diseases in Turner Syndrome

| Diagnoses (ICD-8)  | Observed | Expected | RR (95% CI)        |
|--|----------|----------|--------------------|
| Endocrine diseases, overall (240-258)                      | 51       | 10.47    | 4.87 (3.63-6.41)   |
| Thyroid diseases, overall (240-246)                        | 10       | 4.98     | 2.00 (0.96-3.69)   |
| Thyrotoxicosis (242)                                       | 3        | 1.50     | 2.01 (0.41-5.86)   |
| Hypothyrosis (244)   | 3        | 0.52     | 5.80 (1.20-16.94)  |
| Thyroiditis (245)  | 3        | 0.81     | 16.60 (3.42-48.50) |
| Insulin-dependent diabetes mellitus (249)                  | 9        | 0.78     | 11.56 (5.29-21.95) |
| Noninsulin-dependent diabetes mellitus (250)               | 13       | 2.88     | 4.38 (2.40-7.72)   |
| Miscellaneous endocrine diseases<br>[251-258 (-251.13-15)] | 15       | 1.72     | 8.71 (4.87-14.36)  |
| Parathyroid disease (252)                                  | 1        | 0.14     | 7.25 (0.18-40.37)  |
| Hypoglycemia (251.00)*                                     | 2        | 0.57     | 3.51 (0.43-12.69)  |

\* Based on diagnosis on the 5-digit level.

CI, confidence interval

RR, relative risk

Reprinted with permission from Gravholt C, et al. *J Clin Epidemiol* 1998;51:147-158.

### Growth Failure in Prader-Willi Syndrome Is Secondary to Growth Hormone Deficiency

Thacker and colleagues conducted a retrospective study of 16 children who met the diagnostic criteria for Prader-Willi syndrome (PWS). All had hypotonia during the neonatal and infantile periods, failure to thrive, rapid weight gain after the first year of life, dwarfism, hypogonadism, developmental delay, and hyperphagia. The 15q11-13 chromosomal abnormality had been identified in 13 of the 16. Growth hormone deficiency (GHD) as defined by growth hormone (GH) response <10 ng/mL on a provocative test was present in 12 of the 16. All the nonobese patients were GHD. Seven of the children were treated with recombinant human GH (rhGH) at a dose of 0.3 mg/kg/wk, and rhGH therapy continued for 6 to 23 months (see Table). All 7 children treated with rhGH had significant increases in their growth velocity. The authors state that although their study was retrospective, it suggests that the secretion of GH in PWS children is related to abnormalities of the hypothalamic-pituitary axis rather than to their obesity per se. This is based in part on the association of low insulin-like growth factor 1 (IGF-1) levels in most, which is not attributable to obesity, plus low GH responses to pharmacologic stimulation.

Thacker M, et al. *Hormone Res* 1998;49:216-220.

### Annualized 6-Month Pretreatment and Posttreatment Growth Velocities (GV) in Prader-Willi Syndrome Children Treated With Recombinant Human Growth Hormone at a Dose of 0.3 mg/kg/wk

| Patient No. | Pretreatment<br>GV (cm/y) | Posttreatment<br>GV (cm/y) |
|-------------|---------------------------|----------------------------|
| 5           | 4.5                       | 10.00                      |
| 9           | 5.16                      | 13.92                      |
| 10          | 3.90                      | 13.20                      |
| 11          | 3.60                      | 12.00                      |
| 14          | 0.00                      | 6.76                       |
| 15          | 1.80                      | 12.30                      |
| 16          | 2.50                      | 7.66                       |

Reprinted with permission from Thacker M, et al. *Hormone Res* 1998;49:216-220.

**Editor's comment:** This interesting report describes another clinical syndrome, PWS, in which GHD plays a major role. The children in the study were well characterized both by clinical and laboratory criteria. These investigators have made a worthy retrospective analysis of a potential etiology of GHD and rhGH

treatment in PWS. The lack of a response to provocative stimuli and subsequent increases during rhGH therapy underscore the importance of evaluating these children when seen in the clinical situation.

William L. Clarke, MD

## Growth Hormone Treatment of Children With Prader-Willi Syndrome Affects Linear Growth and Body Composition Favorably

Lindgren et al studied 29 prepubertal children with Prader-Willi syndrome (PWS), all of whom had a paternal deletion or a maternal disomy of chromosomal region 15q11-13, hypotonia, hypogonadism, hyperphagia, obesity, short stature, psychomotor retardation, behavioral abnormalities, and dysmorphic features. In addition, 10 control healthy obese prepubertal children were studied. Growth hormone (GH) was sampled every 30 minutes for 24 hours and plasma insulin-like growth factor 1 (IGF-1), glycosylated hemoglobin, and fasting insulin and glucose were determined. Body mass and body mass index were calculated. Fat-free mass was determined by bioelectrical impedance and by dual energy X-ray absorptiometry. Fifteen of the 29 children with PWS were treated with GH at a dose of 0.1 IU/kg/d (0.23 mg/kg/wk) SC for 1 year. The others served as a second control group.

The obese control children were tall and had normal increased serum IGF-1 levels, whereas the PWS children were short with normal or low IGF-1 levels. However, 24-hour GH secretion was low in both PWS and obese control children. During the 1 year of treatment, significant increases in height velocity were observed. Serum IGF-1 levels increased as well. Body mass index decreased and a 25% reduction in fat mass and a 30% increase

in fat-free mass were observed. Fasting insulin levels increased significantly in the treated group, but fasting glucose and glycosylated hemoglobin levels were unchanged throughout the study. The authors state that these studies demonstrate that the majority of these patients were GH deficient and that GH deficiency is part of the hypothalamic dysfunction observed in this disorder.

Lindgren A, et al. *Acta Paediatr* 1998;87:28-31.

**Editor's comment:** This description of the beneficial effects of GH in children with PWS supports those of Thacker et al recorded in the previous abstract. The children in the current study did not undergo stimulation tests, and thus their findings are not entirely biochemically comparable to those of Thacker et al. However, the growth responses of both groups were similar, and the additional finding of increased fat-free mass and decreased fat mass in the current study (Lindgren) demonstrates an additional important benefit of GH in these children.

William L. Clarke, MD

Thacker M, et al. *Hormone Res* 1998;49:216-220.

## Fathers and *FGFR3* Mutations

Achondroplasia is the prototype of short-limb dwarfism and is by far the most common form of dwarfism in humans. It results from activating mutations of the gene encoding fibroblast growth factor receptor 3 (*FGFR3*). The vast majority of cases result from new mutations, which in all cases involve nucleotide 1138 in exon 10 of this gene. This nucleotide is thus one of the most mutable nucleotides in the entire human genome.

The association of sporadic cases of achondroplasia with advanced paternal age has been recognized for years, suggesting that mutations at this site preferentially occur during spermatogenesis. The Wilkin group has now demonstrated this to be true.

Wilkin et al studied 97 families in which a child with achondroplasia had been born to parents of normal stature. They first identified a DNA polymorphism near the mutation site in the *FGFR3* gene. This enabled them to potentially determine if a mutation in a given case had occurred on the maternal or paternal *FGFR3* allele. The analysis was informative in 40 of the 97 families, revealing that the mutation occurred on the paternal allele in all 40 cases. In other words, the mutation had virtually always occurred in the *FGFR3* gene inherited from the father.

The authors discuss differences between spermatogenesis and oogenesis, noting that meiotic errors can accumulate to a much greater extent in the former because male germ cells divide much more often than do female germ cells. However, they acknowledge that the reasons why mutation of this particular nucleotide is so much more common during spermatogenesis are unknown.

Wilkin DJ, et al. *Am J Hum Genet*. 1998;63:711-716.

### In Future Issues

#### Surfing the Web for Information on Genetic and Hormone Disorders

John A. Phillips III, MD

**Editor's comment:** This paper confirms what has been suspected for years—that achondroplasia mutations of FGFR3 occur primarily, if not exclusively, during spermatogenesis. Unfortunately, the mechanism for the high rate of mutation in male germ cells remains obscure. The authors point out that the mutation occurs in the context of CpG dinucleotide, which is thought to predispose to mutation because of methylation and deamination of the G nucleotide. Other possibilities include defective repair of base mismatches that occur at this nucleotide during DNA replication, which for some reason occurs only during spermatogenesis. Another idea, which is pure speculation, is that such mutations adversely affect the survival of female germ cells so that only male germ cells harboring the mutation survive gametogenesis to contribute the mutations to

offspring. It is interesting that recurrent mutations responsible for thanatophoric dysplasia occur in FGFR3 nucleotides that neighbor nucleotide 1138. This suggests that the underlying mechanism operates not just on the one nucleotide but also on the surrounding area, making it a very hot spot for mutation.

William A. Horton, MD

**2nd Editor's comment:** In GGH 1997;13(4):49-54, Dr. Horton wrote an enlightening lead article entitled, "Molecular Genetics of Human Chondrodysplasias," which can profitably be read in conjunction with the abstract and editor's comments above.

Robert M. Blizzard, MD

## Celiac Disease and Turner Syndrome

The authors initially observed 2 of 26 patients with Turner syndrome (TS) who did not experience increased growth as expected when given recombinant human growth hormone (rhGH). These two GH-resistant patients were then diagnosed as having celiac disease (CD) antibodies, a characteristic of CD. Both patients had subtotal villus atrophy in the gastrointestinal tract, which confirmed the diagnosis. These findings stimulated screening of 35 TS girls, including the 26 receiving rhGH. Four of the patients, including the first 2, were anti-endomysium antibody (EMA) positive. However, 14 of the 35 patients were positive for antigliadin antibodies, suggesting an immunologic phenomenon seen in CD. The authors confirmed the high coincidence of TS and autoimmune thyroid disease in 6 of the 35 patients and overt hypothyroidism in 4.

The authors conclude that the results of the study indicate that gluten sensitivity may be an associated characteristic in TS, and that screening with EMA together with other autoantibodies is advisable in TS at least before starting rhGH treatment.

Bonomico M, et al. *J Pediatr Gastroenterol Nutr* 1998;26:496-499.

**Editor's comment:** The association of autoimmune diseases, particularly thyroid autoimmune disease, has long been recognized. This is the first account known to me of the possible association of CD and TS and should be explored further.

Robert M. Blizzard, MD

## SHOX Mutations in Dyschondrosteosis

The *SHOX* story began about a year ago with the identification of a gene encoding a homeobox-containing transcription factor that maps to the pseudoautosomal region of the X chromosome. The detection of a missense mutation predicted to truncate the protein in a short child suggested that it or, more appropriately, its absence may play a role in the short stature of Turner syndrome (TS). The story has taken a new turn with the finding of *SHOX* mutations and deletions in patients with dyschondrosteosis (DCS).

DCS, or Leri-Weill syndrome, is a relatively mild dwarfing condition that mainly involves the middle segments of the limbs. The major features are shortening of the lower legs and bowing of the radius associated with the Madelung deformity of the wrist. DCS occurs in both males and females and is usually more severe in females. Its inheritance has been considered autosomal dominant based on several examples of male-to-male transmission. In fact, it has long been suspected that the much more severe condition, Langer mesomelic dysplasia, results from homozygosity for DCS.

Two independent groups, Belin et al and Shears et al, carried out very similar studies. Starting with several large families exhibiting dominant transmission of DCS, both groups first established linkage of DCS to gene markers near the *SHOX* locus. Belin et al also linked DCS to a marker within the *SHOX* gene. Next, both groups detected deletions of the *SHOX* gene in DCS patients; Belin and colleagues found deletions in 7 families and Shears and colleagues had detected deletions in 5 families. Finally, point mutations were found in 2 families that segregated with the DCS clinical phenotype. Both groups concluded that the DCS phenotype results from haploinsufficiency for the *SHOX* transcription factor since patients were either missing 1 *SHOX* allele or had mutations predicted to make the transcription factor nonfunctional.

Both groups also provided evidence that Langer mesomelic dysplasia results from the homozygous loss of *SHOX* function. Langer mesomelic dysplasia had been suspected clinically in an infant with 45,XO TS in 1 of the studies by Belin's group. Molecular studies showed that this patient had no *SHOX* alleles; she inherited an X chromosome harboring a *SHOX* deletion



from her mother and lacked a paternal X or Y chromosome. In the other case, a fetus appeared to inherit an X chromosome deleted for *SHOX* from the mother and a Y chromosome with an abnormal *SHOX* gene from the father.

Both groups acknowledge that there are still many unanswered questions, including why DCS is usually more severe in females than males and why the Madelung deformity occurs in some families and not in others.

Belin V, et al. *Nature Genet* 1998;19:67-69.

Shears DJ, et al. *Nature Genet* 1998;19:70-73.

**Editor's comment:** This article brings out several important points. For instance, it delineates the molecular genetics of DCS and probably of Langer mesomelic dysplasia. It provides further insight into the short stature of TS. It also illustrates that male-to-male transmission of a trait does not always indicate autosomal dominant inheritance. In this case, it indicated what might be called "pseudoautosomal" inheritance.

The 2 point mutations were interesting. First, they are very close to one another, converting arginine 195 and tyrosine

199 to stop codons. The protein products are predicted to truncate the protein downstream of the DNA-binding homeobox domain. It is not surprising that such mutations would produce similar clinical phenotypes. Second, the arginine 195 mutation was the same mutation observed in a family with "idiopathic short stature" in the original report of the *SHOX* gene by Rao et al. This implies that the clinical phenotype of DCS can blend into that of idiopathic short stature. Alternatively, the DCS features may have been so mild as to escape detection in the original family. It will be important to determine which is the case. If it is the former, screening for *SHOX* mutations may become an element in the workup of idiopathic short stature.

These reports do not fully resolve the issue of whether short stature in TS is caused by dysfunction of 1 gene, *SHOX*, or more than 1 gene. However, the presence of the Madelung deformity is not an uncommon feature of TS, and suggests that disturbance of *SHOX* function plays an important role in the pathogenesis of this syndrome.

William A. Horton, MD

Rao E, et al. *Nature Genet* 1997;16:54-63.

## Teratogen-Mediated Inhibition of Target Tissue Response to *Shh* Signaling

In the mouse in which Sonic hedgehog (*Shh*) is knocked out, there is severe holoprosencephaly, a developmental anomaly associated with abnormal formation of the brain, eyes, optic nerves, and pituitary. Covalent binding of cholesterol to *Shh* protein is essential for its normal processing and functioning. Experimentally, administration of agents that inhibit cholesterol synthesis to pregnant rats led to holoprosencephaly in their offspring. The present investigators demonstrated that administration of the plant alkaloid jervine, a compound that is structurally similar to cholesterol and inhibits terminal steps of cholesterol synthesis, causes holoprosencephaly in chick embryos with failure of separation of paired midline structures. In vitro in explant cultures of medial neural plate from chick embryos, addition of jervine inhibited *Shh* signaling; similar findings were observed when other inhibitors of cholesterol synthesis were examined in this system. However, further studies revealed that these agents did not inhibit normal processing of *Shh*, although the generated product was unable to induce signaling. The investigators suggest that in addition to inhibition of cholesterol biosynthesis, these compounds may block normal cholesterol movement within cells and interfere with *Shh*-associated proteins that interact with *Shh* in the intracellular signaling pathway that leads to normal morphogenesis. Thus, cholesterol is important for both proper

preparation of *Shh* for its signaling function and for the cellular response to *Shh*.

Cooper MK, et al. *Science* 1988;280:1603-1607.

**Editor's comment:** In humans with loss-of-function mutations of *Shh*, variable forms of holoprosencephaly occur, the most extreme of which is cyclopia (a single large eye) and the mildest fused central incisor teeth. In patients with the Smith-Lemli-Opitz syndrome associated with mutations in 7-dehydrocholesterol reductase, mild forms of holoprosencephaly occur. The clinical and experimental data indicate that cholesterol influences developmental signaling pathways. In target cells, *Patched* is a protein that binds to and is necessary for *Shh* signaling; *Patched* contains a cholesterol recognition domain. It has been hypothesized that if a cell is deficient in cholesterol, *Patched* may not bind to *Shh* and the signaling pathway is then arrested. Whether these observations bear on the optimal amount of cholesterol that a pregnant woman should ingest is unknown at present.

Allan W. Root, MD

Straus E. *Science* 1998;280:1528-1529.

## Target Height as Predicted by Parental Heights in a Population-Based Study

The authors examined the relationship between the adult stature of 2,402 normal Swedish young adults and that of their parents. As anticipated, there were strong correlations between the heights of the offspring, their parents individually, and particularly their midparental heights (average of mother's height + father's height,  $r=0.59$ ). In further analysis of these data, the investigators

determined equations for calculation of target heights for males and females based on midparental heights that were valid through a range of parental heights. The equations were:

Males: target height =  $45.99 + (0.78 \times \text{midparental height})$   
Females: target height =  $37.85 + (0.75 \times \text{midparental height})$



The 95% predicted interval was  $\pm 10$  cm. When the midparental height was shorter than  $-2$  SDS, these equations overestimated target height by  $+2$  cm. The authors also reported that there was little intergenerational (ie, secular) difference in adult stature ( $+0.7$  cm males,  $+1.0$  cm females), and that even major differences between the heights of the parents did not affect the validity of the equations. The investigators conclude that these equations are to be preferred over the Tanner formulation because they lead to less variation in calculated target height, particularly in very short subjects in whom growth-promoting therapy is to be evaluated.

Luo ZC, et al. *Pediatr Res* 1998;44:563-571.

**Editor's comment:** These data need to be confirmed in other populations, particularly those in which a secular trend toward increasing stature persists. Although knowledge of the target height is useful as a therapeutic goal, the range of  $\pm 10$  cm is still extraordinarily wide. The development of a highly accurate and reliable method to predict adult stature of the untreated short child would be an even more useful advance.

Allen W. Root, MD

## Metabolic Basis of Dysmorphogenesis: Smith-Lemli-Opitz Syndrome

The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive inborn error of morphogenesis. The clinical phenotype includes craniofacial, digital, genital, and visceral abnormalities; microcephaly; mental retardation; and growth deficiency. Elevated levels of 7-dehydrocholesterol (7-DHC) have been found in SLOS patients. Since 7-DHC is the immediate precursor of cholesterol, deficiency of the enzyme responsible for the conversion of 7-DHC to cholesterol,  $\Delta^7$ -sterol reductase, has become a prime candidate mechanism to explain SLOS. Earlier this year, the group headed by Fabian Moebius cloned the cDNA encoding the catalytic subunit for this enzyme. Now they report the cloning of the gene, its mapping in humans to chromosome 11q13, and the identification of mutations in 13 patients with SLOS. Two other groups, working independently, have described mutations in the  $\Delta^7$ -sterol reductase gene in an additional 6 SLOS patients, bringing the total to 19 patients.

As noted in a review by Kelley, 19 different mutations (mutant alleles) have been found to date. Thirteen are missense mutations, 1 is a nonsense mutation, and 5 are frameshift mutations. One mutation, a 134-bp insertion, was found in more than 1 patient. All mutations are predicted to cause loss of enzyme activity; and Fascia et al demonstrated reduced synthesis of enzyme compared with normal for 5 of the missense mutations.

The mechanism by which the biosynthetic block disrupts normal morphogenesis is addressed mainly by Kelley. He points out that in addition to the direct effects on morphogenesis of excessive 7-DHC and deficient cholesterol, the latter may

disturb signaling through hedgehog morphogens. This is because cholesterol is added to sonic hedgehog and probably other hedgehog proteins during their normal processing. The cholesterol mediates attachment of the proteins to the surfaces of nearby cells, thereby restricting their diffusion and consequently their effects on morphogenesis.

Fascia BU, et al. *Proc Natl Acad Sci USA* 1998;95:8181-8186.

Waterham HR, et al. *Am J Hum Genet* 1998;63:329-338.

Wassif CA, et al. *Am J Hum Genet* 1998;3:55-62.

Kelley RIL. *Am J Hum Genet* 1998;63:322-326.

**Editor's comment:** The recent advances in molecular genetics have provided exciting insights into the molecular basis of genetic diseases. However, it is not uncommon to learn of a molecular defect but still not understand how it produces the clinical phenotype. This series of papers is commendable because they go beyond the description of the genetic and even protein defect to put forth a mechanism to explain the pathogenetic actions of the defect.

It is ironic, as pointed out by Kelley, that despite the insights provided by these papers, the mainstay of diagnosis will continue to be measurement of biochemical parameters, such as 7-DHC, because they predict clinical severity and because the diversity of mutations makes them difficult to detect. Finally, it is noteworthy that anecdotal evidence suggests patients with SLOS improve substantially when their cholesterol deficiency is treated.

William A. Horton, MD

## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

## Growth in Children With Craniopharyngioma Following Surgery

Tiulpakov et al attempted to determine why some children rendered growth hormone deficient (GHD) secondary to surgery for craniopharyngioma continue to grow normally postoperatively. Twenty-five patients (14 boys, 11 girls), aged 3.8 to 18.9 years, were studied between 0.5 and 10.0 years after surgery. At the time of the study 22 were receiving thyroxine replacement, 13 glucocorticoid replacement, and 23 desmopressin for diabetes insipidus. None were receiving growth hormone (GH) or sex steroids. All children were prepubertal height. Height velocities were recorded as SDS values. Body mass index (BMI) and bone ages also were determined. Following an overnight fast, insulin-like growth factor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3), IGFBP-1, and prolactin were measured. In addition, GH was measured following stimulation with oral clonidine and after 1 mg/kg GH-releasing hormone (GHRH) intravenously. Serum insulin levels were measured during an oral glucose tolerance test (OGTT).

Height SDS for chronologic age ranged from -4.7 to 0.6. However, 4 of the patients had height SDS values (-1.8 to -1.2) that were within normal limits. Height velocity for chronologic age in the patients under 12 years of age ranged from -4.5 to 8.4. BMI ranged from 14.5 to 38.3, and there was a significant correlation between BMI and height SDS ( $r=0.37$ ,  $P=0.03$ ), but not between BMI and height velocity SDS. Thirteen patients showed hyperinsulinemia during OGTT. Only 2 of the 25 children had significant GH responses to oral clonidine with peaks  $>1.0$   $\mu\text{g/L}$ . Maximal GH after GHRH was  $<5$   $\mu\text{g/L}$  in all but 1 subject. Mean fasting IGFBP-1 was  $104.5 \pm 53.7$   $\mu\text{g/L}$ . Only 8 of 31 measurements of fasting IGF-1 were within normal limits. IGF-1 SDS correlated significantly with both height SDS ( $r=0.77$ ,  $P=0.0002$ ) and bone age SDS ( $r=0.5$ ,  $P=0.03$ ). Fasting IGFBP-3 levels were within the normal range for 12 subjects and correlated significantly with height SDS, but not with height velocity SDS. IGFBP-3 SDS correlated significantly with the log of the insulin area under the curve (AUC) ( $r=0.56$ ,  $P=0.002$ ). Basal prolactin concentrations were slightly elevated in 5 subjects. Forward stepwise regression analyses of height SDS and height velocity SDS were performed. The following variables were included in the initial model: log time after surgery;

tumor location; log BMI; midparental height SDS; log insulin AUC; log GH level after clonidine; log GH after GHRH; prolactin; IGFBP-1; IGFBP-3 SDS; and IGF-1 SDS. IGF-1 SDS was the single most important predictor for height SDS ( $r=0.33$ ,  $P=0.001$ ), while log time after surgery was most strongly associated (negatively) with height velocity SDS.

The authors note that the correlation between height SDS and BMI shows that obese subjects maintain a higher growth rate after surgery than nonobese patients. Hyperprolactinemia and hyperinsulinemia and normal IGF-1 were the most frequent findings in the fast-growing GHD patients. However, the correlation between log of insulin AUC and height SDS indicates that hyperinsulinemic patients maintain higher integrated growth rates after surgery compared with children with low or normal insulin. Significant correlations between the insulin AUC and IGF-1 SDS and IGFBP-3 SDS were observed. The authors concluded that the growth phenomenon in children following craniopharyngioma usually accompanied by obesity is likely to be associated with IGF-1 bioavailability, which may be modulated by insulin.

Tiulpakov AN, et al. *Clin Endocrinol* 1998;49:733-738.

**Editor's comment:** This study of a relatively large number of individuals provides further information concerning the growth of children following craniopharyngioma surgery. The data are particularly interesting since not all of the 25 patients grew and since a large amount of data were examined. The fact that IGF-1 SDS was the single most important predictor of height SDS is not surprising. That IGF-1 levels might be modulated largely by insulin supports clinical observations. Pediatric endocrinologists need to continue to keep careful records with regard to auxologic and hormonal changes that occur in their patients following craniopharyngioma surgery. It would be of particular interest to know whether obese children remain obese once GH therapy is initiated and what changes occur in their growth velocity during that therapy.

William L. Clarke, MD

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**GROWTH, Genetics, & Hormones Volume 15, Number 1**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The concept presented in this article is:
  - a) IUGR infants usually have gross chromosomal defects.
  - b) SGA infants usually do not catch up in their growth until late childhood.
  - c) Increased prenatal growth is usually a genetic or metabolic defect.
  - d) Reduced prenatal growth frequently is related to a postnatal endocrinopathy.
2. Approximately \_\_\_\_ of infants as defined in this article are SGA.
  - a) 10%
  - b) 3%
  - c) 1%
  - d) 5%
3. This percent of infants is in accord with most definitions of SGA in the literature.
  - a) Yes
  - b) No—10% of newborns are usually included as being SGA by definition
4. SGA infants often catch up with normal infants in respect to auxology. \_\_\_\_ are usually in the normal range by \_\_\_\_ years of age in respect to height.
  - a) 90%/2 years
  - c) 60%/8 years
  - b) 70%/5 years
  - d) 50%/10 years
5. Which of the following features are stated to often be associated with SGA?
  - a) An increased incidence of GHD
  - b) Hyperandrogenism
  - c) Insulin resistance
  - d) Addison's disease
  - e) Reduced testicular function

**Answer Key:** 1. d. 2. b. 3. b. 4. a. 5. a. b. c. e

**CME Accreditation Statement**

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.



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The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. de Zegher, Francois, Ibanez, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

Vol. 15 No. 2

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## Surfing the Net for Information on Genetic and Hormone Disorders

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### INTRODUCTION

New findings are reported at an ever increasing rate in a growing variety of journals. Access to current information on clinical and laboratory findings and on who performs genetic tests cannot be found in a single journal or text. Electronic databases are now providing medical professionals rapid access to a wealth of current information and data. These electronic databases can be searched in an interactive way for symptoms and signs that permit generation of differential diagnoses that will often include rare or recently discovered disorders and that professionals probably have never encountered. This access enables these professionals to diagnose cases more frequently and be aware of the subtleties that differentiate alternative diagnoses. These are excellent reasons to use electronic databases. This article attempts to enlighten interested professionals on how to surf the Net for information about genetic and hormone disorders.

### ONLINE MENDELIAN INHERITANCE IN MAN

The Online Mendelian Inheritance in Man (OMIM) electronic database is maintained by the National Center for Biotechnology Information, or NCBI.<sup>1</sup> It is available without charge at <http://www.ncbi.nlm.nih.gov/omim>. It is updated daily. As of March 1999, 10,227 entries were included. A wealth of information is presented on the history, signs, symptoms, diagnosis, and management of, and research findings on, these 10,227 disorders, as are detailed gene and disease-focused maps. Access through "hyperlinks" to a variety of Web sites is another important strength. These hyperlinks include MED

LINE™, the Alliance of Genetic Support Groups, the Cardiff Human Gene Mutation Database (HGMD), and databases on genes that cause retinal diseases, mitochondrial diseases, and a variety of other locus-specific databases. The utility of using these databases and their hyperlinks in clinical applications is discussed below.

### FREQUENTLY USED TERMS RELATED TO THE WEB

Knowing the definitions of terms helpful in using the Web is essential as a first step in surfing the Net.<sup>2</sup>

**E-mail:** An acronym for electronic mail; the use of a network to send and receive messages.

**HTML:** Acronym for hypertext markup language, which enables authors to insert hyperlinks. Clicking on a hyperlink displays another HTML document. Therefore, in a hypertext system, one can navigate by clicking on hyperlinks, which produces a display of another document that also contains hyperlinks.

**Http:** The Internet standard supporting exchange of information on the World Wide Web. Http enables the embedding of hyperlinks in Web documents. Http defines the process by which a Web client uses a Web browser program to originate a request for information

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and send it to a Web server, which is a program designed to respond to Http requests and provide the desired information.

**Hypertext:** A computer text form that allows readers to click on hyperlinks to display another document that also may contain hyperlinks to still other documents.

**Internet:** The worldwide system of linked computer networks that facilitates data communication services such as remote log on, file transfer, E-mail, the World Wide Web, and news groups. The Internet assigns every connected computer a unique Internet address so that any 2 connected computers can locate each other on a network and exchange data.

**Log on/Log off:** The processes of establishing (log on) and terminating (log off) a connection with a network or computer.

**Netscape Communicator™:** A package including a popular Web browser called Netscape Navigator™ that is available for Microsoft Windows™, Macintosh™ computers, and a variety of Unix™ workstations.

**Online information service:** America Online™ (AOL) is a for-profit firm that makes current news, stock quotes, and other information available to subscribers over standard telephone lines.

**Surfing the Net:** Exploring the World Wide Web by following a series of links of interest to the surfer.

**URL:** Acronym for uniform resource locator. On the Web, URLs are strings of characters that precisely identify an Internet's resource types and locations. The following *fictitious* URL identifies a Web document (<http://www.genetic.edu>). (1) (<http://>) indicates the domain name of the computer on which it is stored; and (2) ([www.genetic.edu](http://www.genetic.edu)) fully describes the document's location. In addresses, small letters ([www](http://www) and [http](http://)) are used. In abbreviations not pertaining to addresses, capital letters may be used ([WWW](http://WWW) and [Http](http://Http) or [HTTP](http://HTTP)).

**Web (World Wide Web, or WWW):** A global hypertext system that uses the Internet-linked computer network to facilitate data communication.

**Web browser:** A program that runs on an Internet-connected computer and provides access to the World Wide Web.

**Web server:** A program that accepts Http-formatted requests for information. The server processes these requests and sends the requested document.

**Web site:** A set of related documents making up a hypertext presentation on the World Wide Web. A Web

site usually has a welcome or home page that serves as the initial document.

## GENERATING DIFFERENTIAL DIAGNOSES FOR A DYSMORPHIC NEWBORN

Some form of dwarfism is suspected in a newborn. Ventriculomegaly and short limbs, as detected by fetal ultrasound, were noted at 20 weeks gestation. Chromosome studies based on amniotic fluid revealed a 46,XY pattern with no abnormalities noted. The fetal head size at 30 weeks gestation as noted on ultrasound was stated to be 35 weeks. The ventriculomegaly had resolved, and the limb lengths were those expected for a 29-week fetus. Physical examination detected macrocephaly, macroglossia, downward slanting palpebral fissures, cataracts, and syndactyly of the second and third fingers. Blood glucose was low at 28 mg/dL.

The attending neonatologist believes the baby "looks funny" and wants to know if you, the consultant physician, think the baby has a syndrome. He wants to know if the infant has this syndrome, how to confirm it, and what is the expected prognosis.

You as the consultant physician are unaware of this constellation of clinical findings and/or what syndrome might be present but must solve the problem. A **key-word search** is desirable. Since the World Wide Web might contain helpful information, one needs to initiate a search beginning with the OMIM database. The computer in the nursery is turned on and you or the operator opens Netscape Communicator, America Online, or whatever Web browser the computer has, and type in the OMIM URL or address—<http://www.ncbi.nlm.nih.gov/omim>. The OMIM home page appears on the computer screen and you click on "Search the OMIM Database," and then enter "macrocephaly" as a search term and press the "enter" key. Eighty-one disorders in the OMIM database have macrocephaly listed. This is too many items to consider, so you repeat the search using "cataract" as a finding, and 183 matching entries appear. This number of disorders is impossible to consider, so you search by using "syndactyly." The search produces 168 matching entries—again too many. A decision is then made to find out how many disorders have both macrocephaly and cataract by typing in both macrocephaly and cataract (macrocephaly AND cataract) as a search string. Only 7 entries are listed as having both of these findings. These are (1) #109400 Basal Cell Nevus Syndrome, BCNS; (2) #30700 Hydrocephalus Due to Congenital Stenosis of Aqueduct of Sylvius, HSAS1, HSAS, HYCX; (3) #301050 Alport Syndrome, X-Linked, ATS; (4) #156550 Kniest Dysplasia; (5) \*231680 Glutaricaciduria IIA; (6) #312870 Simpson Dysmorphia Syndrome, SDYS; and (7) \*231675 Glutaricaciduria IIC. The entry num-

## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

bers are in hypertext (color) on the screen, and clicking on the number will open each corresponding entry. To further focus, you add "syndactyly," entering macrocephaly and cataracts and syndactyly as a search string. Only 1 OMIM entry is listed for all 3 of these findings—#312870 Simpson Dysmorphia Syndrome, SDYS.

You have participated in a remarkable accomplishment. In less than 2 minutes, a large electronic database of genetic disorders was searched and a successive series of progressively refined differential diagnoses was generated. Open the matching file (#312870 SDYS)<sup>3</sup> by clicking your mouse on it, and the first page of information appears on the screen for review (Figure 1, page 20). The clinical synopsis under Table of Contents or the complete text of this entry can then be reviewed. Figure 1 is only the first of 7 pages of information on SDYS. Note in Figure 1 that the Database Links below the Table of Contents provide immediate access to other databases, including MEDLINE™. The diagnosis needs to be confirmed, and the HELIX™ database is a directory of laboratories that provide testing for genetic disorders (see list of selected Web sites

on page 21). You register with HELIX and are given a password for professional users. This is used first to access and search the HELIX database for SDS or SDYS or the Simpson-Golabi-Beckel syndrome, and then to find matching labs that provide either clinical or research testing for this disorder. In a matter of a very few minutes, you have generated a working diagnosis (SDYS) and obtained information concerning the pathogenesis, mode of inheritance, and the findings associated with SDYS and access to a lab that can help confirm the working diagnosis. Obviously, after expending just a few minutes one feels much better prepared to talk with the neonatologist and the baby's parents, who are waiting for your opinion.

## GENERATING DIFFERENTIAL DIAGNOSES FOR A FAMILY HAVING UNUSUAL ENDOCRINE PROBLEMS

A 15-month-old male is referred to you by his pediatrician, who suspects he has growth hormone deficiency. The child weighed 3.2 kg at full term following an uncomplicated pregnancy, labor, and vaginal delivery. The height SDS is now -3.2, and the length since age 5 months has become progressively retarded. The child is proportionate and the height and weight are commensurate with each other. The extremities appear normal in length, and no kyphosis, limitation of joint motion, or dysmorphic features are present. His bone age is delayed by more than -2 SD. You see no skeletal abnormalities. Serum thyroxine is abnormally low, but the electrolytes, glucose, urea nitrogen, bicarbonate and anion gap, calcium, phosphorus, and urine pH are all within normal limits. A 16-year-old full sister reportedly received growth hormone (GH) for presumed panhypopituitarism. The blood tests on the 15 month old reveal low serum levels of gonadotropins and thyroxine. Combined pituitary hormone deficiency is logical, as there is no response to GH-releasing hormone, thyrotropin-releasing hormone, and luteinizing hormone-releasing hormone, and the magnetic resonance imaging study reveals a hypopituitary.

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Figure 1

**OMIM Page Showing the First of 7 Pages of Information on Simpson Dysmorphia Syndrome. Note Database Links Below Table of Contents That Provide Immediate Access to Other Databases, Including MEDLINE.**

|           |        |          |
|-----------|--------|----------|
| OMIM Home | Search | Comments |
|-----------|--------|----------|

**#312870 SIMPSON DYSMORPHIA SYNDROME; SDYS**  
*Alternative titles; symbols*

BULLDOG SYNDROME  
 DYSPLASIA GIGANTISM SYNDROME, X-LINKED; DGSX  
 GOLABI-ROSEN SYNDROME  
 SIMPSON-GOLABI-BEHMEL SYNDROME; SGBS  
 SGB SYNDROME


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| • CONTRIBUTORS | • CLINICAL SYNOPSIS |

**Database Links**

|          |         |              |        |
|----------|---------|--------------|--------|
| MEDLINE  | PROTEIN | DNA          | Genome |
| Gene Map | Coriell | Nomenclature |        |

Gene Map Locus: [Xq26](#)

Note: pressing the  symbol will find the citations in MEDLINE whose text most closely matches the text of the preceding OMIM paragraph, using the Entrez MEDLINE neighboring function.

**TEXT**

A number sign (#) is used with this entry because of evidence that the Simpson-Golabi-Behmel overgrowth syndrome is caused by mutations in the gene for glypican-3 (300037).

In 2 males, sons of sisters, *Simpson et al.* (1975) observed a 'new' dysmorphism with the following features: broad stocky appearance, distinctive facies (large protruding jaw, widened nasal bridge, upturned nasal tip), enlarged tongue, and broad, short hands and fingers. Intelligence was normal. The family referred to the appearance as 'bulldog'-like. In infancy hypothyroidism was suggested, but this was excluded by laboratory tests. Close linkage with the Xg blood group locus was excluded. *Kaariainen* (1981) told me of a tall (192 cm) 40-year-old man with operated pectus excavatum, ventricular septal defect, central cleft of the lower lip, peculiar cup-shaped ears with knobiness and nodularity, short clubbed terminal phalanges, low-pitched voice, and cataracts developing at age 35. The parents, who came from different parts of Finland, were 170 and 160 cm tall. A brother, height 180 cm, died at age 18 years of ventricular septal defect and pulmonary hypertension. He looked like the surviving brother and quite different from other members of the family. *Kaariainen* (1982) concluded that the disorder is the same as that described by *Simpson et al.* (1975). Cleft of the lower lip was .....

A keyword search is valuable. Since the World Wide Web may contain some information on familial hormone deficiencies, a search of the OMIM database is indicated. To do this, one logs on to the OMIM home page by entering the URL—<http://www.ncbi.nlm.nih.gov/omim>. Then one clicks on "Search the OMIM Database" with the mouse and enters "gh and thyroid" as a search term. Note that "gh" is used as a search term rather than growth hormone because the latter is 2 words. Use of "gh" gives 36 hits, while "growth AND hormone" gives 271 hits. By using "gh AND thyroid," 7 disorders appear on the screen (Figure 2, below). The first entry (\*173110 POU DOMAIN, CLASS 1, TRANSCRIPTION FACTOR 1; POU1F1)<sup>4</sup> is about the PIT1 transcription factor and includes an interesting paragraph:

*Mutations of the POU1F1 gene in the human and Pit1 in the mouse are responsible for deficiencies of growth hormone, prolactin, and thyroid-stimulating hormone, while the production of ACTH, LH, and FSH are preserved. In contrast, patients with combined pituitary hormone deficiency due to homozygosity or compound heterozygosity for inactivating mutations of PROP1 (601538) cannot produce LH and FSH at a sufficient level and do not enter puberty spontaneously (Wu et al, 1998).<sup>5</sup>*

The case under consideration seems to fit the findings reported for *PROP1* mutations, so it is appropriate to review the article by Wu et al. On the Web, you can obtain a copy of the abstract by clicking the mouse on

**Figure 2**  
**OMIM Page Showing Results of Search Initiated Using "gh AND thyroid" as a Search String.**

|           |        |
|-----------|--------|
| OMIM Home | Search |
|-----------|--------|

Select Entries from OMIM—  
**Online Mendelian Inheritance in Man**  
 7 entries found, searching for "gh and thyroid"

---

\*173110 POU DOMAIN, CLASS 1, TRANSCRIPTION FACTOR 1; POU1F1  
 #262500 PITUITARY DWARFISM II  
 \*139320 GUANINE NUCLEOTIDE-BINDING PROTEIN, ALPHA-STIMULATING ACTIVITY POLYPEPTIDE 1; GNAS1  
 #118450 ALAGILLE SYNDROME; AGS  
 \*262600 PITUITARY DWARFISM III  
 \*188545 THYROTROPIN-RELEASING HORMONE RECEPTOR; TRHR  
 \*600239 G PROTEIN-COUPLED RECEPTOR 1; GPR1



## SELECTED WEB SITES THAT CONTAIN INFORMATION ON GROWTH AND HORMONE DISORDERS

### **Summaries of Disorders for Professionals, Educators, and Laypeople**

**MEDLINE PubMed and Internet Grateful Med** (<http://www.nlm.nih.gov/databases/free.html>): Provides access to a cornucopia of scientific and medical publications in a searchable format.

**Alliance of Genetic Support Groups, Inc.** (<http://www.geneticalliance.org>): Genetic support groups voice the common concerns of children, adults, and families living with, and at risk for, genetic conditions.

**American Diabetes Association** (<http://www.diabetes.org>): For professionals and laypeople; <http://www.childrenwithdiabetes.com> is the online community for kids, families, and adults.

**Chromosomal Variation in Man** (<http://www.wiley.com/products/subject/life/borgaonkar/access.html>): A catalog of chromosomal variants and anomalies that includes citations on all common and rare chromosomal alterations, phenotypes, and abnormalities in humans. The database is organized by variations and anomalies, numerical anomalies, and chromosomal breakage syndromes.

**Cytogenetic Resources** (<http://www.kumc.edu/geneinfo.html>): Database of normal and abnormal karyotypes, empiric risks for chromosome abnormalities, and maps of genes on chromosomes.

**Endocrine Society** (<http://www.endo-society.org>): Information on the Endocrine Society, fellow societies, organizations, and patient education groups as well as resources for scientists and physicians.

**GeneMap'98** (<http://www.ncbi.nlm.nih.gov/genemap98>): Includes the locations of more than 30,000 genes and provides an early glimpse of some of the most important pieces of the human genome.

**Genetic Conditions/Rare Conditions Support Groups & Information Page** (<http://www.kumc.edu/gcc/support>): For professionals, educators, and individuals seeking information on genetic disorders, birth defects, and chromosomal disorders.

**Genetic Web Sites National Society of Genetic Counselors** (<http://www.kumc.edu/gcc/geneinfo.html>): Covers cancer, cytogenetics, genetics, hyperlipidemia, neurogenetics, single gene disorders, and support groups.

**Helix** (<http://healthlinks.washington.edu/helix>): Helix is a directory of laboratories providing testing for genetic disorders. Information on both research and diagnostic laboratories is included and labs are listed by disease name.

**HGMD** (<http://www.uwcm.ac.uk/uwcm/mg/index.html>): Links to locus-specific mutation databases available on the World Wide Web.

**Human Growth Foundation** (<http://www.genetic.org/hgf>): A lay organization established for parents and friends of children with various sorts of growth disturbances, including overgrowth, growth hormone deficiency, Turner syndrome, etc.

**Idiogram Server** ([http://www.pathology.washington.edu/cytopages/idiogram\\_select.html](http://www.pathology.washington.edu/cytopages/idiogram_select.html)): Includes figures of human chromosomes and translocations.

**Information for Genetic Professionals** (<http://www.kumc.edu/gcc/geneinfo.html>): Covers genetic conditions; clinical genetics resources, clinical genetics centers, departments and clinics; genetics education centers; genetics courses, lectures, and educational materials; ethical, legal, and social implications of the Human Genome Project; and genetic computer resources.

**International Society for Pediatric and Adolescent Diabetes** (<http://www.ispad.org>): Presents news, membership rosters, and meeting dates.

**Lawson Wilkins Pediatric Endocrine Society** (<http://lwps.org>): Features news, job listings, etc.

**Magic Foundation** (<http://www.magicfoundation.org>): A lay organization established for parents and friends of children with various sorts of growth disturbances, including overgrowth, growth hormone deficiency, Turner syndrome, etc.

**March of Dimes** (<http://modimes.org>): Information for professionals and families on birth defects.

**National Association for Rare Disorders (NORD)** (<http://www.rarediseases.org>): Rare disorder database includes information on symptoms, etiology, diagnostic tests, and treatment for families and professionals.

**National Human Genome Research Institute (NHGRI)** (<http://www.nhgri.nih.gov>): Contains information on the Human Genome

Project and Ethical, Legal and Social Implications Policy.

**Neurofibromatosis Home Page** (<http://nf.org>): Contains information about neurofibromatosis and contacts for related resources.

**NLM Site Map** (<http://www.nlm.nih.gov/sitemap.html>): Includes MEDLINE, TOXNET, biotechnology information, cancer information, and links to DNA databases.

**OMIM** (<http://www.ncbi.nlm.nih.gov/omim>): Online Mendelian Inheritance In Man contains textual information, pictures, and reference information on genes and genetic disorders, including clinical findings, references, and gene maps. OMIM has many links to NCBI's Entrez database of MEDLINE articles and sequence information and many links to other databases.

**Peds Endocrine Cedars Sinai Medical Center** (<http://www.csdc.edu/pediatrics/default.html>): Available for sharing cases, asking questions, and disseminating information to pediatric endocrinologists.

**Policy Statements From the American Academy of Pediatrics** (<http://www.aap.org>): Contains policy statements and guidelines on the diagnosis and treatment of genetic disorders as well as newborn screening.

**Policy Statements From the American College of Human Genetics** (<http://www.faseb.org/genetics/acmg/pol-menu.html>): Contains a variety of policy statements about genetic diseases, genetic testing, and treatment of genetic disorders.

**Primer on Molecular Genetics** (<http://www.ornl.gov/hgmls/publicat/primer/intro.html>): A Department of Energy site that contains information on molecular genetics, genetic testing, and the Human Genome Project.

**Quackwatch** (<http://www.quackwatch.com>): Information on health fraud and quackery as well as alternative treatments such as nutritional supplements for Down syndrome.

**Rare Genetic Diseases in Children** (<http://mcrcr2.med.nyu.edu>): Intends to publicize, educate, and refer those interested in or concerned with the various lysosomal storage diseases.

**Simulated Genetic Counseling Session** (<http://www.kumc.edu/gcc/gcslm.html>): Online simulated session that illustrates the process of genetic counseling.



**Figure 3**  
**NCBI/PubMed Page Showing Results of Search Initiated Using "gh AND thyroid AND gonadotropin AND familial" as a Search Term. Note That 7 Publications Match All 3 Items in the Search String and That The First 2 of These Contain Essential Information.**

PubMed

PubMed QUERY

Docs Per Page: 
Entrez Date limit:

8 citations found

for the articles selected (default all).

documents on this page through Loansome Doc

☐
Rosenbloom AL, et al. [\[See Related Articles\]](#)  
Clinical and biochemical phenotype of familial anterior hypopituitarism from mutation of the *PROP1* gene.  
J Clin Endocrinol Metab. 1999 Jan;84(1):50-7.  
PMID: 9920061; UI: 99116789.

☐
Fofanova OV, et al. [\[See Related Articles\]](#)  
Rarity of PIT1 involvement in children from Russia with combined pituitary hormone deficiency.  
Am J Med Genet. 1998 Jun 5;77(5):360-5. Review.  
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☐
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PMID: 9462743; UI: 98122575.

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Screening for PIT1 abnormality by PCR direct sequencing method.  
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Watanabe S, et al. [\[See Related Articles\]](#)  
Risk factors for leukemia occurrence among growth hormone users.  
Jpn J Cancer Res. 1989 Sep;80(9):822-5.  
PMID: 2513298; UI: 90094016.

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either of the following: (1) the Wu et al, 1998 hypertext at the end of the paragraph cited above; or (2) the Wu et al, 1998 hypertext in either the PIT1 (173110) or *PROP1* (601538) entries, and then click on the PubMed ID (9462743) that follows the reference that appears. You then will see the abstract of the reference

on your screen and you can print it. Since this is a PubMed document, you also can save it as a file on your computer, as shown at the bottom of the page, or you can order a complete copy through Loansome Doc™ as shown at the top. Alternatively, you can obtain copies of articles by clicking on MEDLINE under "Database Links" that are just below the TABLE OF CONTENTS of each entry (Figure 1, page 20). If you do a PubMed search for "gh AND thyroid AND gonadotropin AND familial," you will immediately find 8 related articles, the first 3 of which contain information that you may find helpful in the further evaluation and treatment of your new patient (Figure 3). As in the first case, you can carry out a HELIX search to find a lab that can conduct a molecular analysis of the *PROP1* gene.

Now there is a working diagnosis (*PROP1* defects), information on the pathogenesis, mode of inheritance, and the findings associated with the disorder, and a way to find a lab that helps confirm the working diagnosis. Obviously, after this you feel better prepared to talk with your patient's parents and to answer their questions.

## CONCLUSIONS

The World Wide Web is here to stay. It offers an expedient way to find the answers to complex diagnoses and problems that previously had not been possible. It behooves every physician to capitalize upon the aids that the World Wide Web and computer technology provide. As is true for most things in life, some effort has to be put forth to develop the expertise needed to master the Web. Hopefully, this introduction will help you succeed.

**ADDENDUM:** Since this article was solicited and received, an article by Dr. Phillips regarding [On Use of the WEB to DIAGNOSE GENETIC AND ENDOCRINE CASES](#) has been placed on the Web. You can find it at the URL-[http://bret.mc.vanderbilt.edu/genetics/html/frame\\_education.htm](http://bret.mc.vanderbilt.edu/genetics/html/frame_education.htm).

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# Effect of Growth Hormone Treatment on Adult Height of Children With Idiopathic Short Stature

Of 121 children with idiopathic short stature (ISS) entering the study to receive GH, 80 have reached adult heights after 3.5 to 10 years of GH therapy. The children were randomly assigned to either an observational control group or to treatment (0.1 mg/kg/3x weekly). The change in adult height relative to initial predicted height achieved with GH was compared with 2 groups of normal children, including 291 children with initial height SDS >-1 and bone age (BA)  $\leq$ 10 years and 37 with height SDS <-1. In addition, the change in height of the 80 was compared with the change in height in untreated ISS patients (initial height SDS <-2).

The baseline and final characteristics of the 80 are recorded in Table 1, and the height SDS  $\pm$  SEM (for chronologic age [CA]) versus treatment years are recorded in Figure 1. The mean baseline height SDS for the 121 ISS children was -2.7. The mean height SDS for age in the 69 treated for 5 years increased to -1.4. The 29 children treated for 7 years reached a mean height SDS of -1.0. Mean adult height also increased in the 80 compared with their initial predicted height. Sixty-five of the 80 (81%) had an increase. The mean ( $\pm$  SD) changes in heights from predicted heights of the treated boys and girls were 5.0  $\pm$  5.1 cm and 5.9  $\pm$  5.2 cm, respectively. Neither the control group of boys from the Fels Longitudinal Growth Study with basal height SDS of <-1 nor the control group of untreated ISS boys with basal height SDS <-2 reached their mean initial predicted height (mean change in height of -1.7  $\pm$  4.2 cm and -4.2  $\pm$  7.7 cm, respectively). Thirty-eight of 48 boys (79%) treated with GH exceeded their initial predicted adult height compared with only 2 of 11 (18%) of the untreated ISS boys. In addition, 24 of the 48 boys (50%) had a clinically important (>5 cm) increase in adult height as compared with only 1 of the 11 untreated boys. These results are summarized in Table 2 on page 24, as are the similar studies for girls.

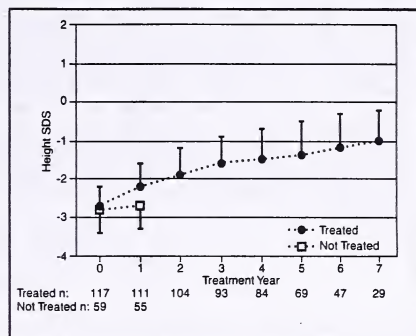
**Table 1**  
Baseline and Final Characteristics in 80 Children With Idiopathic Short Stature Treated With Growth Hormone Until Final Height\*

|  | Boys (n=57)               | Girls (n=23)    |
|--|---------------------------|-----------------|
| Baseline chronologic age                 | 10.4 $\pm$ 1.8            | 9.7 $\pm$ 2.1   |
| Baseline bone age                        | 8.7 $\pm$ 1.6             | 8.2 $\pm$ 1.9   |
| Baseline height SDS                      | -2.8 $\pm$ 0.5            | -2.7 $\pm$ 0.4  |
| Duration of growth hormone therapy (yrs) | 6.0 $\pm$ 1.7             | 5.5 $\pm$ 1.7   |
| Final height (cm)                        | 165.5 $\pm$ 7.2           | 153.1 $\pm$ 4.8 |
| Pretreatment predicted adult height (cm) | 160.6 $\pm$ 6.4           | 147.2 $\pm$ 5.1 |
| Final height minus pretreatment PAH (cm) | 5.0 $\pm$ 5.1             | 5.9 $\pm$ 5.2   |
| Midparental target height (cm)           | 170.7 $\pm$ 4.5<br>(n=54) | 159.0 $\pm$ 3.4 |
| Chronologic age at final height (yrs)    | 18.1 $\pm$ 1.6            | 17.2 $\pm$ 2.0  |

\*Values are given as means  $\pm$  SD.

Reprinted with permission from Hintz RL, et al. *N Engl J Med* 1999;340:502-507.

**Figure 1**  
ISS Treated With GH: Height SDS  $\pm$  SEM for Chronologic Age Versus Treatment Year for Treated and Untreated Controls



Reprinted with permission from Hintz RL, et al. *N Engl J Med* 1999;340:502-507.

Since approximately one half of ISS children treated with GH had a >5-cm increase in adult height, as compared with initial predicted height, an important question must be addressed: Is prediction of the end result possible? In this respect, no relationship was found between the increase in adult height and the CA, BA, height age, predicted adult height, peak GH response, 12- or 24-hour integrated GH levels, or any of the insulin-like growth factor-related peptides at initiation of therapy. There also was no correlation of ultimate gain with the growth response in the first 12 or 24 months of GH treatment or the total length of treatment.

After a cogent discussion of the variability of responses and the ethics of treating ISS children, the authors concluded:

*If there were no long-term benefit of treatment with GH in ISS, there would be no reason to treat and thus no ethical problem. However, our study demonstrates that there is a significant potential benefit of GH treatment. Thus the decision to treat must involve a difficult judgment of relative benefits, risks, and the cost of treatment.*

Hintz RL, et al. *N Engl J Med* 1999;340:502-507.

**Editor's comment:** Much has been written in the literature regarding the possible treatment of ISS with GH. Many articles, both pro and con, regarding this have been abstracted in GROWTH, Genetics, & Hormones (Vol 11[4]:8; Vol 12[1]:14; Vol 15[1]:7-8). The study by Hintz et al abstracted here is as close to a definitive study that has been done. Each interested reader of this abstract should review the entire article in the New England Journal of Medicine and possibly others before deciding whether in his/her opinion GH treatment is justifiable. As for myself, I judge each instance on the characteristics and merits of the case and cost.

Fortunately, the creation of the Genentech Foundation for Growth and Development has made it possible for several psychological studies to be initiated. These will be reported subsequently regarding the psychological importance of a possible modest gain in height among such patients as reported here. Unfortunately, these studies will not be reported for another 2 to 3 years.

Robert M. Blizzard, MD

**2nd Editor's comment:** This interesting report provides very good data regarding the use of GH for the treatment of children with ISS. Although these children showed an increase in final adult height, they did not exhibit a major improvement even after 10 years of GH therapy. The advantage of 2 or 3 inches gain in final height has to be correlated with the cost and the long-term duration of treatment needed to achieve such a modest gain. However, we now have clear data to present and to discuss with those patients and their families when evaluating the need for GH treatment.

Fima Lifshitz, MD

Table 2  
Change in Final Height in ISS Treated With GH  
Compared With the Change in 2 Untreated  
Control Groups (cm)

|   | Boys                 | Girls                |
|---|----------------------|----------------------|
|   | Mean (95% CI)        |                      |
| △ Ht: ISS treated with GH<br>(48 boys, 21 girls)                | 5.0<br>(3.6 to 6.3)  | 5.9<br>(3.7 to 8.1)  |
| △ Ht: ISS treated with GH minus<br>△ Ht: controls (Ht SDS < -1) | 6.7<br>(3.7 to 9.6)  | 2.3<br>(-0.6 to 5.1) |
| △ Ht: ISS treated with GH minus<br>△ Ht: controls (Ht SDS < -2) | 9.2<br>(5.5 to 12.8) | 5.7<br>(2.1 to 9.4)  |
| Ht = height   |                      |                      |
| △ Ht = final height minus initial predicted adult height        |                      |                      |

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## A Study of Females With Deletions of the Short Arm of the X Chromosome

A clinical and molecular study of 25 females with deletions of the short arm of the X chromosome was undertaken. The deletion breakpoints, the parental origin, and the activation status of the deleted X chromosomes were determined.

The short stature observed in Turner syndrome (TS) and in patients with terminal Xp deletions results from the deletion of a homeobox gene, *SHOX*, which escapes X-inactivation and is located in the pseudoautosomal region of Xp. In determining parental origin of the X chromosome with deletions, the paternal X chromosome was defective in the vast majority. Parental disomy of the X chromosomes was excluded in 24 of the 25 deleted X chromosomes.

In all cases where the breakpoint was in or proximal to Xp22.1, the deleted X was late replicating in all cells observed.

Of the 25 patients, 23 had short stature resulting from the deletion of the *SHOX* gene in the pseudoautosomal region of Xp. None of the patients in this study had a webbed neck, which is lymphomatous in origin, although 49% of 45,X individuals in a recent review (Ogata and Matsuo. *Human Genetics* 1995;95:607-629) had neck webbing. The studies suggest that a gene (or genes) responsible may reside on Xq or very proximal on Xp. Six of 18 patients examined were found to have a low hairline posteriorly. The most distal breakpoint in such a patient was Xp22.31. Congenital edema of the extremities was described in 2 cases with breakpoints in Xp22.3 but not in any of the other cases, even when breakpoints occurred in the very proximal part of Xp. None of the patients with breakpoints distal to Xp11 had any cardiovascular problems; however, breakpoints in or proximal to Xp11 were sometimes associated with aortic valve abnormalities.

The authors concluded that the apparently normal ovarian function seen in 2 cases of females with a single copy of the *DFRRX* gene suggests that the ovarian failure seen in cases of 45,X TS may result from a mechanism other than haploinsufficiency of *DFRRX*. The absence of neck webbing in any of the patients studied suggests that the gene for this feature may lie on Xq or very proximal Xp. There were no consensus breakpoints for the other stigmata associated with lymphatic abnormalities, skeletal abnormalities, or cardiac and renal abnormalities. The presence of excess melanocytic nevi is associated with breakpoints proximal to Xp22. There is some evidence for the presence of a gene for some of the soft features of TS, located in Xp22.3, and subject to X-inactivation. In cases of distal terminal deletions, the deleted X may remain active in a proportion of cells, resulting in a Turner-like phenotype, dependent on the degree of mosaicism and the tissue specificity.

James RS, et al. *Hum Genet* 1998;102:507-516.

**Editor's comment:** This presentation of a very detailed clinical and cytogenetic study contributes significantly to a better understanding of the locations of the genes on the X chromosome. We have come a long way since Ferguson-Smith determined in 1965 that deletions of the whole short arm of the X chromosome in females are associated with short stature, gonadal dysgenesis, and the classic stigmata of TS. However, 34 years have passed since then. With our ever-advancing technology, probably we will know the complete genome in the next decade. We then will understand many more intricacies of gene interaction, and the time interval to learn all this will be short.

Robert M. Blizzard, MD



## Calcium Metabolism and Growth During Early Treatment of Children With X-Linked Hypophosphatemic Rickets

The authors administered calcitriol (20 to 40 ng/kg/d) in 2 divided doses and phosphate 40 to 60 mg/kg/d in 5 divided doses to 8 infants (1 male) with X-linked familial hypophosphatemic rickets (XLHR). This syndrome is a result of an inactivating mutation in *PEX*, a gene that encodes an endopeptidase whose substrate may be the elusive "prephosphotonin." The subjects ranged in age from 3 to 12 weeks at diagnosis and initiation of therapy and were followed for 1 to 4 years.

Treatment led to a decline to normal or nearly normal serum concentrations of alkaline phosphatase and urinary excretion of hydroxyproline, but little rise in serum phosphate concentrations. Secondary hyperparathyroidism developed occasionally, usually when phosphate intake exceeded 50 mg/kg/d. Nephrocalcinosis (grades 1 and 2) appeared in 3/8 infants without compromise of renal function. The infants grew reasonably well, with lengths maintained within the normal ranges in 6/8 patients at the conclusion of the study (see Figure). Genu varum developed in 3 children while genu valgum developed in 1. The authors conclude that early treatment with calcitriol at a daily dose of at least 30 to 40 ng/kg and phosphate at a maximal daily dose of 40 to 50 mg/kg improves mineral metabolism and seems to minimize severe growth delay and leg deformities.

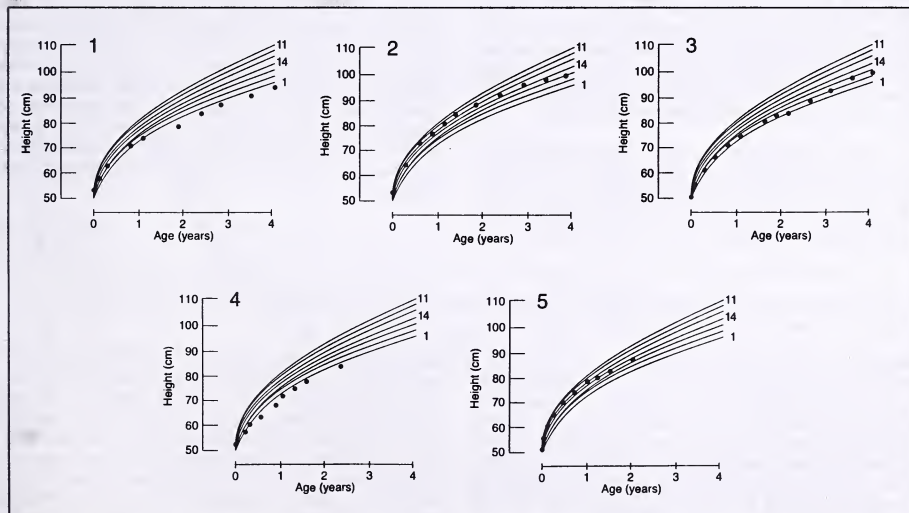
**Editor's comment:** Most children with XLHR have significant retardation of linear growth and deformities of the lower extremities despite administration of appropriate therapy with calcitriol and phosphate. In many instances this is the result of noncompliance with the arduous therapeutic regimen of multiple daily doses of phosphate. Treatment is not only difficult but also hazardous, with distressingly frequent periods of hypercalciuria (leading to nephrocalcinosis), hypercalcemia, and secondary hyperparathyroidism that necessitate frequent chemical and radiographic monitoring.

Several investigators have reported the beneficial effects of rhGH on the growth of children with XLHR. The experience of this writer with rhGH also has been encouraging and has seemingly made the management of calcitriol and phosphate dosing less difficult. The identification of "phosphotonin" is awaited as it may offer a more specific therapeutic (and hopefully less toxic) agent for this illness than is now available.

Allen W. Root, MD

Kruse K, et al. *Eur J Pediatr* 1998;157:894-900.

Figure  
Growth Charts of 5 Study Patients With XLHR Treated the Longest With Phosphate and Calcitriol



Reprinted with permission from Kruse K, et al. *Eur J Pediatr* 1998;157:894-900.



## Efficacy and Safety of Lovastatin in Adolescent Males With Heterozygous Familial Hypercholesterolemia: A Randomized Controlled Trial

Stein et al report the experience of 132 patients recruited from 14 different centers to participate in a study evaluating the safety and efficacy of lovastatin treatment in children with heterozygous familial hypercholesterolemia (HeFH). Adolescent boys aged 10 to 17 years who had followed the American Heart Association (AHA) pediatric diet for at least 4 months were recruited in this randomized, placebo-controlled study. The inclusion criteria for HeFH were: (1) low-density lipoprotein cholesterol (LDL-C) values of at least 4.9 mmol/L (189 mg/dL) and no more than 13.0 mmol/L (503 mg/dL) and at least 1 parent had an LDL-C value of at least 4.9 mmol/L (189 mg/dL); or (2) LDL-C values of at least 5.7 mmol/L (220 mg/dL) and no more than 13.0 mmol/L (503 mg/dL) and a parent who had died of coronary artery disease (CAD) with no available lipid values. These LDL-C entry criteria had to be met prebaseline. Both groups in the study were similar as to age, height, weight, smoking rate, blood pressure, testicular volume, and baseline serum lipid profile.

Prior to randomization, all eligible participants received placebo during the last 4 weeks of an 8-week stabilization period in addition to the AHA diet. After randomization, they were administered either placebo or lovastatin immediately prior to the evening meal during the following 24 weeks (period 1). Lovastatin was started at 10 mg/d and increased at 8 and 16 weeks to 20 and 40 mg/d, respectively. During the second 24-week period (period 2), lovastatin was given at 40 mg/d. Those who received placebo during period 1 continued receiving placebo during period 2. Subjects were followed every 4 weeks during period 1 and every 6 weeks during period 2. Clinical assessment, including growth assessment and serum cholesterol, triglyceride, LDL-C, high-density lipoprotein cholesterol, apolipoprotein B (Apo B), transaminases, and creatine kinase levels, were determined at every visit. Total protein, albumin, ferritin, glucose, and vitamins A, D, and E serum levels also were determined to evaluate nutritional status.

There was a positive family history for familial hypercholesterolemia in 74% of the 132 randomized subjects. In 56% the mother was affected while in 44% the father was affected. The mean age of onset of CAD of the affected parents was 37 years. Lovastatin had no significant effect on growth parameters

throughout the study. Sexual maturation progressed similarly in both groups. Baseline total cholesterol, LDL-C, and Apo B serum concentrations were elevated, as expected in individuals with HeFH. Lovastatin reduced total cholesterol and LDL-C levels at all dosages and Apo B at 40 mg/dL during period 1 (Apo B was not measured during treatment with lower dosages). Continued therapy with lovastatin reduced LDL-C and Apo B levels 25% and 22%, respectively, during period 2. Although a gradual upward trend from baseline levels in alanine aminotransferase was noted, no significant differences were found between groups at week 48. Biochemical measurements to assess nutritional status did not show differences at baseline or after 48 weeks of lovastatin treatment except for a reduced tocopherol level.

The authors concluded that lovastatin did not affect growth and effectively reduced serum LDL-C and total cholesterol levels.

Stein EA, et al. *JAMA* 1999;281(2):137-144.

**Editor's comment:** Familial hypercholesterolemia is a worrisome genetic condition that requires aggressive lipid-lowering therapy. Failure to control hypercholesterolemia has been associated with high mortality and frequent life-threatening cardiovascular episodes, often seen early in life. Thus, this interesting report demonstrates the importance of these therapeutic interventions in the pediatric population. The study itself demonstrates modest reductions (21%) in serum lipid level after 48 weeks of intense therapy. This reduction occurred with the help of a low-fat, low-cholesterol diet without alterations in growth and development while other nutritional indexes remained intact. This is important since dietary restrictions to lower serum cholesterol may result in a decreased growth rate. This paper also remarks on the significance of the potential prevention of the high morbidity associated with increased serum cholesterol levels. However, long-term studies are necessary to corroborate the safety and effectiveness of lipid-lowering therapies for prolonged periods beginning in childhood.

Fima Lifshitz, MD

## Weight Control and Risk Factor Reduction in Obese Subjects Treated for Two Years With Orlistat: A Randomized Controlled Trial

A randomized, double-blind, placebo-controlled study using orlistat, a gastrointestinal lipase inhibitor, to promote weight loss is reported by Davidson et al. A total of 1,187 obese subjects older than 18 years with a body mass index of 30 to 43 kg/m<sup>2</sup> and absence of weight loss in the previous 3 months were recruited from 18 different research centers in the United States. Qualified subjects received a controlled-energy diet that provided 30% of energy intake as fat during a 4-week lead-in period. Individual energy intake was calculated according to the estimated daily maintenance energy requirement (1.3 x calculated basal metabolic rate) minus 2,100 to 3,360 kJ/d. After the 4-week lead-in period, 892 subjects, who

had a treatment compliance rate of  $\geq 75\%$ , were randomly assigned to receive placebo (25% of the subjects, n=224) or orlistat 120 mg (75% of the subjects, n=668) for 52 weeks. Subjects who received placebo in the first year who had a  $\geq 70\%$  compliance rate received placebo for an additional 52 weeks. Orlistat-treated subjects who completed 1 year with a compliance rate of  $>70\%$  received placebo, orlistat 120 mg, or orlistat 60 mg for an additional 52 weeks. Medical history, body weight determination, clinical chemistry, thyroid function, fasting lipid levels, fasting glucose and insulin, and 3-hour glucose tolerance tests were performed at the time of randomization and at the end of years 1 and 2.

A weight loss of approximately 2.3% of the initial body weight was noted during the 4-week placebo lead-in period. Mean age, weight, and body mass index were similar in both groups after randomization. Individuals who received orlistat had significantly greater weight loss ( $8.8\% \pm 0.4\%$ ) compared with controls (see Figure). Subjects treated with orlistat 120 mg during year 1 and who received orlistat 120 mg during year 2 regained significantly less of their first-year weight loss (35.2% regain) compared with those who received orlistat 60 mg (51.3% regain) or placebo (63.4% regain). Systolic and diastolic pressure significantly decreased with orlistat 120 mg versus placebo. Decrease in mean waist circumference was greater in the orlistat-treated group compared with the placebo group after 2 years of therapy. Serum cholesterol and low-density lipoprotein cholesterol (LDL-C) levels decreased 8% during the 4-week placebo lead-in period. Serum cholesterol and LDL-C levels declined in the orlistat-treated group during year 1. Fasting serum glucose levels increased less and fasting serum insulin levels decreased in the orlistat-treated group over 2 years versus placebo. There were more undesirable gastrointestinal events (ie, flatulence, oily spotting, fecal urgency, oily evacuation, fecal incontinence, and increased defecation) associated with orlistat treatment.

The authors concluded that orlistat promoted weight loss, lesser weight regain, and improved fasting cholesterol, LDL-C, and insulin levels.

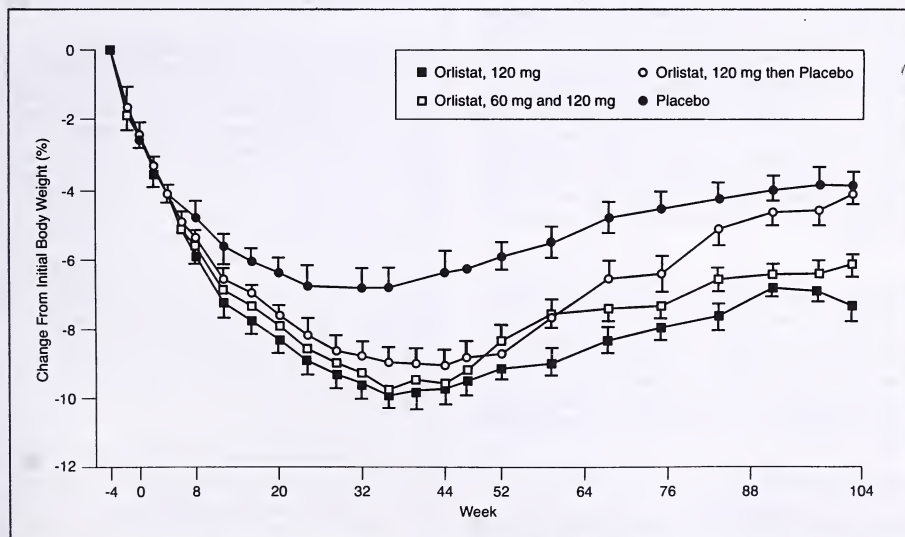
Davidson MD, et al. *JAMA* 1999;281(3):235-242.

**Editor's comment:** Obesity has reached epidemic proportions, especially in developed countries. Obesity and its metabolic abnormalities are associated with very high morbidity and mortality. This interesting study supports the European data reported by Sjostrom et al (*Lancet* 1998;352:167-173) in which orlistat promoted weight loss, reduced weight regain, and lowered serum lipids and insulin levels over a 2-year period. Orlistat, a gastrointestinal lipase inhibitor, exerts its action by producing fat malabsorption without being absorbed or metabolized. Thus, it would not have the potentially life-threatening side effects (ie, valvular heart disease and primary pulmonary hypertension) described for fenfluramine-phentermine (*N Engl J Med* 1997;337:581-588).

Even though much literature has been published regarding pharmacotherapy for obesity in adults, reports concerning pediatric experience are limited. Therefore, it will be interesting and important to have more information on the use of orlistat in treating severe obesity in the pediatric population, where obesity usually begins. Both pharmaceutical firms and the FDA are urged to support such studies.

Fima Lifshitz, MD

**Figure**  
**Mean Body Weight Change ( $\pm$  SEM) During 2 Years of Double-Blind Treatment**



Reprinted with permission from Davidson MH, et al. *JAMA* 1999;281:235-242.

## A Gene Encoding a Transmembrane Protein Is Mutated in Patients With Diabetes Mellitus and Optic Atrophy (Wolfram Syndrome)

Wolfram syndrome is an autosomal recessive neurodegenerative disorder defined by young-onset, nonimmune insulin-dependent diabetes mellitus (IDDM), progressive optic atrophy, and diabetes insipidus. It is also called the DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) syndrome. Most patients with this progressive disorder eventually develop all four cardinal manifestations and die prematurely with widespread atrophic changes throughout the brain. Onset of IDDM occurs at a mean age of 6 to 8 years. The pancreatic islets are atrophic and insulin-producing beta-cells are selectively absent. The disease is believed to account for 1 of every 150 patients with young-onset IDDM.

The investigators confirmed the localization of the gene to chromosome 4p and isolated a gene (*WFS1*) from this region with 8 exons encoding a probable cell membrane protein with 890 amino acids that is related to the prenyltransferase  $\alpha$ -subunit. Hydrophilic amino and carboxyl terminal regions encompass a central hydrophobic core. Further analysis suggested that the protein had approximately 10 transmembrane domains. The function of *WFS1* protein was not identified but

its mRNA was expressed in pancreatic islets, brain, heart, skeletal muscle, kidney, and placenta. Inactivating homozygous and compound heterozygous mutations in exon 8 of *WFS1* were found in all affected members of 6 families, but not in control subjects, including deletions, insertions, and nonsense and missense mutations. The authors conclude that mutations in *WFS1* are related to Wolfram syndrome and suggest that the *WFS1* protein is important in the maintenance of islet cell and brain function.

Inoue H, et al. *Nature Genet* 1998;20:143-148.

**Editor's comment:** Further proof of the relationship of *WFS1* to Wolfram syndrome awaits the characterization of the phenotype in a mouse model in which the homologous gene has been "knocked out." Identification of the biologic function of *WFS1* protein will be of great interest as in its absence pancreatic islets and beta cells, as well as the brain, atrophy. Thus, the *WFS1* protein may be involved in the regulation of organ-specific apoptosis. Perhaps it is even an "antiaging" agent!

Allen W. Root, MD

## Bone Age in 116 Untreated Patients With Turner's Syndrome Rated by a Computer-Assisted Method (CASAS)

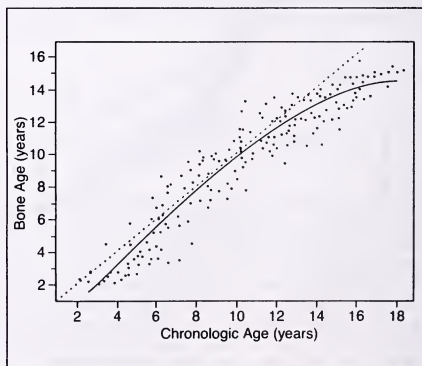
The investigators employed the computer-assisted skeletal age system (CASAS) to examine skeletal maturation in 265 radiographs of 116 untreated patients with Turner syndrome (TS). CASAS is based on the TW2-RUS (Tanner-Whitehouse 2—Radius, Ulna, Short Bones) method of bone age (BA) determination. In this procedure, the operator identifies on the radiograph the bones of the wrist and hand to be scored; the computer program then digitizes and analyzes the images and computes a score that is translated into a BA. There is apparently good reliability between the CASAS BA and that interpreted by human operators (Tanner JM, et al. *Hormone Research* 1994; 42:487).

In this study, the investigators reported that in untreated TS subjects the BA was delayed relative to chronologic age (CA) from the 3rd to the 6th years of life, advanced rapidly between 6 and 7 years of age, was similar to CA between 7 and 12 years of age, and was delayed after 12 years of age; epiphyseal fusion was not achieved until after 17 years of age (see Figure). The authors provided normative data for BA in TS and compared their data with those reported using the Greulich and Pyle and TW2-RUS atlases.

The authors concluded:

*In this study a reference curve was presented for bone age progression in TS for use in clinical practice. Knowledge of bone age and consequently accurate bone age rating at diagnosis and during longitudinal follow-up is essential in order to be able to counsel the patient, advise on therapy,*

Figure  
Plot of Bone Age Ratings Versus  
Chronologic Age of 116 Untreated Patients  
With Turners Syndrome:  
A Regression Curve to the  
Data Was Fitted by Polynomial  
Regression Analysis.



Reprinted with permission from Schwarze CP, et al. *Acta Paediatr* 1998;87:1146-1150.



initiate treatment and monitor development during treatment. The determination of bone age using a computer-assisted system proved a valid and reliable method. This will compensate for the additional time that needs to be invested. Future studies evaluating the effect of growth-promoting treatment in TS, by growth hormone or other means, should use such a computerized method for the determination of bone age.

Schwarze CP, et al. *Acta Paediatr* 1998;87:1146-1150.

**Editor's comment:** There are currently 2 computer programs for the analysis of BA, both based on the TW2-RUS system: (1) CASAS, and (2) the Royal Orthopedic Hospital Skeletal Aging System – in which the digitized image can be obtained from a radiograph or directly from files and the bones are recognized

by their position on the image (Aicardi G, et al. *Acta Med Auxol* 1998;30:121-127). In addition, investigators at the Universities of Genoa and Florence are in the preliminary stages of developing computer programs for BA determination. Apparently, computer programs utilizing the Greulich and Pyle atlas or other methods for BA determination have yet to be developed. One wonders if current technology permits the computerized construction of 3-dimensional images of the wrist, hands, and other epiphyses that might afford further insight into the developmental changes that accompany growth and development of the skeleton and perhaps even better assessment of skeletal maturation—perhaps by determination of bone volume or other measurement. Regardless, more widespread utilization of CASAS is important for consistency within and among institutions.

Allen W. Root,

## Difference in Height Associated With a Translation Start Site Polymorphism in the Vitamin D Receptor Gene

Because calcitriol (1,25-dihydroxyvitamin D<sub>3</sub>) is an important regulatory factor in the differentiation and proliferation of chondrocytes, the investigators speculated that various isoforms of the vitamin D receptor (VDR) might influence the effect of calcitriol on these processes and ultimately on linear growth. The VDR is polymorphic, in part because of 2 possible "start sites" in exon 2, one encoding a 427 amino acid peptide, the other a 424 amino acid molecule. The 2 isoforms are designated T and C, respectively, for the polymorphic alleles ATG/ACG at the first start site.

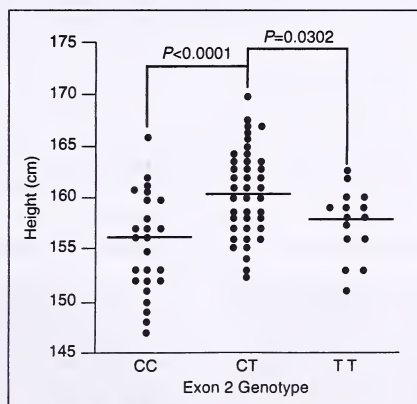
The authors examined the relationship between the presence/absence of the 2 variants of the VDR and the adult height in 90 healthy Japanese females (Figure), the height at age 13 years of 159 healthy Japanese children, and the height of 24 children aged 6 to 10 years with constitutional short stature (<1.5 SD), mostly with parents of normal height. They found that adult females with the CT genotype (ie, heterozygotic subjects with both the long and short forms of the VDR) were 4.4 cm taller than those with the CC genotype (ie, homozygous for the short form of the VDR) and 2.7 cm taller than those with the TT genotype (ie, homozygous for the long form of the VDR). There was no relationship between VDR genotype and age at menarche or between height and age at menarche in this population. Among the children (87 female, 72 male), height SDS also was greatest in those with the CT genotype. The frequency of the 3 VDR genotypes was CT 0.51, CC 0.37, and TT 0.12 in 249 normal subjects. Among constitutionally short children, the distribution of genotypes was CT 0.21, CC 0.62, and TT 0.17.

Thus, the frequency of the CT genotype was lower in children with constitutional short stature than in the general population.

The investigators suggest that the VDR genotype very possibly influences the growth of children and the height of adult females in the population studied, although the mechanism by which this effect might act is unknown, as are the complementary roles played by the polymorphic variants of the VDR.

Minamitani K, et al. *Pediatr Res* 1998;44:628-632.

Figure  
Exon 2 Polymorphism and Adult Height  
in 90 Female Subjects



Reprinted with permission from Minamitani K, et al. *Pediatr Res* 1998;44:628-632.

### Please Send Correspondence to:

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Charlottesville, VA 22903



**Editor's comment:** It would have been interesting to learn if the sex of the subject influenced the height-promoting effect of the polymorphic forms of the VDR, but no data were provided on the relationship of the VDR genotype to the adult height of Japanese men or to the group of children studied. In addition to the exon 2 polymorphism of the VDR, there are several other polymorphic variants of the VDR. Suarez et al reported that absence of the BsmI endonuclease site in intron 8 (homozygous for the presence of the endonuclease site designated BB) was associated with greater length and weight in females at 2 years of age than those with the genotype bb (homozygous for the presence of the endonuclease site), while the size of those with genotype Bb (heterozygous for the presence of the endonuclease site) was intermediate. On the other

hand, 2-year-old BB males were smaller than those with either the Bb or bb genotype. Subsequently, these investigators observed that there was an interactive effect upon growth in male infants between the polymorphic genotypes of the estrogen receptor and the VDR, although the mechanism remains enigmatic. The polygenic mechanisms that affect growth and stature are slowly being deciphered. In addition to the effects of SHOX and LHB on growth, we may now add the VDR and the ER genes.

Allen W. Root, MD

Suarez F, et al. *J Clin Endocrinol Metab* 1997;82:2966-2970.

Suarez F, et al. *J Clin Endocrinol Metab* 1998;83:3563-3568.

## Role of Nonexercise Activity Thermogenesis in Resistance to Fat Gain in Humans

The authors studied the mechanisms by which some individuals are able to prevent substantial weight gain when ingesting excessive calories while others cannot so do. For 8 weeks, 16 healthy, nonobese young adults (12 males, 4 females) were fed a diet that had 1,000 calories in excess of that necessary for weight maintenance. Body composition was measured by dual energy X-ray absorptiometry. Total energy expenditure (TEE) was determined by quantitating carbon dioxide production. TEE is composed of basal metabolic rate (BMR), postprandial thermogenesis (PPT), and physical activity thermogenesis. BMR was assessed by indirect calorimetry to measure oxygen consumption and carbon dioxide production. PPT also was measured by indirect calorimetry. Thermogenesis due to total physical activity was calculated as TEE minus the sum of BMR and PPT. The latter was subdivided into volitional exercise, which was maintained at low and constant levels and assessed by pedometer, and nonexercise activity thermogenesis (termed NEAT), which was calculated as the difference between total physical activity and volitional physical activity. They found: (1) individual fat gain varied 10-fold (0.36 to 4.23 kg); (2) fat gain was inversely related to TEE; (3) on average, 43% of the excess calories were stored, and 53% were expended; (4) a 5% increase in BMR accounted for 8% of the excess energy ingested; (5) a 14% increase in PPT; (6) NEAT increased on average by 66% during overfeeding, but there were wide interindividual differences in NEAT (-98.3 to +692 kcal/d); and (7) NEAT was inversely related to the gain in fat mass. The investigators concluded that "activation of

NEAT" explained individual variability in weight/fat increase during overfeeding.

Levine JA, et al. *Science* 1999;283:212-214.

**Editor's comment:** These investigators have NEATly documented what we have been taught for many decades—that obese subjects expend far fewer calories than nonobese subjects in everyday activities of living such as sitting, standing, and "existing." Obese individuals sit and stand in an impassive, almost motionless manner that conserves every possible calorie. They choose to sit rather than stand whenever possible. Now, the question is, How does one "activate" NEAT, that is, can we induce fidgeting? Since NEAT appears to be a familial trait, it is likely to be of multifactorial/genetic origin. Among the factors that may influence NEAT might be stimulation of the sympathetic nervous system, leading to uncoupling of oxidative phosphorylation in brown fat and synthesis of or response to leptin or other appetite and energy expenditure regulating agents. Interestingly, Levine and colleagues found that the 4 female volunteers had the lowest increases in NEAT. The significance of this gender-related difference awaits further evaluation. The accompanying commentary (Ravussin E, Danforth E Jr. *Science* 1999;283:184-185) also is recommended reading.

Allen W. Root, MD

## Cellular Therapy May Be Successful for Severe Osteogenesis Imperfecta

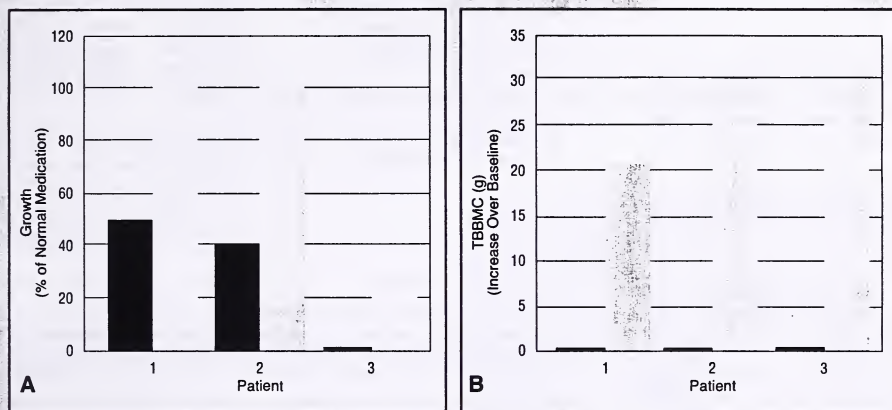
Osteogenesis imperfecta (OI) is a relatively common autosomal dominant disorder of connective tissues. It results from mutations of genes encoding the  $\alpha 1$  or  $\alpha 2$  chain of type I collagen that lead to varying degrees of generalized osteopenia with fractures, progressive deformities, and growth deficiency. Until the recent introduction of the bisphosphonate compound, pamidronate, which shows promise as an agent for increasing bone density, there has been no therapy for OI other than symptomatic treatment. However, Horwitz et al now report encouraging results from the transplantation of bone marrow-derived mesenchymal stem cells in 3 children with severe OI.

The children ranged in age from 13 to 32 months. They had clinical phenotypes consistent with OI type III and documented mutations of COL1A1 or COL1A2. After bone marrow ablative chemotherapy, each received a bone marrow infusion from an HLA-matched sibling (partial or complete). The children were evaluated extensively for at least 6 months.

Two of the children showed complete hematopoietic engraftment, while the third exhibited partial engraftment. Osteoblasts cultured from iliac bone of the first 2 children after transplantation showed 1.5% to 2% donor cells. Comparison of pretrans-

Figure

(A) Growth rates of the patients during the 6 months immediately before (■) and after (□) transplantation. The values are percentages of the median growth of unaffected children of the same age and sex. (B) Increase of total body bone mineral content (TBBMC) at approximately 100 days after transplantation (□). Dual energy X-ray absorptiometry scans, obtained just before transplantation, served as the baseline. The predicted increase of TBBMC (■), based on an increase (if any) in the child's weight, is shown for comparison.



Reprinted with permission from Horwitz EM, et al. *Nature Med* 1999;5:309-313.

plantation to posttransplantation bone histology revealed changes consistent with enhanced bone formation. Similarly, bone mineral content increased considerably in all 3 patients following transplantation. All 3 children showed substantial clinical improvement. Growth velocity, which had been below normal before transplant, increased dramatically, especially during the first 100 days (Figure). Fracture rates dropped in all 3 children.

The authors concluded that marrow derived-osteoblastic precursor cells are capable of populating bone tissues of transplant recipients, where they attenuate the biochemical, structural, and clinical abnormalities associated with OI. They were unable to fully explain how such a small proportion of cells can produce such a substantial improvement in 3 parameters. Both the authors of this paper and of an accompanying editorial caution that questions remain regarding the long-term viability of this therapy.

Horwitz EM, et al. *Nature Med* 1999;5:309-313.

Gerson SL. *Nature Med* 1999;5:262-264.

**Editor's comment:** OI has long been considered a good potential candidate for cellular therapy because of the accessibility and the dynamic nature of trabecular bone. In theory, donor cells that home to bone marrow have ready access to the trabecular surfaces on bone, and constant remodeling provides a

means to capture donor cells within trabeculae. This report demonstrates that at least in the short term, osteoblastic precursors can be transferred by transplantation and that these theoretical considerations hold up clinically. Of course, more time and more patients will be needed to validate this therapeutic strategy. Nevertheless, the results are very promising.

William A. Horton, MD

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**GROWTH, Genetics, & Hormones Volume 15, Number 2**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

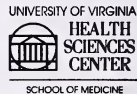
1. Access to information on the signs and symptoms of rare or recently discovered disorders can be located by surfing the Web.
  - a) True
  - b) False
2. OMIM is an acronym for:
  - a. Online Mendelian Inheritance in Man
  - b. Online Medical Information for Man
  - c. Ontime Mendelian Inheritance in Man
  - d. Ontime Medical Information for Man
3. When searching the OMIM database, only one search term is accepted at a time.
  - a) True
  - b) False
4. By searching the OMIM electronic database, you can obtain information regarding:
  - a. the pathogenesis and mode of inheritance of a disorder
  - b. the findings associated with a disorder
  - c. accessing a lab that can help confirm the working diagnosis of a disorder
  - d. (a) and (b)
  - e. all of the above

5. At the recent endocrinology meeting you heard someone mention that mutations in the *PTEN* gene caused thyroid disease, along with breast and ovarian tumors in some families. Which of the following conditions is caused by *PTEN* mutations?
  - a) Carney complex
  - b) Congenital adrenal hyperplasia
  - c) Cowden disease
  - d) Marfan syndrome
  - e) Multiple endocrine neoplasia

Answer Key: 1. a 2. a 3. b 4. e 5. c

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Drs. Phillips, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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### Adult Consequences of Pediatric Endocrine Disease, I: Congenital Adrenal Hyperplasia

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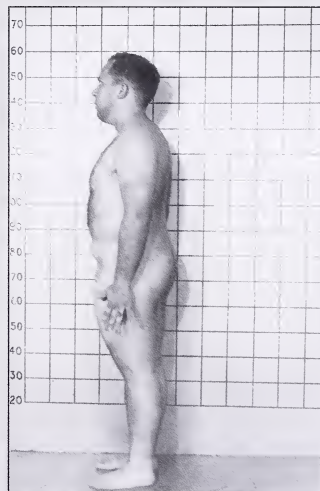
Before 1950, congenital adrenal hyperplasia (CAH) was characterized by marked virilization in females and sexual precocity in males. Salt-wasting with adrenal crisis was common. Wilkins et al (1950) and Barter et al (1951) independently reported that cortisone therapy placed the adrenals at rest and androgenic hormones fell to appropriate levels.<sup>1,2</sup> Salt-retaining corticosteroids usually controlled the salt-wasting.

The adult consequences of CAH in infants and children now can be evaluated. The purpose of this article is to relate what used to happen to CAH patients, what happens to infants who are treated with glucocorticoids, what happens to patients treated late in childhood, what difference early and adequate treatment may make when patients reach adulthood, and what possible alternative therapeutic approaches now can be considered and evaluated.

#### WHAT USED TO HAPPEN TO PATIENTS WITH CAH?

The salt-losers usually died. Consequently, it was the non-salt-losers who lived to adulthood. Exemplary of this is C.L., who as a newborn was thought to be a male with bilateral cryptorchidism and hypospadias. Following diagnosis of CAH at chronologic age (CA) of 6 years, height age (HA) of 9 3/12 years, and bone age (BA) of 12 years, an oophorectomy and hysterectomy were performed. In 1955, he appeared to be a very virile adult male (Figure 1), although his chromosomal karyotype was 46,XX. His genitalia was that of an adult male, although the testes were prostheses. C.L. married and had a heterosexual relationship compatible with his

Figure 1  
**C.L.: A 46,XX CAH Patient Raised as a Male**



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#### In This Issue

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phenotype. When last seen, he regarded himself as a male in every respect. This is an extreme example of what happened to CAH patients who were not treated until late.

A second example is Andrea (A.J.) at age 10 years (Figure 2, on the left), who subsequently was Andrew (Figure 2, on the right). CAH was diagnosed shortly after birth. Her poor rural parents were given cortisone to treat her, but they never returned to the doctor's office until she was referred at age 10. A.J.'s playmates questioned whether she was a male or a female. This exceedingly shy individual would talk with none of us except occasionally with Dr. John Money, the psychologist whose help was invaluable and historic. Dr. Money talked with her over a period of 6 months, and she was given the choice to decide whether she wished to remain a female or become a male. One day, A.J. wrote a note to each of her parents stating, "I gotta be a boy." And so he was. A phallic urethra was created and testicular prostheses were put in place. The last time he was seen, he was functioning as a male (Figure 3), although at 24 years of age he professed not to be sexually active.

These are the things that used to happen to patients with CAH. A tremendous amount has been learned from untreated patients such as these 2 and many others, particularly as they reached adulthood.

#### WHAT HAPPENS TO CAH PATIENTS WHO ARE TREATED WITH GLUCOCORTICOIDS WHEN INFANTS?

One of the first patients treated early was M.L., who was the firstborn child of tall parents. She had ambiguous genitalia and salt-wasting, and received cortisone in the first month of life starting in 1952. M.L. was appropriately

Figure 2  
A.J.: A 10-Year-Old 46,XX CAH Patient Whose Sex of Rearing Was Changed at 10.5 Years



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treated—both surgically and medically. Surgically, the clitoris was removed and a vaginal orifice separate from the urethral orifice was created. The urinary 17-keto steroids were consistently in accord with adequate suppression. On physical examination there was nothing to suggest Cushing's syndrome, which could have stunted growth and resulted from overtreatment with cortisone. Her BA was consistent with her CA, but her HA throughout childhood was approximately 2 SD below the mean. She experienced no salt crises, indicating adequate

#### CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

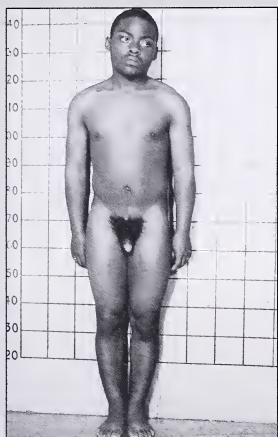
**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Figure 3  
A.J. as 24-Year-Old Adult Male



Reprinted with permission from R. Blizzard, MD.

therapy with deoxycorticosterone acetate and salt. Regardless, she did not grow in accord with her genetic potential. As an adult, she is 160 cm tall, and is the smallest of her 3 unaffected female siblings, all of whom are very close to or above the midparental height (MPH) of 175 cm.

Thelarche occurred when M.L. was 13 years old. Significantly, menarche did not occur until 19.5 years. Her uterus was determined to be very small at age 19 years. Menses were not established with any regularity until she was 25 years of age. At age 35, M.L. married, but had been having unprotected intercourse for 10 years. She was not enthusiastic about assuming parental responsibilities, but did not avoid the possibility.

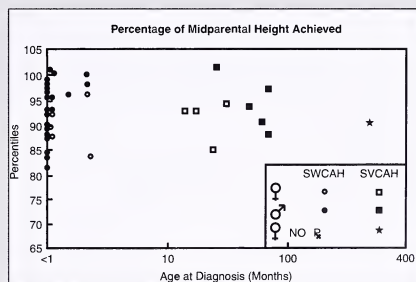
### AUXOLOGIC CONSIDERATIONS OF THE CAH SYNDROME

The heights of CAH patients who are diagnosed early and treated in accord with what is believed to be "good treatment" are less than ideal or expected. Di-Martino-Nardi et al<sup>3</sup> summarized reports from 9 clinics regarding the ultimate height of patients with salt-wasting CAH (SWCAH) and simple virilizing CAH (SVCAH). Nearly all male and female patients who were studied ended up being shorter than expected. The percentages of MPHs achieved according to the age at diagnosis are plotted in Figure 4. Surprisingly, the age at diagnosis did not

markedly affect the outcome, and there was little difference between patients with SWCAH and SVCAH. Conclusions reached by this group and others include the following: (1) The age at diagnosis did not correlate with ultimate height; (2) the quality of metabolic control did not seem to affect the ultimate height; and (3) SVCAH and SWCAH patients were equally below their target heights as adults and less than their first height prediction as a child. In another study, Mulaikal et al<sup>4</sup> reported that 40% (32 of 80 patients) were below the 5th percentile for height (Figure 5 page 36). Multiple authors have reported similar reductions in ultimate heights.<sup>5-14</sup>

The experience of some but not all reporting investigators<sup>12,13</sup> is that untreated SVCAH children are slow growing in the first year of life. The data of Clayton<sup>8</sup> demonstrate that the adolescent growth spurt is dwarfed. This has been confirmed in more recent studies. Decreased adult height has been correlated with increased body weight and body mass index (BMI) during childhood.<sup>13,15-17</sup> Clayton's data<sup>8</sup> indicate that both boys and girls with CAH are usually overweight for height.

Figure 4  
Adult Heights of CAH Patients  
Diagnosed at Different Ages



SVCAH, simple virilizing CAH  
SWCAH, salt-wasting CAH

Adapted with permission from DiMartino-Nardi, et al.<sup>3</sup>

Data regarding bone mineral density (BMD) are limited. Data from 2 reports concerning adults indicate no decrease in BMD among CAH patients who were receiving glucocorticoid therapy.<sup>18,19</sup> Guo et al<sup>15</sup> reported that BMD at various sites was not decreased although adult height was. In a third report,<sup>19</sup> BMD was decreased in adults on treatment (Figure 6 page 37). These authors evaluated 32 adult patients. BMD was evaluated in the left femoral neck and in the lumbar area with dual X-ray absorptiometry. BMD Z score was -0.52 ( $P=0.045$ ) at L2-4

and -0.83 in the femoral neck ( $P < 0.001$ ). For both sites, there was significantly negative correlation between mean glucocorticoid doses and BMD (Figure 7 page 38). The comparable effects of various corticosteroids were reported. Hydrocortisone was preferable as therapy to prednisone, prednisolone, and dexamethasone.<sup>20</sup>

## INTELLIGENCE AND BEHAVIOR IN CAH PATIENTS

The intelligence of CAH patients was initially reported in the 1960s to exceed genetic expectations. However, subsequent studies have found no increased intelligence. In 1991, Nass and Baker<sup>21</sup> reported that SWCAH patients have a lower IQ (107 vs 117) than SVCAH patients. They reported a greater incidence of learning disabilities among female patients than nonaffected females. A greater incidence of learning disabilities exists in normal male than in normal female children. It has been postulated that this is attributable to intrauterine exposure to androgens. The verbal performance IQ discrepancy (10.1) for 18 female patients was significantly greater than for 27 unaffected female sibs. A wider discrepancy exists in normal males than females. The data are compatible with intrauterine exposure to androgens by the CAH females.

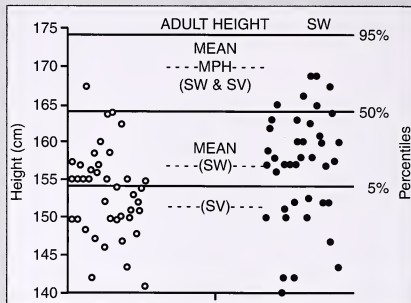
CAH males are more likely to show aggressive behavior than CAH females. In a well-controlled study with 3 different approaches, control males had higher aggression scores than control females, as predicted, but the difference was statistically significant only at adolescence and in adulthood. The scores of CAH females were more in accord with normal males than with normal female controls.

## GENDER IDENTITY, GENDER ROLE, AND PSYCHOSEXUAL PREFERENCE

Gender identity, gender role, and psychosexual preference are not necessarily the same. Gender identity relates to whether one considers oneself a male or a female. Gender role relates to whether one acts as a male or a female regardless of what one's self-image is in respect to gender identification. An individual may have a gender identity of a female but play the role of a male in a modest sense, such as being a tomboy, or in a full sense, such as being masculine in characteristics and playing the male part in a lesbian relationship. A tomboy is defined as a girl who prefers to wear jeans to dresses, play football rather than jump rope, fish rather than play dolls, be dusty rather than clean, and climb trees rather than go shopping. Psychosexual orientation or preference is reflected by homosexuality, heterosexuality, or bisexuality.

To best study gender identity, gender role, and psychosexual orientation in CAH patients, it was necessary to study not only patients treated with glucocorticoids since infancy but also those who were not treated until much

Figure 5  
Heights of 80 CAH Females in Adulthood



MPH, midparental height  
SV, simple virilizing  
SW, salt-wasting

Reprinted with permission from Mulaikal, et al.<sup>4</sup>

later. Exemplary were patients who were not treated until late childhood or adulthood, reported in 1968 by Ehrhardt, Evers, and Money.<sup>22</sup> These authors reported on the influence of androgens on various aspects of sexually dimorphic behavior in women with CAH who were treated late, ie, after 8 years of age. The purpose of their study was to see if exposure to androgens fetally and/or in childhood and/or later produces sexually dimorphic behavior. Twenty-three women met the sampling criteria. Their ages ranged from 8 to 47 years, with a mean of 26 years. A majority had lived for many years with the stigma of heavy virilization and some with uncorrected genital morphology. Most lacked female secondary sexual characteristics such as breast development. With initiation of therapy in this older group, breast development and menstrual periods began, usually within 3 to 6 months. There are, however, exceptions (see M.L. case). Their sex drive and sexual practices, gender identification, gender role, and psychosexual orientation were quite revealing. Ten of the 23 were married. Eight of these were heterosexual and 2 bisexual. Twelve (approximately 50%) had engaged in heterosexual activities exclusively, although 5 of these reportedly had homosexual fantasies, homosexual dreams, or both. Sex drive was high in 11 of 23. Orgasm had occurred in 4, even though the clitoris had been removed. The incidence of homosexual inclinations, the erotic response to visual material, and their personal freedom in sexual activity indicated behavior more often found in males than in females. The authors concluded that the androgens in utero must be responsible for the male-like activity since normal female fetuses who were exposed to placental transfer of synthetic progesterones, which were derivatives of



testosterone, had ambiguous genitalia and gender roles similar to the patients with CAH. This paper<sup>22</sup> was one of the first to suggest that male behavior traits were increased in female CAH patients. Many other publications up through 1997 have confirmed these findings, including an outstanding article by Zucker et al.<sup>23</sup>

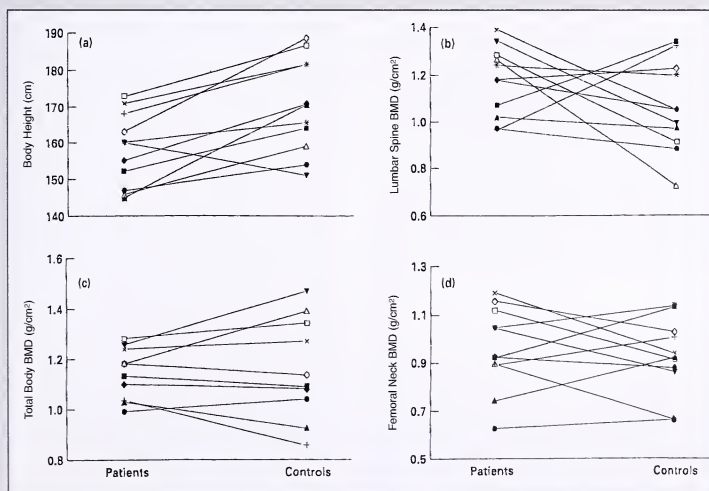
In 1984, Money et al<sup>24</sup> wrote about adult heterosexual status in relation to fetal hormonal masculinization and demasculinization. This excellent study compared 30 46,XX CAH patients who were treated at CA >8 years and a control group of 27 consisting of 15 patients with androgen insensitivity and 12 46,XX females with the Rokitansky-Küster-Hauser syndrome, meaning congenital absence of the uterus.

In Table 1 (page 39), 37% of the individuals with CAH were rated as bisexual or homosexual in contrast to the 7% incidence in the controls. The 7 who were noncommittal are suspected of having a bisexual or homosexual orientation, as they would not commit themselves to a response. If these 7 are eliminated from the statistics and the data based on the 23 remaining patients with CAH who committed themselves in their responses, homoerotic arousal imagery by age 20 was present in 48%, in contrast to 7% of the controls and to 15% of the general

female population sample from the Kinsey report. Twenty-two percent of the 23 had had a homoerotic partner contact by age 20 in contrast to 4% of the androgen insensitivity patients. Data in this report confirmed and extended earlier data reported by Ehrhardt and colleagues.<sup>22,25,26</sup> Dittmann et al<sup>18,27-29</sup> demonstrated that prenatally androgenized CAH patients, even when treated soon after birth, differed significantly from sisters of CAH patients and from controls. These CAH children, although identifying primarily as females in respect to their gender identity, were much more oriented towards activities that interest boys than girls. Comparisons were remarkable when these patients were compared with their normal sisters. Sixteen of 29 single items used for testing suggested significant differences between the 2 groups of girls. In all CAH patients, there was a stronger male orientation than in their unaffected sisters. The patients with SVCAH were less male oriented than the SWCAH patients, and similar although not as markedly feminine as their unaffected sisters. The salt-wasters were oriented markedly towards the male end of the scale. Since androgens are present in utero in both SWCAH and SVCAH patients, the difference between these 2 groups is not explained readily.

Dittmann et al<sup>18</sup> compared 34 adult CAH patients to 14 sister controls, focusing on the psychosexual development

Figure 6  
**Bone Mineral Density (BMD) of CAH Patients on Therapy Versus Controls.**  
Various Symbols Reflect Paired Patients With Controlled Patients for Size, Age, Etc.



Reprinted with permission from Guo CB, et al.<sup>15</sup>



and sexual orientation of the 2 groups. Significantly fewer patients than sisters had ever experienced love relationships and/or sexual activities with male partners. Twenty percent of the patients and none of the sisters wished for and/or had homosexual relationships. In patients >21 years, 44% expressed this interest. Patients with SWCAH differed significantly more from their unaffected sisters than did the SVCAH females from their unaffected sisters. These results corroborate earlier reports on both delays in reaching psychosexual milestones and increased rates of bisexual/homosexual fantasies and experiences in CAH women.

A study contributing to the concept that 46,XX CAH patients raised as males are male oriented was published in 1976 by Money and Dalery.<sup>30</sup> In this study, the authors compared the gender identities, gender roles, and psychosexual orientation of 3 46,XX CAH patients who had complete differentiation of the external genitalia along male lines and who were raised as males with the same parameters of 4 similar patients who were assigned as females at 6 days, 19 days, 8 weeks, and 1 year of life. The 3 reared as males were last studied as adults. Those raised as females were last studied in late childhood.

Evaluation of the data (Table 2) reveals certain common patient characteristics in the 2 groups during infancy and childhood. The data concerning the 4 raised as females are in the right column and the data concerning the 3 raised as males are in the left column. All 7 played with dolls rarely. They preferred traditional boys' toys such as trucks, guns, and blocks. All 7 had a lack of interest in infant caretaking. All 7 exhibited high-energy expenditure and rough outdoor play. All 7 preferred boys over girls in peer contact, and they liked competition with boys. All were oriented toward clothes appropriate for tomboys. The 4 raised as girls, although tomboyish, did not have an overt desire to be boys. All 7 were able to mix with and be accepted without

difficulty by their age-mates in all settings. Study of the 3 raised into adulthood as males revealed all functioned as males sexually. Two of the 3 were married and the third anticipated marrying. The wives of the 2 already married reported being satisfied with their partner's sexual function. None reported any erotic behavior or fantasies toward men. More recent studies<sup>13,14,31,32</sup> confirm that 46,XX CAH females have a greater than expected propensity to accept or request being raised as males, even some at adolescence or post adolescence.

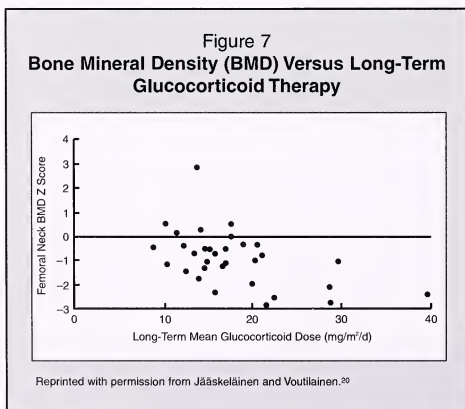
If androgen exposure is interrupted at birth—as in the case of the 4 girls reported above who were assigned to the female sex, who were reared as girls, whose genitalia was surgically corrected to female, and who experienced a feminizing puberty—establishment of a female gender identity occurs. However, the gender role and psychosexual preference is often male oriented. Both the fetal hormonal milieu and the postnatal environment, including sex assignment, may play important roles in gender identity. Very rarely does a 46,XX CAH patient raised from early infancy as a male elect to have the sex of rearing changed to that of a female.

Gender identity, gender role, and psychosexual preference of 46,XY individuals with CAH are uniformly in accord with those of most 46,XY males, as might be expected from intrauterine exposure to excessive androgens.

## PREGNANCY IN CAH FEMALES

The incidence and prevalence of pregnancy in SWCAH females is very low compared with that in SVCAH patients. Mulaikal et al<sup>4</sup> reported on 80 adult female CAH patients (40 with SWCAH and 40 with SVCAH). Twenty-five pregnancies were reported among 15 women with SVCAH, but only 1 pregnancy was recorded in 40 patients with SWCAH. In the Cardiff experience<sup>5</sup> of 16 patients (11 SWCAH, 5 SVCAH), a 40% ovulation rate, as measured by salivary progesterone, was found. Three of 5 patients with SWCAH and 2 of 3 patients with SVCAH who had both an adequate introitus and were sexually active produced 8 pregnancies. This study highlights the potential for improved fertility in compliant patients who are treated early, who have adequately reconstructed genitalia, and who are followed closely during pregnancy for progesterone, 17 $\alpha$ -hydroxyprogesterone, and testosterone levels. A 1998 review by Garner,<sup>16</sup> entitled "CAH in Pregnancy," provides an overview of CAH in both mothers with and without CAH and in potential CAH fetuses in utero. More information on the frequency of pregnancy in SWCAH is very much needed, but the pregnancy rate certainly is low from all data presented to date.

What are the possible causes of the difference in pregnancy rates in SVCAH and SWCAH? The first possibility is that therapeutic noncompliance is greater among SWCAH patients. The second possibility is that there is



**Table 1**  
**Data Regarding 30 46,XX CAH Patients**  
**Diagnosed at >8 Years and a Control Group**  
**of 15 Androgen Insensitivity (AIS) Patients**  
**and 12 46,XX Females With**  
**Rokitansky-Küster-Hauser Syndrome (RS)**

| Comparison of Homoerotic Incidence in CAH, AIS/RS,<br>and Kinsey's Sample at Age 20 |             |              |                |                    |
|---|-------------|--------------|----------------|--------------------|
|   | CAH<br>N=30 | CAH<br>N=23* | AIS/RS<br>N=27 | Kinsey's<br>Sample |
| Homoerotic arousal<br>(imagery)   | 37%         | 48%          | 7%             | 15%                |
| Homoerotic partner  | 17%         | 22%          | 4%             | 10%                |

\*Omitting 7 noncommittal subjects.

Reprinted with permission from Ehrhardt and Meyer-Bahlburg.<sup>28</sup>

a higher frequency of menstrual irregularity among SWCAH patients, reflecting less ovulation in this group. Third, there is a lower incidence of marriage in the SWCAH patients than in the SVCAH patients and, therefore, there is less opportunity for pregnancy. Fourth, the vaginal introitus is more frequently inadequate in the SWCAH group compared with the SVCAH group (53% vs 18%).<sup>15</sup> Another possibility is that SWCAH patients engage in heterosexual activity less frequently.

The necessity for cesarean section<sup>15</sup> in patients with SVCAH is very high. Of the 15 females with SVCAH experiencing 25 pregnancies, 13 carried to term. Nine of these 13 required cesarean sections because of pelvic disproportion. This is not surprising because of the constrictive anatomy that often is present postoperatively.

In respect to the necessity for a clitoris or phallus to be present to effect orgasm, it appears that the clitoris is not essential although probably desirable. Ehrhardt et al documented this in 1968,<sup>22</sup> and my observations with several adult female CAH patients who have had total clitoridecomy confirmed that orgasm does occur in some patients in the absence of the clitoris. Meyer-Bahlburg and colleagues<sup>31</sup> and others<sup>33</sup> have confirmed that the clitoris is desirable but not essential for orgasm. Vaginal sensitivity seems to be adequate for orgasm to occur in many.

## THERAPEUTIC CONSIDERATIONS

Therapeutic approaches are designed to ameliorate the adult consequences of CAH. As in the past, most current therapy is directed toward salt loss in SWCAH and toward controlling the excessive production of adrenal androgens and correcting reduced cortisol production with glucocorticoids. The question whether continued glucocorticoid therapy is necessary in 46,XY males who reach adulthood is often raised. In some male patients,

stopping therapy produces development of testicular tumors (adrenal rests). The tumors histologically appear as testicular tissue but do not have Reinke crystals. These crystals are found in testicular tissue and differentiate Leydig cells from adrenal cells. Orchiectomies have been performed unnecessarily by uninformed physicians. Adrenal rest tumors of the ovary also occur. Adequate glucocorticoid therapy prevents the development of adrenal rest tumors.

Gonadal failure and infertility have been documented in males when glucocorticoid therapy was stopped. Some untreated patients with resultant gonadal suppression by adrenal androgens take more than 2 years to develop spermatogenesis. Urban et al,<sup>6</sup> however, noted that 19 of 20 adult males, 7 untreated at the time, had normal sperm counts. Variation among patients probably occurs in relation to the extent of hyperandrogenicity.

Adrenal tumors,<sup>17</sup> some of which are malignant, have been reported. These result from hyperactivity in the non-suppressed gland. In addition, hyperandrogenicity may produce overt aggression, hostility, violent temper explosions, and inability to handle the slightest frustration in males; one case in association with an adrenal rest tumor of the testes also has been reported.<sup>17,34</sup> All symptoms disappeared with adequate glucocorticoid treatment and reappeared when compliance waned.

There is ample evidence that for many reasons treatment should be continued in male and female CAH patients throughout adulthood.<sup>35</sup>

**Table 2**  
**Data Regarding 7 46,XX Females**  
**(3 Raised as Males and 4 as Females From Birth):**  
**Characteristics of the 2 Groups**

| Characteristic                    | Rearing |        |
|-----------------------------------|---------|--------|
|                                   | 3 as ♂  | 4 as ♀ |
| Rarely played with dolls          | 3       | 4      |
| Played with guns, trucks          | 3       | 4      |
| Interest in infant and child care | 0       | 0      |
| High energy at play               | 3       | 4      |
| Preferred male peers              | 3       | 4      |
| Preferred male clothing           | 3       | 4      |
| Wished to be opposite sex         | 0       | 0      |
| Accepted by playmates             | 3       | 4      |

Reprinted with permission from Money and Dalery.<sup>30</sup>

Innovative approaches recently have been considered, including total adrenalectomy. Van Wyk et al<sup>36</sup> suggest this approach in at least some female patients for the following reasons:

- Many CAH patients have adrenals that are deficient in producing hydrocortisone and aldosterone.
- Infertility and hirsutism may be related to increased adrenal production of progesterone and/or androgen even when endogenous or exogenous hydrocortisone levels and urinary ketosteroids are normal.
- Medical treatment that is successful requires physiologic doses of hydrocortisone and not the pharmacologic doses now usually used in attempting to control androgen production. Decreased hydrocortisone treatment as a result of adrenalectomy could produce greater height.
- In the opinion of some authorities, the mortality risk for CAH patients could be less than with medical treatment.

At least 20 of 158 female patients receiving medical treatment at The Johns Hopkins Hospital died of their disease, which is an impressive number. Recent personal communication with Van Wyk indicates that several respected pediatric endocrinologists have successfully utilized adrenalectomy in selected patients as a form of therapy. Personally, I believe this therapy has a role in selected cases and potentially in many more than currently are being treated in this manner.

In respect to alternative medical therapy, Laue et al<sup>37</sup> at the National Institutes of Health (NIH) have proposed the combined use of flutamide (an androgen receptor blocker), testolactone (an aromatase inhibitor), and decreased doses of hydrocortisone. While theoretically logical, I agree with Dr. C.J. Migeon of Johns Hopkins that we need less therapy because we need more compliance. More medications will usually lead to less compliance. Therefore, I am not optimistic about the NIH proposal, although further studies currently being carried out need to be completed for appropriate interpretation.

Arguments that are presented against performing adrenalectomy include: (1) Medical treatment is less invasive; (2) medical treatment very possibly is less threatening to life than adrenalectomy; (3) preserving the adrenals provides potential for other types of treatment in the future; and (4) medical therapy as proposed by Laue et al<sup>37</sup> may provide a better approach to therapy than those we now have.

Dexamethasone treatment of the female CAH fetus in utero, via the pregnant mother, is still debated after 18 years of use. A recent debate<sup>38</sup> by several prominent pediatric endocrinologists offers an excellent summation of current thinking. The contributors were Dr. M. Forest, who is a promoter of this form of therapy; Dr. W. Miller, who is conservative in considering this therapy;

and Dr. M. Ritzen, who gave a favorable commentary on its use.

The results of treatment as reported by Forest are as follows: (1) Treatment is effective and has been used in more than 110 mothers; (2) maternal side effects are bothersome in approximately 9% to 30% of mothers receiving dexamethasone for this purpose, but the etiology of many of these side effects may be the pregnancy itself; (3) no teratogenic effects have been noted; (4) no inhibition of growth in utero, in childhood, or adolescence has been reported to date; and (5) regarding the effects of dexamethasone on the brain and its development, the only possible effect that has been reported is mild temperament alterations in a few patients.

The stated arguments<sup>38</sup> against the use of dexamethasone treatment were fourfold:

- (1) Only 1 of 8 fetuses treated initially in utero are treated long term but all 8 are exposed to dexamethasone at a critical time in utero. These statistics exist because 4 of 8 fetuses treated will be males; the other 4 will be females but only 1 of these will have CAH and require continuing treatment. Unfortunately, treatment has to be given before the sex of the fetus is known, and, therefore, all fetuses must be treated starting at a very early age before differentiation of the external genitalia occurs.
- (2) The dexamethasone dose is pharmacologic, not physiologic.
- (3) The effect of dexamethasone on the fetal brain may be unknown.
- (4) The safety of acute withdrawal of dexamethasone in 7 of the 8 fetuses after 4 to 5 weeks of treatment is of concern.

Ritzen<sup>38</sup> countered in his commentary, noting that the risk for increased abortion is not significant; intrauterine and postnatal growth are normal; the effects on the fetal brain are not significant, as reported to date; and, while there are some mild maternal side effects during pregnancy, no significant long-term effect has been reported. He agrees with Miller that well-controlled, prospective, multicenter studies are needed and that patients should be treated only under a well-designed protocol.

## CONCLUSION

Forty-eight years of study of CAH patients have passed since CAH was first effectively treated. Many challenging questions have been raised, and many have been answered. These cover the transition of patients with CAH from early fetal development to infancy, to adolescence, and then to adulthood. This review is presented to historically record the effect of CAH and its treatment on the mature individual with CAH. A second goal is to emphasize that challenges still exist for clinical investigators.



This manuscript is dedicated to Lawson Wilkins, whose intellectual curiosity stimulated so many of his colleagues and students to continue to ask questions and find the answers to the pathophysiology and treatment of congenital adrenal hyperplasia.

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An extended list of references will be supplied upon request. Address to: GGH, Dr. R. Blizzard, 1224 West Main Street, Suite 701, Charlottesville, VA 22901. Telephone: 804-977-8192. Fax: 804-977-9450. E-mail: rblizzard@compuserve.com.

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## Abstracts From the Literature

### Reconstructing a Human Limb

The vertebrate limb is a very complicated structure whose development is extremely complex. Much has been learned in recent years; however, most articles, even review articles, are not written for clinicians. Bamshad and colleagues at the University of Utah have now assembled a clinician-oriented review that provides many important insights into how human limbs develop and how disturbances that lead to limb defects may arise.

The review covers many areas. It describes how the limb originates in the early embryo and how it grows to its final form. It presents the major actors in the process, many of which have been recently identified because mutations have been detected in patients with limb defects. A few examples include Greig's cephalopolysyndactyly and Pallister-Hall syndromes, which are due to mutations of *GLI3*; ulnar-mammary and Holt-Oram syndromes, which result from *TBX3* and *TBX5* mutations, respectively; Hunter-Thompson and Grebe syndromes, which are caused by *CDMP1* mutations; and Aarskog syndrome due to *FGD1* mutations.

Particular attention is given to 3 groups of genes. *HOX* genes encode transcription factors that contain a 60 amino acid DNA-binding domain called a homeodomain. There are 39 *HOX* genes in humans; these are organized into 4 clusters. *HOX* gene products partly control patterning of many different embryonic structures, including the limbs, where they may subdivide the limb into specific domains and activate downstream genes contingent upon the position of the domain along different axes.

*TBX* genes also encode transcription factors that share a DNA-binding domain called a T-box. At least 5 *TBX* genes are differentially expressed in the developing limb. In the chick and mouse, *Tbx5* and *Tbx4* are exclusively expressed in the forelimb and hindlimb, respectively, suggesting that they specify or at least influence limb identity. *GLI3* encodes a zinc-finger transcription factor. It seems to have both transcriptional activator and repressor activities. The clinical phenotypes that result from *GLI3* mutations are thought to reflect the combination of these activities that are disturbed. Finally, the authors address the evolutionary aspects of limb development.

Bamshad M, et al. *Pediatr Res* 1999;45:291-299.

**Editor's comment:** This is a very complete review of the last decade of progress in vertebrate limb development. This review is for the clinician and nonclinician alike and provides considerable insight into the physiology and pathophysiology of limb embryogenesis. The latter is key to understanding physical developmental defects.

One can divide limb development into 2 phases: an early phase during which the essential elements of the limb are formed and a later phase during which the limb grows to reach its final form. This review focuses on the former. Hence, the genes that influence patterning produce the syndromes that reflect defective patterning when mutations occur. In contrast, genes involved in the growth phase are those mutated in osteochondrodysplasias (growth phase defects).

William A. Horton, MD



## Growth of Long-Term Survivors of Liver Transplantation

Viner et al performed a retrospective analysis of growth of 105 children who were long-term survivors of liver transplantation. During the 10-year period of the study, triple immunosuppression therapy using cyclosporine, azathioprine, and prednisolone was used. Height was recorded at all clinic visits using a Harpenden stadiometer. Following transplantation, height was recorded every 3 months for the first 18 months, twice a year for the next 3 years, and then yearly thereafter. Height is expressed as height Z scores (height SD scores) standardized against 1990 British growth references. Severe growth retardation was defined as a height below the 0.4 percentile. Continuous variables were analyzed by paired-tests and multiple regression analysis.

The height of patients at transplantation was significantly below that of the general population ( $P < 0.0001$ ), with the height Z score at -1.22. Following liver transplantation, height Z scores fell significantly in the first 6 months ( $P < 0.006$ ) but catch-up growth then occurred and was maximal from 6 months to 2 years. Height Z score at transplantation predicted 64% of the variance in height SDS at 2 years and 53% of the variance in height SDS at 5 years. Of 19 patients who were severely growth retarded, half remained so 4 years posttransplantation. Final height was recorded for only 14 patients, but their mean height SDS was -0.55. Diagnosis was not significantly associated with height Z scores at the time of liver transplantation ( $6.1 \pm 4.4$  years). Subjects undergoing transplantation who were less than 2 years old had significantly greater growth retardation at 6 months following transplantation ( $P < 0.05$ ) and a trend towards greater catch-up growth from 6 months to 2 years. Multiple regression analysis demonstrated that predictors for height Z scores at 6 months were initial Z scores and bilirubin at transplantation and prednisolone dose at 6 months. At 4 years, height Z scores were predicted by height Z scores at transplantation and the cumulative dose of prednisolone at transplantation. Age, sex, diagnosis, liver function at transplantation, cyclosporine dose, and the need for retransplantation were not predictive.

The authors state that this was the first report of long-term growth and final height after liver transplantation in children. They note

that their patients were a heterogeneous group and that transplantation in infancy was not associated with poor height outcome. The average final height after liver transplantation was on the 27th percentile. They suggest that further investigation of the use of corticosteroids prior to transplantation is needed, and that an attempt should be made to transplant children at earlier ages.

Viner RM, et al. *Arch Dis Child* 1999;80:235-240.

**Editor's comment:** This is an interesting and fairly complete retrospective analysis. A summation is in the box that follows. The authors point out 2 very important questions for further study. First, how can corticosteroid doses be minimized prior to the time of transplantation such that the cumulative dose would be less at that time? Second, what is the role of hepatic-derived growth factors in the findings? Whether exogenous growth hormone might have a role in preventing growth retardation prior to transplantation is an additional area for further study and speculation.

William L. Clarke, MD

### Key Findings of the Retrospective Analysis of 105 Long-Term Survivors of Pediatric Liver Transplants

- Average final height after liver transplantation was on the 27th percentile, although those undergoing transplantation as infants can achieve better final heights.
- Height at transplantation is the most important predictor of later height outcome, emphasizing the need for optimal transplant timing and preoperative nutritional management.
- High corticosteroid dose, poor liver function, and the need for a second transplant were associated with poor height outcome.
- Transplantation in infancy was not associated with poorer height outcomes.
- Normal pubertal progress was resumed 3 to 5 years after transplantation.

## Prolongation of Ovarian Lifespan Into Advanced Chronological Age By Bax-Deficiency

In both humans and mice, there is a marked excess of primordial ovarian follicles that degenerate early in life. The remaining oocytes appear to go through apoptosis over the lifetime of the individual female, leading usually during the fifth decade of life to menopause in women and to infertility 3 to 6 months before death in female mice. Bax-deficient female mice (ie, deficient in the normal Bax protein) have been found to have an increased number of primordial follicles in their ovaries compared with wild-type littermates. This excess of follicles is maintained into advanced age. In addition, the Bax-deficient mice possess hundreds of ovarian follicles that are in different stages of development. The aged Bax-deficient females fail to become pregnant. However, it appears that this is due to failure of the pituitary axis rather than nonfunctional oocytes because these mice can be superovulated and oocytes remain that are competent for in vitro fertilization. This suggests that the increased number of oocytes are competent for producing embryos even though assisted reproductive technology may be needed in old age. Careful morphometric analysis

shortly after puberty of the numbers and structure of nonatretic follicles in wild-type and in Bax-deficient mice revealed approximately 3 times the number of nonatretic primordial follicles in the latter, suggesting that Bax-deficient mice do not go through the normal apoptotic process seen in normal mice and normal human females.

Perez GI, et al. *Nature Genet* 1999;21:200-203.

**Editor's comment:** This observation in Bax-deficient mice has enormous ramifications for infertile women and suggests that there may be a mechanism to maintain oocytes in Turner syndrome and some familial types of ovarian degeneration. Perhaps developing a block to the normal Bax protein could maintain normal viable oocytes. Fortunately, this manipulation should be possible in mice and yet have great application in humans.

Judith G. Hall, MD

## Association Between Type I Diabetes and Haemophilus Influenzae Type B Vaccination: Birth Cohort Study

The temporal association between *Haemophilus influenzae* type b (Hib) vaccination and the development of type I diabetes in Finland was studied. The risk of type I diabetes was compared among 3 Finnish birth cohorts: those born within 24 months before the Hib vaccination trial (ie, historical controls); those vaccinated at 3 months of age and with a booster at 14 to 18 months of age; and those vaccinated only at 24 months of age. The unvaccinated cohort included 128,936 children; the 2 vaccine-eligible cohorts totaled 116,352 children. No significant differences were found at any time during the 10-year follow-up in risk of type I diabetes between the children born before the vaccination period and those vaccinated at the age of 24 months only (risk ratio 1.01;  $P=0.228$ ). The difference in the risk between children vaccinated first at the age of 3 months and those vaccinated only at the age of 24 months also was not statistically significant (risk ratio 1.06;  $P=0.545$ ). The authors conclude that "based both on randomized design and on the use of historical controls," it is unlikely that Hib vaccination or its timing is related to type I diabetes in Finnish children.

Karvonen M, et al. *Br Med J* 1999;318:1169-1172.

**Editor's comment:** *Insulin-dependent diabetes mellitus (IDDM) has been increasing in Finland over the last 3 decades. In children under 14 years of age, there has been a 2% to 5% per year increase, with a prevalence of 45/100,000 reached in 1996. This incidence is perhaps one of the highest in the world. During this*

*period, children also were given an increased number of vaccines, including Hib immunizations. However, this study provides ample evidence that the concomitant expansion of Finland's childhood immunization program, at least in regard to Hib, is not responsible for the increased incidence of IDDM. The temporal association between the 2 variables does not seem to indicate that there is a cause-and-effect relationship. Unfortunately, the media, including a major segment on "ABC World News" on September 25, 1998, have reported on the alleged association between Hib vaccine and the development of this disease. I wish that the report by Dr. Karvonen and colleagues would receive equal time so that the public would not be needlessly concerned. The beneficial effects of Hib vaccination are well proven, and it would be unjustified to restrict vaccination because of potential adverse consequences that have not been proven to exist.*

*In addition, other studies have found no increase of type I diabetes in association with various vaccines used in childhood, including measles, BCG, and pertussis vaccines.*

Fima Lifshitz, MD

Blom L, Dahlquist G. *Diabetologia* 1991;34:176-181.

Dahlquist G, Gothefors L. *Diabetologia* 1995;38:873-874.

Heijbel H, et al. *Diabetes Care* 1997;20:173-175.

Parent M-E, et al. *Diabetes Care* 1997;20:767-772.

## Cow's Milk Formula Feeding Induces Primary Immunization to Insulin in Infants at Genetic Risk for Type I Diabetes

Insulin autoantibodies (IAAs) often appear as the first sign of islet cell autoimmunity in prediabetic children. Because cow's milk contains bovine insulin, the authors followed the development of insulin-binding antibodies in children fed with cow's milk formula. Bovine insulin- and human insulin-binding antibodies were analyzed by enzyme immunoassay (EIA) and IAAs were analyzed by radioimmunoassay (RIA) in 200 infants carrying *HLA-DQB1\*0302* but no protective alleles. These children participated in a Finnish population-based birth cohort study. Based on the prospectively registered information, the first 100 infants (group 1) enrolled in the study who were exposed to cow's milk formula before age 12 weeks and the first 100 infants (group 2) enrolled in the study who were exclusively breast-fed for longer than the first 12 weeks of life were selected for the present study. Also studied were 11 children from the 200 infants who had developed at least two diabetes-associated autoantibodies, 98 children with newly diagnosed type I diabetes, and 92 healthy children. The authors reported that the amount of IgG antibodies binding to bovine insulin was higher at age 3 months in infants who were exposed to cow's milk formula than in infants who were exclusively breast-fed before and at 3 months of age (median, 0.521 vs 0.190;  $P<0.0001$ ). The antibodies binding to bovine insulin cross-reacted with human insulin. None of these infants tested positive for IAAs. The levels of bovine insulin-binding antibodies declined in both groups at age 12 and 18 months; in the 11 children with at least two diabetes-associated autoanti-

bodies, the levels increased during the follow-up period ( $P<0.0001$ ). IgG antibodies correlated with IgG2 antibodies binding to bovine insulin ( $r=0.43$ ,  $P=0.004$ ) and IAAs ( $r=0.27$ ,  $P=0.02$ ) in diabetic children, but not in healthy children.

The authors concluded that cow's milk feeding is an environmental trigger of immunity to insulin in infancy that may explain the epidemiologic link between the risk of type I diabetes and early exposure to cow's milk formulas. This immune response to insulin may later be diverted into autoaggressive immunity against beta cells in some individuals, as indicated by these findings in children with diabetes-associated autoantibodies.

Vaara O, et al. *Diabetes* 1999;48:1389-1394.

**Editor's comment:** *Many studies have linked cow's milk consumed by infants to subsequent diabetes. The association is*

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based on animal experiments or indirect evidence derived from studies in which parents of diabetic children tried to recollect when their babies first started drinking milk-based formula.

The Finnish researchers who conducted this study avoided the vagaries of poor recall by studying children from birth. In so doing, they have added to the case against cow's milk. By monitoring infants in diabetes-prone families, namely, those with HLA-DQB1\*0302, the scientists found that infants getting cow's milk formula were more likely to develop the immune reactions associated with insulin-dependent diabetes mellitus (IDDM) than infants fed exclusively human milk.

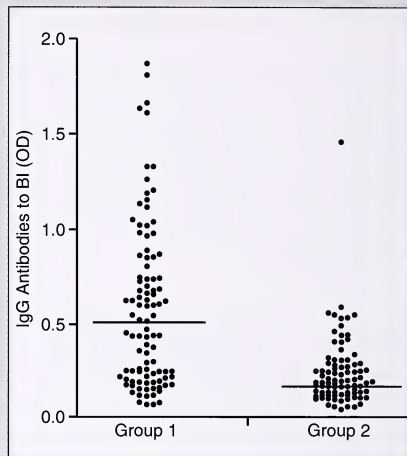
It is known that having one type of autoantibody to insulin indicates that a child has roughly a 40% chance of developing type 1 diabetes within the next decade. Additionally, having more types of these autoantibodies may be a sign of greater risk; having 3 types of autoantibodies imparts an 80% to 90% likelihood of developing type 1 diabetes. However, the precise cause of IDDM remains unclear. The children in the study were genetically predisposed to IDDM, but most will never get the disease. Something in the environment or diet, such as consuming cow's milk during infancy, may be a triggering factor.

This study presents further evidence implicating cow's milk. In Puerto Rico, fewer than 5% of mothers breast-feed their children. Instead, nearly all use formula made from cow's milk. Meanwhile, the IDDM incidence in Puerto Rico is roughly 10 times the rate seen in Cuba, where breast-feeding is nearly universal. Such findings represent circumstantial evidence suggesting that ingestion of cow's milk in the first few months of life plays a very important role in the etiopathogenesis of this disease.

To date, none of the data on cow's milk and IDDM preclude feeding cow's milk formula to infants who do not have the good fortune of being fed human milk.

Fima Lifshitz, MD

Figure  
The Levels of IgG Antibodies to Bovine Insulin (BI) at Age 3 Months in Infants Who Received Cow's Milk Formula Before Age 12 Weeks (Group 1) and in Infants Who Were Exclusively Breast-Fed Until Age 12 Weeks (Group 2)



The median is marked with a line.  $P < 0.0001$ , group 1 versus group 2 (Mann-Whitney  $U$  test).

Reprinted with permission from Vaarala O, et al. *Diabetes* 1999;48:1389-1394.

## A Molecular Pathway Revealing a Genetic Basis for Human Cardiac and Craniofacial Defects

The investigators have identified a gene that is deleted or mutated in patients with the DiGeorge association (DGA) of craniofacial and cardiovascular malformations. Specific defects include interruption of the aortic arch, truncus arteriosus, tetralogy of Fallot, defective immunocompetence, and hypoparathyroidism. These widespread anomalies have been attributed to a defect in the function of neural crest cells important for normal structural development. The vast majority of patients with DGA have a monoallelic microdeletion of chromosome 22q11.2. Noting that mice lacking the transcription factor *dHAND*, a factor necessary for survival of neural crest cells in the branchial arches, aortic arch, and right ventricle, have many of the anomalies present in DGA, these workers examined the genes that this protein regulates. They found it to regulate the human homologue of *Ufd1*, a gene that encodes a protease that degrades proteins linked with ubiquitin that is localized to the critical region of 22q11 associated with DGA. The investigators demonstrated that in mice, *Ufd1* was expressed in those tissues that are adversely

affected in patients with DGA. In all 182 patients with DGA and deletion of 22q11, *UFD1L* was absent. In 1 patient with DGA but no apparent chromosomal anomaly, the investigators demonstrated monoallelic deletion of exons 1 through 3 of *UFD1L* with retention of exons 4 through 12 of this gene. In the same patient, there was partial deletion of an adjoining gene, *CDC45*, a cell cycle protein. However, this gene is widely expressed. Therefore, the authors suggest that monoallelic inactivation of *UFD1L*, either by gross deletion or more subtle mutation, may be responsible for DGA. They hypothesize that the failure to degrade an as yet unidentified, ubiquitinated protein adversely affects the development of those neural crest cells necessary for normal formation of craniofacial bones, heart, thymus, and parathyroid glands.

Yamagishi H, et al. *Science* 1999;283:1158-1161.

**Editor's comment:** Although it remains possible and perhaps even probable that DGA and associated disorders found in



patients with deletion of chromosome 22q11 are the result of loss of contiguous genes, the current report strongly implicates UFD1L as a key gene in DGA. Examination of this gene in additional patients with DGA without visible microdeletions of 22q11 will be important. Ubiquitin is a 76 amino acid peptide that links to and apparently "tags" proteins before they are degraded by proteases associated with the nonlysosomal 20S proteasome.

Recently, the gene that is mutated in Angelman syndrome (UBE3A) has been found to encode a protein (E6-AP) that is a ubiquitin-protein ligase. (Interestingly, E6-AP also is a coacti-

vator for the transcriptional activity of the human progesterone receptor, but this metabolic function of E6-AP is intact in patients with Angelman syndrome.) Thus, Angelman syndrome is likely to be another example of a disease resulting from accumulation of a toxic protein that escapes degradation by the ubiquitin-proteasome pathway of protein degradation. Thus is identified another class of disorders, the "ubiquitinopathies," a name suggested by Dr. A. diGeorge.

Allen W. Root, MD

Fang P, et al. *Hum Molec Genet* 1999;8:129-135.

Nawaz Z, et al. *Molec Cell Biol* 1999;19:1182-1189.

## The Molecular Genetics of Growth Hormone Deficiency

Proctor et al have written an excellent review of growth hormone deficiency (GHD) from a molecular genetic perspective. It is both comprehensive and extremely useful. The GH synthetic pathway is relatively well worked out, as is its relationship to pituitary releasing factors and insulin-like growth factor 1 (IGF-1). Between 5% and 30% of "idiopathic" GHD individuals have a first-degree relative who also is affected, suggesting that there is a genetic etiology for many cases of GHD. The known mutations and genetic forms of GHD are reviewed, including the pituitary-expressed genes that have an effect on GH synthesis and release. In addition, of course, there are primary GH mutations.

The molecular basis of GHD is now being defined in multiple families. More than 30 specific deletions are known. Deletions seem to be particularly predisposed to anti-GH antibody production. At least 10 specific mutations have been described in different parts of the *GH1* gene. Until now, no correlations between mutant genotype and clinical phenotype have been reported.

The GH gene lies in a family of GH-type genes. Their closeness provides potential mechanisms for mutagenesis through slip-page. There are a series of *GH1* gene mutations, including deletions, autosomal recessive mutations, and autosomal dominant splice site and intronic mutations. The human GH (*hGH*) gene cluster includes 2 chorionic somatotropin hormone genes, a chorionic somatotropin pseudogene, and 2 GH genes; *GH1* is

the important functional gene. Evolutionarily in nonprimate mammals, GH is encoded by a single gene.

There are several familial forms of combined pituitary hormone deficiency (CPHD). The *PIT1* gene (*POU1F1*) is associated with autosomal recessive and autosomal dominant inheritance. In addition, *PRO1* gene mutations lead to autosomal recessive CPHD. GH-releasing hormone receptor mutations also have been identified.

Proctor AM, et al. *Hum Genet* 1998;103:255-272.

**Editor's comment:** This is an excellent review. There are 5 pages of references for those individuals trying to research the problem. The delineation of the mutations is complete. As the authors point out, the development of specific mouse models should lead to a better understanding of genotype-phenotype correlation, as well as mechanisms to avoid anti-GH antibody production.

Judith G. Hall, MD

**2nd Editor's comment:** This article is an absolute must to read and digest for both clinicians and researchers involved in the origin of GHD and/or GHD-like syndromes. Drs. Proctor and Cooper of the University of Wales and Dr. Phillips of Vanderbilt University are eminently qualified as world experts on this topic.

Robert M. Blizzard, MD

## Metabolic Effects of Discontinuing Growth Hormone Treatment

The authors serially determined the resting metabolic rate (RMR), fat mass, percent body fat, and total body bone mineral content (BMC) by skinfold measurements and/or dual X-ray absorptiometry after discontinuing the administration of human growth hormone (hGH). The treatment periods ranged from 1.7 to 11.8 years in 11 (4 female) adolescent patients (aged 14.5 to 18.5 years) with isolated GH deficiency (GHD) or multiple anterior pituitary hormone deficiencies (N=8). They found that these measurements were stable during the last year of hGH therapy but that RMR declined within 2 weeks after stopping hGH and remained low through the next year. In GHD patients, fat mass increased within 6 months after cessation of hGH therapy. (In 15

non-GHD control subjects, 5 of whom had been treated with hGH, these measurements did not change appreciably over 1 year.) Six months after hGH administration was halted, there was an inverse relationship between the changes in RMR and fat mass. BMC was normal in the GHD subjects upon completion of hGH treatment and did not change in the ensuing year. The investigators suggest that the short-term decline in RMR after discontinuation of hGH in subjects with childhood-onset GHD may identify those patients with persistent adult GHD who would benefit by reinitiation of hGH therapy.

Cowan FJ, et al. *Arch Dis Child* 1999;80:517-523.



**Editor's comment:** These data demonstrate that discontinuing the administration of hGH in childhood-onset subjects with GHD leads to rapid decrease in RMR. This was predictive of a later increase in body fat content. The data suggest that measurement of RMR shortly after discontinuing hGH administration (Figure) may identify those patients who will benefit by restarting therapy for adult GHD. This hypothesis merits further testing.

Of interest would have been presentation of data on insulin-like growth factor 1, insulin-like growth factor binding protein 3, and acid labile subunit values after discontinuing hGH, and analysis of the relationships of these measurements to change in RMR and body fat composition. The current data also demonstrate that prolonged treatment of GHD children and adolescents with hGH can prevent the osteopenia often associated with hypopituitarism.

Allen W. Root, MD

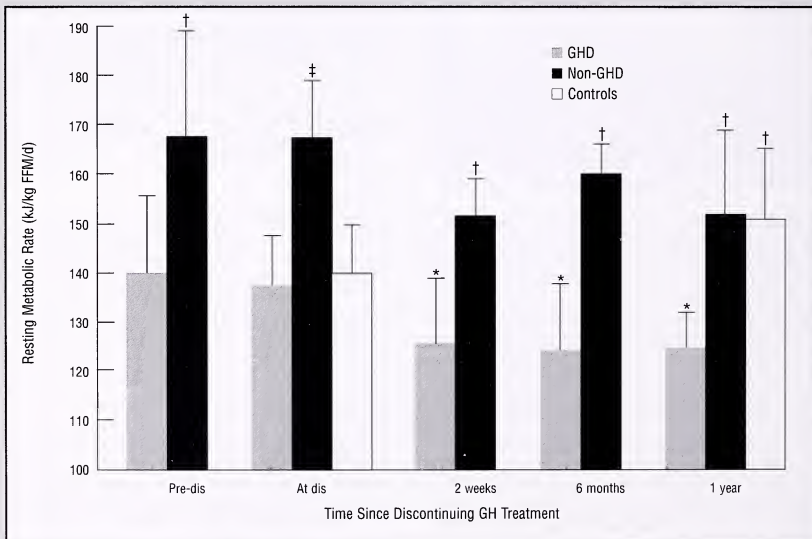
**2nd Editor's comment:** This is an interesting and well-conducted study. The authors have pointed out that the serum

GH criteria for replacing GH in adults is different from that in children, and that many treated children have relatively normal GH secretory responses to pharmacologic stimuli as adults. Thus, it is unclear which individuals should be considered for continuation of therapy.

RMR measurements are not easy to perform, and their determination is not readily available in most clinics. Thus, even though these authors demonstrated some potentially important and very sensitive measurements that might help the physician decide which patient should continue GH therapy, it may not be possible for these data to be collected in many settings. It would be useful for all pediatric endocrinologists to know what happens to children who meet these criteria, and those who do not, who are subsequently treated with GH as adults. Until such data are available, physicians will most likely continue to rely on a myriad of subjective information to determine which individuals to retest and continue on therapy into their adult years.

William L. Clarke, MD

Figure  
Mean (SD) Resting Metabolic Rate Before Discontinuing (Pre-dis) GH Treatment, at Discontinuation (At dis) of Treatment, and 2 Weeks, 6 Months, and 1 Year After Stopping Treatment



Reprinted with permission from Cowan FJ, et al. Arch Dis Child 1999;80:517-523.

## Female Development in Mammals by *Wnt-4* Signalling

Although differentiation of the testes and of male internal and external genitalia have long been known to be actively guided processes, it has been assumed that differentiation of the ovary and of female internal genitalia are "innate" processes that occur by default. Vainio et al demonstrated that in the mouse, ovarian and Müllerian duct differentiation are active processes influenced by the product of *Wnt-4*, a member of a family of extracellular, cysteine-rich glycoprotein signaling molecules (OMIM 603490). The latter influence a number of developmental processes, including normal renal development in the mouse.

*Wnt-4* also is expressed in the embryonic gonadal ridge, the undifferentiated fetal gonad, the fetal ovary (although downregulated in the fetal testis), and in mesenchyme of the Müllerian but not the Wolffian ducts. By crossbreeding animals heterozygous for inactivation of *Wnt-4*, these investigators developed fetuses and newborns that were homozygous for this defect (*Wnt-4*<sup>-/-</sup>).

The male offspring were phenotypically normal, but the female offspring were virilized. The ovary was deformed and the Müllerian ducts were absent. The single gonadal duct resembled an epididymis. The external genitalia of the female offspring were normal. The *Wnt-4*<sup>-/-</sup> female gonad appeared to synthesize and secrete androgens as it expressed both *HSD3B* and *CYP17*. The homozygous fetal ovary had far fewer oocytes than the normal ovary, suggesting that *Wnt-4* is necessary for oocyte maintenance. The investigators conclude that *Wnt-4* is important for normal fetal female sexual differentiation in the

mouse and, by inference, in other mammals, possibly including humans.

Vainio S, et al. *Nature* 1999;397:405-409.

**Editor's comment:** This study demonstrates the essential role that *Wnt-4* plays in Müllerian duct differentiation and ovarian function. It is of interest that in the *Wnt-4*<sup>-/-</sup> females, Müllerian duct inhibitor factor from the Sertoli cells was not necessary for regression of the Müllerian ducts!

It has been suggested that DAX1 (dosage sensitive sex reversal on the X chromosome—Xp21) may be important for ovarian differentiation. The role of DAX1 in sexual differentiation has been demonstrated by the sex reversal of 46,XY males in patients with duplication of this gene, thus suggesting that this transcription factor is involved in ovarian determination and repression of testicular function. Yu et al (Role of Ahch in Gonadal Development and Gametogenesis. *Nature Genet* 1998;20:353-357) generated a mouse model in which Dax1 has been inactivated. In these animals, ovarian differentiation and fertility are normal, but spermatogenesis and testosterone secretion in the male are disrupted. Thus, Dax1 affects testicular function in a dose-dependent manner; normally, it supports spermatogenesis and Leydig cell function, but when duplicated leads to inhibition of the testis-determining effects of SRY and SOX9. Embryologic sex differentiation becomes more complex as we learn more and more about gene control. We used to be able to explain sexual differentiation on a hormonal basis, but we cannot do this in 1999.

Allen W. Root, MD

## COL9A3: A Third Locus for Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) refers to an autosomal dominant clinical phenotype characterized by mild to moderate short stature associated with painful joints and precocious osteoarthritis. Historically, MED has been divided into the severe (or Fairbank) form and the mild (or Ribbing) form, although the phenotypes merge to form a gradient of severity. MED is a genetically heterogeneous condition.

MED mutations were first found in the gene encoding cartilage oligomeric matrix protein, COMP. This locus, which also harbors mutations responsible for pseudoachondroplasia, was designated *EDM1*. It was clear soon after the *EDM1* locus was identified that, in some families, MED does not map to the *COMP-EDM1* locus.

A second locus, designated *EDM2*, was identified in 1996 through positional cloning. It encodes the alpha 2 chain of type IX collagen, an extracellular matrix protein of cartilage and skeletal growth plate. This led to the view that type IX collagen and COMP interact functionally in growing bones and that disturbances of this interaction occur in pseudoachondroplasia and MED.

The existence of a third *EDM* locus was anticipated from linkage studies that excluded *EDM1* and *EDM2* in some families with MED phenotypes. The genes encoding the other 2 chains of type IX collagen, *COL9A1* and *COL9A3*, were the strongest candidates. *COL9A3* has now been identified as *EDM3*. Paasilta et al first established linkage of a relatively mild form of MED to a genetic marker in *COL9A3*. They next demonstrated a mutation predicted to disrupt splicing of *COL9A3* mRNA transcripts, leading to deletion of 12 amino acids near the amino-terminal end of the collagen chain. The mutation resembles the *COL9A2* mutation found in other cases of MED. Not surprisingly, the manifestations are similar in the families with type IX collagen mutations.

Paasilta P, et al. *Am J Hum Genet* 1999;64:1036-1044.

**Editor's comment:** It is now evident that genes encoding cartilage matrix proteins are a very rich source of mutations that cause human chondrodysplasias. This reflects the importance of these proteins to endochondral bone growth. Since these proteins interact with each other to form a functional extracellular matrix, it makes sense that disturbances of different elements of this matrix lead to relatively similar clinical

phenotypes. However, the mechanisms by which such disturbances interfere with bone growth remain poorly understood. Potential mechanisms include: (1) disturbances of the mechanical properties of cartilage, which must serve as a template for bone growth; (2) disturbances in the diffusion, sequestration, and/or presentation of growth factors; and (3) disturbances of direct interactions of the matrix with cartilage cells. As emphasis evolves in the post genome era from finding gene loci and detecting mutations to elucidating how mutations act, the mechanisms relevant to cartilage matrix protein mutations should be unveiled.

William A. Horton, MD

**2nd Editor's comment:** MED must be thought of clinically in the presence of unexplained short stature (normal or

abnormal proportions) and/or joint pains (particularly of the knees). Osteoarthritis results and frequently necessitates hip and knee replacement in adult life. In the current 4-generation family described by Paassilta et al, none of the 8 affected adults were outside the normal range for height. All had knee pain, often dating to childhood, and some had hip pain. A few had involvement of other joints. Clinical investigation should include radiologic examination, particularly of the knees and hips. X-ray studies of adults may or may not show abnormalities after epiphyseal fusion has occurred. Family history and investigation of short children and/or children with knee pain in the family may prove the diagnosis in the adult with suspected MED (by familial association).

Robert M. Blizzard, MD

## 10 Years of Genomics, Chromosome 21, and Down Syndrome

Despite being the smallest of human chromosomes, chromosome 21 occupies a prominent place in human genetics. The long arm of this chromosome is approximately 37 mb in length and constitutes about 1% of the human genome. Trisomy for chromosome 21 is the most common aneuploidy at birth in humans; it results in the most common form of mental retardation, occurring in 1/700 live births. To celebrate the 10th birthday of the journal *Genomics*, Stylianos Antonarakis has written a comprehensive review covering the last decade of progress involving chromosome 21 and Down syndrome. The review covers diverse topics, including a comparison of different types of chromosome 21 maps; genes that may be responsible for the clinical phenotype in Down syndrome; genes that contribute to the pathogenesis of other disorders, including mouse models of Down syndrome; and the mechanisms that cause trisomy 21. The review also provides a good reference

list of achievements in this area and insight into future progress that can be expected.

Antonarakis SE. *Genomics* 1998;51:1-16.

**Editor's comment:** This 16-page article is an excellent review, especially for nongeneticists and geneticists who do not work in this field. It not only provides considerable information about chromosome 21 but also serves as a good tutorial on gene mapping strategies. Especially interesting is a discussion of how the many different types of genetic maps of chromosome 21 are related and how they are constructed. This review article is highly recommended to all our readers.

William A. Horton, MD

## Familial Defects in X-Inactivation

The molecular mechanism by which X-inactivation occurs is beginning to be elucidated. The process of X-inactivation is under the control of the X-inactivation center (XIC), which initiates and proliferates inactivation along the X chromosome. The active X chromosome *Xist* gene encodes and produces RNAs that coat the opposite X chromosome and seems to keep it inactive. Lee et al have shown there also is a *Tsix* element, which apparently lies within the XIC region and is expressed on the active X chromosome. High transcription levels of *Tsix* and *Xist* appear to be mutually exclusive. Interestingly, the transcript seems to overlap the *Xist* genes. How *Xist* and *Tsix* interrelate is not yet clear.

Naumova et al studied X-inactivation in normal women in families that are not known to have any genetic disease. They found quantitative differences among families with strong sister-to-sister correlations as to the degree of skewing from the expected 50% inactivation of each X chromosome. Interestingly, there is a lack of correlation between mothers and daughters. Lymphocytes were used for these studies, and it is certainly possible that other tissues might yield other

results. The sister-to-sister correlation is consistent not only with a hereditary aspect of X-inactivation but also with the possibility that their phenotype is controlled by cis-acting gene. Also intriguing is the possibility that familial X-inactivation skewing involves differences in *Xist* and *Tsix* expression.

Heard E, et al. *Nature Genet* 1999;21:343-344.

Lee JT, et al. *Nature Genet* 1999;21:400-404.

Naumova AK, et al. *Eur J Hum Genet* 1998;1018:552-562.

**Editor's comment:** X-inactivation is beginning to be unraveled. It is a wonderful model for gene control, identifying the mechanisms that may apply to both genomic imprinting and time-specific expression in tissues. The interesting correlation between sibs, but not mothers and daughters, in skewed X-inactivation families suggests that there is "cross talk" between the 2 X chromosomes. We all have been taught to expect 50-50 inactivation of the X chromosomes, but that appears to have been a generalization rather than a reality.

Judith G. Hall, MD



## A Novel Skeletal Dysplasia With Developmental Delay and Acanthosis Nigricans Is Caused By a LYS650MET Mutation in the Fibroblast Growth Factor Receptor 3 Gene

Mutations that cause the achondroplasia group of human chondrodysplasias map to a small number of codons in the fibroblast growth factor receptor 3 (*FGFR3*) gene. For example, almost everyone with typical achondroplasia has a mutation of codon 380, and all infants with the type II variant of thanatophoric dysplasia (TDII) have mutations at codon 650. This genetic homogeneity contrasts with the dispersion of mutations through host genes in many disorders that involve extracellular matrix proteins. There is now a new twist regarding *FGFR3* mutations.

Groups from California and Maryland have identified a novel clinical phenotype associated with a mutation of *FGFR3* codon 650 that is distinct from TDII. In TDII, the mutation changes the normal lysine at position 650 to a glutamic acid. A methionine residue is substituted for lysine 650 in the new disorder. This single amino acid difference produces substantial differences in manifestations.

Four unrelated patients were reported by Tavormina and colleagues. They all exhibited growth deficiency comparable to the type I variant of TD. However, they survived past infancy without prolonged life-support measures. The patients developed extensive areas of acanthosis nigricans beginning in early childhood, and they all suffered from severe neurologic impairment. The authors refer to the clinical phenotype as SADDAN (Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans). Lysine 650 resides in the activation loop of the tyrosine kinase domain of *FGFR3*, where it helps to regulate kinase activity in response to fibroblast growth factor ligand binding to the receptor. The kinase phosphorylates intracellular substrates, thereby initiating signals that influence bone growth. The TDII mutation has been shown to activate kinase activity in absence of ligand binding. A similar constitutive activation of kinase activity was demonstrated for the SADDAN mutation. In fact, the level of activation was higher for the SADDAN mutation than for TDII and achondroplasia mutations. The authors suggest that the SADDAN mutation may do more than activate the receptor in the absence of ligand. For example, it may affect downregulation of the activated receptor. They also suggest that the different amino acid substitution in SADDAN versus TDII may

alter the specificity for substrate-signaling molecules that transmit *FGFR3* signals inside cells.

Tavormina PL, et al. *Am J Hum Genet* 1999;64:722-731.

**Editor's comment:** *The FGFR3 story continues to unfold. This report highlights the importance of the kinase region of the receptor, lysine 650 in particular, in understanding the pathogenesis of the achondroplasia group of disorders. It also underscores the importance of delineating differences in clinical phenotypes so that the functional consequences of mutations can be defined.*

*The report raises some interesting questions. For example, is acanthosis nigricans a consequence of the SADDAN and not the TDII mutation? Or would TDII infants develop the skin lesions if they survived longer? Why is codon 650 so mutable? Will other mutations be found in FGFR3, or have most or nearly all the mutations already been found, as is commonly believed?*

William A. Horton, MD

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## A Comparison of Target Height Estimated and Final Height Attained Between Swedish and Hong Kong Children

The investigators compared the target height (TH) equations of Luo et al, the final parental height method, derived for a Swedish population (see *GGH* 1999;15:13-14), and those of Tanner (derived from an English population) in Chinese subjects living in Hong Kong in whom adult stature was 10 to 12 cm less than that of the Swedish subjects. They found that on average the Tanner equations (corrected midparental height) underestimated adult stature by 4.5 cm, whereas the Luo equations gave values close to achieved mean adult heights in both males and females. However, there were wide ranges ( $\pm 10$  cm) of calculated TH for both sets of equations. The discrepancy between the 2 sets of TH

prediction equations was exaggerated in subjects with low mid-parental heights. The authors conclude that the Luo equations are superior to the Tanner equations for estimation of TH.

Luo ZC, et al. *Acta Paediatr* 1999;88:248-252.

**Editor's comment:** *This article is brought to your attention as only a limited number of pediatricians know about the Luo equations (final parental height) method for estimation of TH, which may more accurately evaluate the effect of growth-promoting agents on the growth of pediatric patients. These equations have now*



been validated in a population in which adult height is far less and there is a secular trend for increased adult stature compared with that of the (stable) Swedish population. If the validity of the Luo equations is further confirmed, they will be more widely utilized and could replace the corrected midparental height of Tanner. Those of you who are particularly interested and/or concerned

about the auxologic tools we use in clinical practice and in research will appreciate this article.

Allen W. Root, MD

Luo ZC, et al. *Pediatr Res* 1998;44:563-571.

## Effects of Thyroxine as Compared With Thyroxine (T<sub>4</sub>) Plus Triiodothyronine (T<sub>3</sub>) in Patients With Hypothyroidism

The authors studied 33 patients receiving either replacement T<sub>4</sub> therapy for chronic lymphocytic thyroiditis (CLT) or suppressive therapy after near-total thyroidectomy because of thyroid cancer. Sixteen had CLT and 17 had thyroid cancer. Mean age was 46±13 years and mean T<sub>4</sub> dose was 175 ± 53 µg/d at baseline. After randomization, patients were assigned to receive T<sub>4</sub> alone for 5 weeks followed by T<sub>4</sub> + T<sub>3</sub> for 5 weeks or vice versa. On the last day of each 5-week period, thyrotropin, thyroid hormones, cholesterol, triglycerides, and sex hormone-binding protein (SHBP) were measured. Physiologic measurements, including pulse, blood pressure, electrocardiogram, sensory threshold, and Achilles tendon reflex, were recorded. Psychological assessment included cognitive function and psychological state.

Significant higher serum T<sub>4</sub> and free T<sub>4</sub> levels were found after T<sub>4</sub> treatment, compared with the combined treatment group. Significantly lower SHBP levels and heart rates also were observed during T<sub>4</sub> treatment. Conversely, after combined treatment, patients showed higher serum total SHBP levels and heart rates. However, those values remained within normal limits in both groups. Serum thyroid-stimulating hormone (TSH), cholesterol, triglycerides, blood pressure, sensory threshold, and Achilles tendon reflex relaxation half-time were similar with both treatment regimens. Significantly higher scores on the digit symbol test indicated better incidental learning, and the higher scores on the digit span test indicated improved mental flexibility and attention. After receiving T<sub>4</sub> + T<sub>3</sub>, patients tended to be less depressed and experi-

enced less fatigue-inertia, depression-dejection, and anger-hostility. At the end of the study, 20 patients preferred T<sub>4</sub> + T<sub>3</sub> treatment, 2 preferred T<sub>4</sub> alone, and 11 had no preference.

The authors concluded that patients with hypothyroidism may benefit from partial substitution of T<sub>3</sub>, improving their mood and neuropsychological function.

Bunevicius R et al. *N Engl J Med* 1999;340:424-429.

**Editor's comment:** Triiodothyronine treatment has been proven to be effective in several conditions, including myxedema coma and selective pituitary thyroid hormone resistance. In addition, Escobar-Morreale et al demonstrated that tissue euthyroidism and normal serum concentrations of T<sub>3</sub>, T<sub>4</sub>, and TSH were achieved in rats only with the administration of a combination of thyroid hormones (*Endocrinology* 1996;137:490-502). In the present study, Bunevicius et al add data to support the potential significance of adding T<sub>3</sub> to the conventional T<sub>4</sub> therapeutic regimen in hypothyroidism. The authors demonstrated not only increases in serum T<sub>3</sub> levels but also improvements in mood and neuropsychological function without total suppression of TSH concentrations. However, long-term studies are necessary to establish the effectiveness of combined treatment with T<sub>4</sub> and T<sub>3</sub>, in particular the long-term effects on bone mineralization and cardiovascular function.

Fima Lifshitz, MD

## Gene Mutations With Characteristic Deletions in Cord Blood T Lymphocytes Associated With Passive Maternal Exposure to Tobacco Smoke

The risks for cancer, heart disease, and other chronic illness are well known for adults who use tobacco, as are the risks for growth deficiency for fetuses exposed prenatally to tobacco smoke. Now there is evidence that prenatal exposure to tobacco increases the risk for childhood malignancy. Finette et al used *HPRT* as a reporter gene to study the genetic consequences of tobacco exposure in utero. They analyzed *HPRT* mutations in cord blood T cells from newborn infants of mothers who had been exposed passively to tobacco smoke and of mothers with no known exposure. They searched especially for differences in types of mutations. The results showed the smoke-exposed infants harbored a higher frequency of a genomic deletion commonly associated with early childhood leukemias and lymphomas. The deletions are referred to as "illegitimate" mutational events because they are mediated by V(J)D recombinase activity, which normally mediates genomic rearrangements responsible for T-cell receptor and immunoglobulin diversity. The authors emphasized that the frequency of mutations was not statistically

different between the 2 patient groups; rather, it was the type of mutations that differed. They noted that tobacco-derived nitrosamine derivatives from O6-methylguanine adducts have been detected in fetal cord blood of leukocyte DNA of primates and raise the possibility that these adducts could be related mechanistically to the mutations.

Unfortunately, too few T-cell clones were isolated from infants whose mothers had smoked to be included in the analysis. The authors, as well as the authors of an accompanying editorial (Sozzi G, et al. *Nat Med* 1998;4:1119-1120) cautioned that the results need to be confirmed by other studies.

Finette BA, et al. *Nat Med* 1998;4:1144-1151.

**Editor's comment:** This is an intriguing article because of its clinical implications. The following is abstracted from the editorial by Sozzi et al appearing in the same issue:

"Although epidemiologic studies have suggested that maternal and paternal passive smoke exposure increases cancer risk in children, the Finette study is the first demonstration of smoking-induced genetic damage in utero. It is noteworthy that a recent study by Hecht and colleagues (presented in August at the American Chemical Society meeting) found that urine from 19 of 31 neonates born to mothers that smoked during pregnancy contained metabolites of NNK (4-methylnitrosamino-1-(3-pyridyl)-1-butanone), a carcinogen found only in tobacco smoke. Metabolites were not found in urine samples from any infants born to non-smoking mothers.

Given the small sample size of the Finette study, additional investigations of the transplacental effects of passive smoke in newborns are required. These studies should include analysis of transplacental exposure of preterm infants and newborns to 'active' as well as passive cigarette smoke. (In adults a similar spectrum of p53 mutations in lung tumors from passive and active smokers has been found.) In addition, measurement of the 'rate' of tobacco consumption in actively smoking mothers as well as a more precise

quantitation of passive smoke exposure in non-smoking mothers should be obtained. V(D)J-recombinase-mediated HPRT deletions also occur spontaneously, thus the comparison of these changes in exposed and in unexposed groups is critical.

This study provides incontrovertible genetic evidence of the devastating effects of tobacco smoke particularly among the young, who suffer a greater risk from environmental toxicants, such as tobacco smoke, not only because of their smaller size but also because of their physiological immaturity. The time has come to proclaim an end to the exposure of preterm infants, newborns, and children of all ages to tobacco smoke."

My opinion is possibly a little more cautious than that of Sozzi et al. I agree the results must be confirmed. The causative nature of the deletions needs to be established before drawing firm conclusions. If the risk turns out to be true, the article provides an additional reason not to expose fetuses to tobacco smoke.

William A. Horton, MD

## Monthly Measurements of IGF-1 and IGFBP-3 In Healthy Prepubertal Children: Characterization and Relationship With Growth: The 1-Year Growth Study

Gelander et al studied 65 prepubertal healthy children (38 boys and 27 girls) between the ages of 8 and 11 years (mean,  $9.1 \pm 0.85$  years) with monthly determinations of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3). In addition, measurements of height, weight, and lower leg length (using a knemometer) were recorded monthly by the same person between 0800 and 1000 hours. All biochemical analyses for each child were performed in the same assay. Additional data, including recent illness, food intake, and the daily mean temperature and number of hours of sunshine, also were recorded. Since concentrations of IGF-1 and IGFBP-3 are age dependent, the values were converted to SDS using prepubertal reference values.

Mean levels of IGF-1 in the children were significantly higher for the girls than for the boys ( $P < 0.05$ ). By multiple stepwise regression analysis, height SDS, gender, and height velocity were significant parameters that explained 45% of the variance in IGF-1 SDS. The mean coefficient of variation for IGF-1 adjusted for age for each child was 13.9%, with a mean difference between samples taken at 3 monthly intervals from -0.4 to +0.3. These changes were correlated with changes in body mass index, but also were influenced negatively by illness and positively by outdoor temperature. Maximum changes over 3 months were related only to changes in temperature. The mean serum concentration of IGFBP-3 was comparable in boys and girls and correlated with the height SDS, weight SDS, height velocity, and weight gain. By using multiple regression analysis, 33% of the level of IGFBP-3 could be explained by gender, height SDS, and weight gain. The mean coefficient of variation for IGFBP-3 was 9.7%, and changes in IGFBP-3 were not related to recent illnesses and changes in body mass index. However, the changes in IGFBP-3 over 1 and 3 months correlated with season, evaluated as either changes in the outdoor temperature or hours of sunshine.

The authors noted that their data demonstrate considerable monthly variation in both IGF-1 and IGFBP-3 of such a magnitude that it exceeds the analytical precision of the measurements. This infor-

mation needs to be carefully considered when evaluating a single IGF-1 or IGFBP-3 concentration in a child who is not growing or whose growth is being evaluated. If repeated IGF-1 concentrations are to be used to evaluate treatment, the changes must exceed -0.4 to +0.4 SDS, whereas the changes for IGFBP-3 must exceed -0.6 to +0.3 to reflect a significant treatment effect. The data also demonstrate the importance of following more than 1 auxologic or biochemical variable. The seasonal variation in growth also has been demonstrated with these changes in IGF-1 and IGFBP-3.

Gelander L, et al. *Pediatr Res* 1999;45:377-383.

**Editor's comment:** This is an interesting, well-conducted study. The type of carefully collected information that Gelander and colleagues have provided can be of significant use in interpreting biochemical growth variables in short children, even those not receiving exogenous growth hormone. Knowing the coefficient of variation around the child's IGF-1 or IGFBP-3 level enhances the physician's ability to determine whether changes in these parameters are of biologic significance. Finally, it is of interest to have verification of the frequently observed finding that children grow up better when the weather is warmer.

William L. Clarke, MD

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**GROWTH, Genetics, & Hormones Volume 15, Number 3**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Gender identity determines
- gender role.
  - psychosexual preference.
  - the pregnancy rate in females with SVCAH.
  - self-image.
2. There is almost full agreement among investigators regarding which one(s) of the following:
- that estrogen in utero influences determination of the gender role in females.
  - that testosterone influences in utero the gender role.
  - that testosterone in utero affects the incidence of learning disabilities.
  - that Müllerian inhibiting factor affects gender identity.

4. Data suggest that CAH patients who undergo adrenalectomy as treatment will grow taller than those medically treated.
- true
  - false
5. The pregnancy potential in CAH females can be monitored by using
- progesterone serum levels to estimate libido.
  - 17-ketosteroids to determine libido.
  - progesterone levels to modulate whether ovulation has occurred.

Answer Key: 1.d 2.b,c 3.d 4.b 5.c

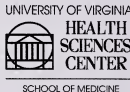
3. Growth in CAH patients correlates directly with which one(s) of the following:
- basal metabolic rate.
  - birth weight.
  - midparental height.
  - time of onset of menarche.
  - none of the above.

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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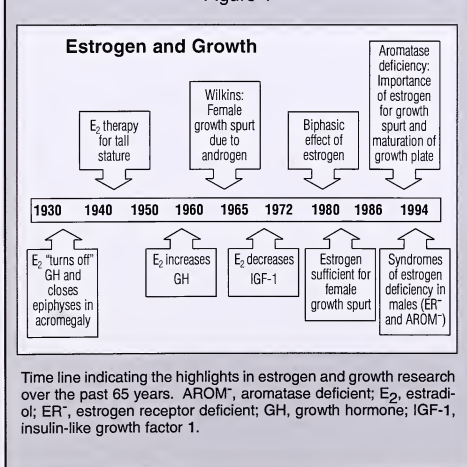
## Growth and Estrogen

**Graeme R. Frank, MD**  
*Department of Pediatrics*  
*Albert Einstein College of Medicine*  
*New Hyde Park, New York*

### INTRODUCTION

During the past 5 years we have gained great insight into the critical role that estrogen plays in growth. This article reviews highlights of growth and estrogen research of the past 65 years (Figure 1), points out a number of earlier misconceptions, and culminates in the identification of "experiments of nature" that have revolutionized our understanding of the role that estrogen plays in linear growth.

Figure 1



### GROWTH-INHIBITING EFFECTS OF ESTROGEN: THE EARLY YEARS

Children with precocious puberty have short stature as adults as a result of premature epiphyseal closure. Furthermore, in the absence of gonadal steroids, the epiphyses remain open and growth continues. The conclusion from these observations was that gonadal steroids were responsible for closing the epiphyses. Animal studies performed by Zondek in the 1930s revealed that estrogen had growth-inhibiting and growth hormone (GH) antagonistic properties.<sup>1</sup> As a result, it was concluded that estrogen, in addition to closing the epiphyses, "turned off" GH secretion.

As an extension of the assumption that gonadal steroids were responsible for turning off GH secretion, it was assumed that children would have more circulating GH than adults, the notable exception being patients with acromegaly. Beginning in the 1930s, in an attempt to inhibit growth and turn off GH secretion, patients with acromegaly were treated with gonadal extracts<sup>2,3</sup>; then, when pure steroid hormones became available in the 1940s, patients with acromegaly were treated with estrogens and androgens. The results with testosterone were disappointing, but estrogen proved to be highly effective, which was taken as proof that estrogen turned off GH secretion.<sup>4</sup>

### In This Issue

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As an extension of the estrogen treatment of acromegals, the late 1940s and early 1950s saw the start of estrogen treatment of excessive growth in adolescent girls.<sup>5</sup> A number of preparations have been used, including diethylstilbestrol, conjugated estrogens, injectable estrogen esters, and ethinyl estradiol. Ethinyl estradiol is the most widely used, and doses have decreased from 500 µg in the 1960s to 200 to 300 µg in the 1970s to 100 µg more recently.<sup>6</sup> Comparing studies is difficult because of the different preparations used, the different doses of estrogen administered, the varied duration of therapy, and the bone age at the start of treatment. Most studies show a growth-inhibiting effect that is inversely correlated with bone age at start of therapy (Figure 2).<sup>6</sup>

## ESTROGEN DOES NOT "TURN OFF" GH SECRETION

It was only in the early 1960s that the radioimmunoassay was developed to measure physiologic levels of GH.<sup>7,8</sup> and it was a great surprise that young adults had higher levels of GH than children. Women also were noted to attain higher levels than men. Finally, in 1964 Rabkin and Frantz demonstrated that estrogen increases GH.<sup>9</sup> Therefore, the earlier assumption that gonadal steroids turned off GH secretion was incorrect.

The immediate question that then arose was, "How, in an individual with open epiphyses, can estrogen slow growth while increasing GH?" The answer to this question came in 1972 from the elegant work of Wiedemann and Schwartz, who demonstrated that in acromegals estrogen therapy (0.5 to 1.0 mg) caused a rapid fall in insulin-like growth factor 1 (IGF-1) but not GH. IGF-1 rose again when estrogen therapy was stopped.<sup>10</sup> In addition, they demonstrated that in patients with GH deficiency (GHD), estrogen therapy aborted the rise in IGF-1 that follows GH therapy.

Up until this point, only growth-inhibiting actions of estrogen had been demonstrated. In fact, in the Third Edition of Lawson Wilkin's textbook of endocrine disorders, published in 1965, the following statement appears: "Since estrogens have little or no effect upon nitrogen retention and in large doses may even inhibit it, *it is probable that the adolescent growth spurt in females is due to adrenal androgens rather than to estrogen.*"<sup>11</sup>

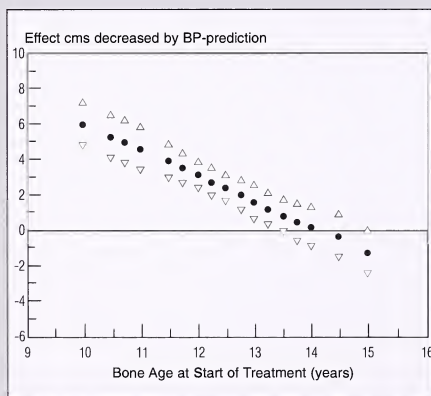
## GROWTH-PROMOTING EFFECTS OF ESTROGEN

Children with Turner syndrome lack estrogen and also lack a pubertal growth spurt. This observation prompted Ross, in 1983, to study the growth of patients with Turner syndrome in response to different doses of estrogen.<sup>12</sup> She studied 19 girls with Turner syndrome who received estradiol for 4 weeks in doses of 0, 50, 100, 200, 400, and 800 ng/kg/d in a double-blind manner. Patients received up to 3 monthly studies per year, and there was a 3-month washout period between monthly studies. She demonstrated a biphasic effect of estrogen on growth (Figure 3A). At low doses (100 ng/kg/d), there was a marked stimulatory effect on ulnar growth that disappeared at doses of 400 ng/kg/d and higher. This maximal stimulatory dose of estradiol (100 ng/kg/d) has been shown to increase the pulse amplitude of GH without affecting the pulse frequency.<sup>13</sup> However, despite the increase in GH secretion, there is no significant increase in the IGF-1 level at this growth-stimulating low dose.<sup>12,13</sup> Regarding longer duration of therapy, 5 µg ethinyl estradiol therapy daily in Turner syndrome (131 to 192 ng/kg/d) for up to 14 months resulted in increased growth velocity, again with no change in the IGF-1 levels.<sup>14</sup> It is only at the increased doses of estrogen, which have no effect on growth rate, that the IGF-1 levels rise (Figure 3B). It therefore appears that there also is a biphasic response of IGF-1 to estrogen in that intermediate levels of estrogen increase IGF-1 and high doses decrease IGF-1.<sup>14</sup>

## THE FEMALE GROWTH SPURT: THE ROLE OF ESTROGEN

Given that estrogen can stimulate growth in females, it was important to question the assumption that the female growth spurt was due to androgen. Differentiating the roles that estrogen and androgen play in growth is very difficult

Figure 2



Adjusted effect of estrogen therapy in adolescent females with tall stature. This representative study included 247 girls aged 12.7 ± 1.2 years. There were 88 controls and 159 treated subjects (90% were treated with ethinyl estradiol 200 µg). Duration of therapy was 1.9 ± 0.6 years. Mean length of follow-up was 10.9 years. Solid dots regression line; open triangles represent 95% confidence interval. Adjusted effect of estrogen (cm) = 20.22 - 1.44 × bone age (years).<sup>30</sup>

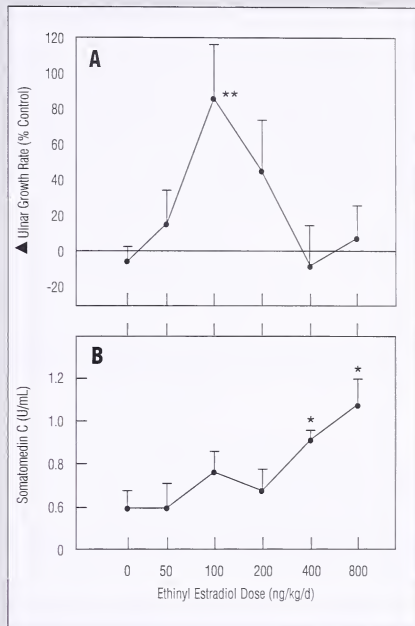
Reprinted with permission from de Waal WJ, et al.<sup>30</sup>

since estrogen and androgen are present in each sex. Testosterone is an obligatory intermediate in estradiol biosynthesis and aromatase, the enzyme that catalyzes the conversion of testosterone to estradiol, is found in males as well as females. In order to evaluate the role that estrogen plays in growth, without any influence from androgen, Zachmann (in 1986) studied 8 patients with androgen insensitivity.<sup>15</sup> These were individuals with disruption of their androgen receptors, and therefore they had only 1 functional sex steroid receptor. The pubertal peak height velocity occurred at a mean age of 12.7 years, closer to that of normal girls (12.4 years) than normal boys (13.9 years).<sup>16</sup> Mean peak height velocity was 7.4 cm/y, the same as in normal girls (7.3 cm/y) and lower than that in normal boys (9.3 cm/y). Therefore, estrogen alone, in the absence of androgens, is able to support a normal female pubertal growth spurt, both in magnitude and timing.

### CAN ANDROGEN SUPPORT NORMAL PUBERTAL GROWTH IN FEMALES?

The answer to this question came in 1994 when Conte described a female patient with aromatase deficiency.<sup>17</sup> As a result of this deficiency, she had high levels of androgens and low levels of estrogens. At age 14 years, she had no breast development and no menarche. She had Tanner stage IV pubic hair, abundant axillary hair, acne, and an enlarged clitoris. Despite elevated androgens sufficient to produce virilization, she was short (height SDS of -1.5), had no growth spurt and, most remarkably, her bone age was delayed (10 years at chronologic age 14 years). With replacement therapy of 20 µg ethinyl estradiol, there was a striking decrease in her levels of androgens and gonadotropins, and she had a 13-cm pubertal growth spurt. Therefore, the assumption that the female pubertal growth spurt was due to androgens appeared to be incorrect.<sup>11</sup>

Figure 3



Relationship between dose of ethinyl estradiol and ulnar growth rate and serum somatomedin C.<sup>12</sup> \* $P < 0.05$ ; \*\* $P < 0.025$

Reprinted with permission from Ross JL, et al.<sup>12</sup>

### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

## THE ROLE OF ESTROGEN IN MALES

Since pubertal peak height velocity occurs early in girls and late in boys, at times when estradiol levels are low and quite similar, it has been suggested that low concentrations of estradiol are important for the pubertal growth spurt in both boys and girls.<sup>18</sup> Further evidence supporting the role of estrogen in the male growth spurt came from the work of Caruso-Nicoletti and colleagues, who demonstrated that a 4-day infusion of estradiol 4 µg/d increased the ulnar growth velocity in 5 prepubertal boys.<sup>18</sup> The most convincing data supporting the importance of estrogen in the male pubertal growth spurt came from patients with familial male precocious puberty, in whom there is autonomous production of androgen from the testes. In these patients, therapy with an androgen antagonist alone is not sufficient to revert skeletal growth to a prepubertal rate. Once an aromatase inhibitor is added to the androgen antagonist to block conversion of androgen to estrogen, a prepubertal growth rate is once more achieved.<sup>19</sup> Since androgen antagonist therapy alone was not sufficient to slow the growth rate, this supported the notion that a major portion of the androgen-induced growth in boys was likely to be mediated via aromatization to estrogen.

### ARE THERE ANY CONSEQUENCES TO LIFE WITHOUT ESTROGEN?

Up until 1994 it was impossible to conclude that estrogen played a major role in the growth of males since there were no human male models that lacked estrogen action. However, in 1994, a man with complete estrogen resistance, caused by a disruptive mutation in the estrogen receptor gene,<sup>20</sup> was described. For the first time we were provided with the unique ability to evaluate the role played by androgen alone, in the absence of estrogen action. The man with estrogen resistance experienced normal prepubertal growth and normal onset of secondary

sexual characteristics. He achieved his midparental target height (5 ft 10 inches) at age 16 years. Despite full masculinization, however, epiphyseal fusion had not occurred and, consequently, he continued to grow. At 28 years, he was 6 ft 8 inches with a bone age of 15 years, and he was growing at a growth velocity of approximately 1 cm/y.<sup>20</sup> One year later, a man with the identical phenotype was described.<sup>21</sup> The striking feature of this 24 year old also was tall stature and continued linear growth as a result of delayed skeletal maturation. He was 6 ft 8 inches with a bone age of 14 years at chronologic age 24 years. This man had a disruptive mutation in the aromatase gene and an inability to convert androgen to estrogen. Within 6 months of therapy with conjugated estrogens (0.3 mg/d increased to 0.75 mg/d over the first year), linear growth ceased and his epiphyseal growth plates fused.<sup>22</sup> A second male with aromatase deficiency, with the same skeletal phenotype, also has been described (Table).<sup>23</sup>

It is clear from the syndromes of estrogen deficiency that androgen, in the absence of estrogen, is relatively ineffective in epiphyseal maturation.<sup>24</sup> However, it appears that androgen, in the environment of severe estrogen deficiency, is able to sustain linear growth despite arrested skeletal maturation. The estrogen-resistant and 2 aromatase-resistant males achieved their genetic potential for height at a normal age of 16 to 17 years, rather than at a later age, as would be expected with hypogonadal individuals. A possible explanation for the observed growth is that androgen, if not aromatized to estrogen, can stimulate growth directly at the level of the epiphyseal chondrocyte. In support of this are the observations made by Keenan and colleagues. They demonstrated that in short boys with delayed puberty, 5-dihydrotestosterone, a metabolite of testosterone and nonaromatizable androgen, induced and maintained an accelerated growth rate in spite of a 50% decline in integrated GH concentration and no change in IGF-1 level.<sup>25</sup>

All 3 males who lacked estrogen action have eunuchoid body proportions (Table), indicating relatively poor spinal growth (which is largely dependent on sex steroids) compared with limb growth. While it is tempting to speculate about the growth spurt of these individuals, there is insufficient longitudinal growth data on any of them to comment on the presence or absence of a growth spurt. Recently, a male infant with aromatase deficiency was described, and it will be highly informative to carefully follow his growth during his pubertal years.<sup>26</sup>

### ESTROGEN ACTION AT THE GROWTH PLATE

As our clinical understanding increases, there is still much to learn about the mechanism of estrogen action at the growth plate. The growth plate is made up of chondrocytes, which are organized into layers. At the distal epiphyseal ends, the chondroblast progenitor cells occur singly or in small clusters to form

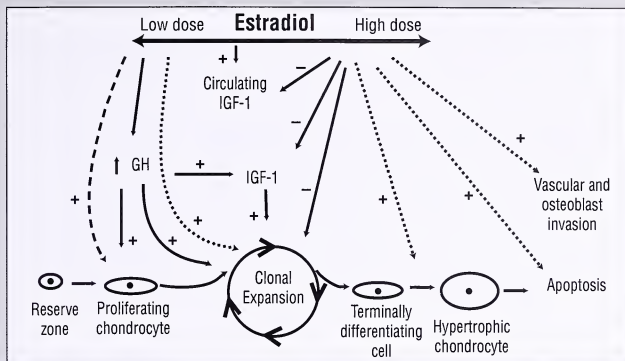
Table  
Syndromes of Estrogen Deficiency in Males

|                         | Estrogen Resistance<br>Case <sup>20</sup> | Aromatase Deficiency<br>Case 1 <sup>21</sup> | Case 2 <sup>23</sup> |
|-------------------------|---|--|----------------------|
| Age (y)                 | 28  | 24   | 38                   |
| Height (cm)             | 204                                       | 204  | 190                  |
| Upper/lower ratio       | 0.88                                      | 0.84   | 0.85                 |
| Tall stature            | yes                                       | yes  | yes                  |
| Continued linear growth | yes                                       | yes  | yes                  |
| Eunuchoid habitus       | yes                                       | yes  | yes                  |
| Bone age                | delayed                                   | delayed                                      | delayed              |
| Bone mineral density    | reduced                                   | reduced                                      | reduced              |



Figure 4

Proposed mechanism of action of estrogen at the level of the growth plate. The solid arrows represent data that have been demonstrated and the interrupted arrows represent other possible effects. Refer to text in article. GH, growth hormone; IGF-1, insulin-like growth factor 1.



the reserve zone. The next zone is the proliferative zone, in which the chondrocytes undergo clonal expansion and form discrete columns. The proliferative chondrocyte then undergoes terminal differentiation to form the hypertrophic chondrocyte. The hypertrophic chondrocytes secrete matrix, which undergoes mineralization. The hypertrophic chondrocytes then undergo apoptosis, and finally there is vascular and osteoblast invasion, which reduces the size of the growth plate.

Figure 4 represents some of our understanding of estrogen's role in growth. Low-dose estrogen increases GH secretion and stimulates growth. According to the dual effector theory of Green and associates,<sup>27</sup> GH primes the resting chondrocyte, preparing it for clonal expansion under the influence of IGF-1. However, GH receptors are not confined to the resting chondrocytes in the reserve zone, and GH and IGF-1 have been shown to exert their effect at each stage of differentiation.<sup>28</sup> The presence of estrogen receptor  $\alpha$  in all populations of chondrocytes suggests that estrogen may have a direct role on the chondrocyte to stimulate growth.

High doses of estrogen inhibit growth by decreasing IGF-1 and inhibiting cell proliferation in the hypertrophic zone. The inhibition of clonal expansion by estrogen is not overcome by the addition of GH or IGF-1, suggesting that it is mediated directly by estrogen.<sup>29</sup> High-dose estrogen also may inhibit growth by inducing terminal differentiation of proliferating chondrocytes, apoptosis of hypertrophic chondrocytes, and vascular and osteoblast invasion into the growth plate.

## CONCLUSION

Estrogen is only one of many important factors involved in chondrocyte growth and differentiation. Other critical factors include androgens, thyroid hormone, vitamin D, Indian hedgehog protein, and parathyroid hormone receptor protein. Despite our limited knowledge of the mechanisms at the level of the growth plate, we now appreciate the critical role that estrogen plays in the growth of both females and males.

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## A Letter to Our Readers

March 15, 1985

Dear Colleagues:

The Editorial Board is pleased to introduce the inaugural issue of *GROWTH, Genetics & Hormones*, a publication for academicians and practicing physicians who are interested in these important areas of medical practice. We are pleased to welcome you as a reader and invite you to participate as a reader and as a correspondent.

Normal and abnormal growth, genetically determined conditions, and the overall development of children are important aspects of pediatric practice. Hormonal production is essential in growth and development. It is probable that these areas will assume even greater prominence because pediatricians are showing greater interest in growth and development as immunizations and antibiotics diminish the incidence of infectious disease, as greater numbers of children with leukemia and other cancers enter sustained remissions, and as growing numbers of handicapped infants with congenital anomalies survive.

It is also well recognized that the literature concerning these topics is voluminous. Therefore, *GROWTH, Genetics & Hormones* was developed, primarily to provide a close look at current—and often controversial—topics in endocrinology, genetics, and metabolism and their potential clinical applications. To ensure that this goal is met now and in the future, several nationally and internationally respected authorities in genetics, endocrinology, anthropometrics, pediatrics, pharmacology, and metabolism have agreed to serve on the Editorial Board.

The eminent investigators who have agreed to serve as Associate Editors are: Dr. Jürgen Bierich of the University of Tübingen, West Germany; Dr. Judith Hall of the University of British Columbia Medical School; Dr. Fima Lifshitz of Cornell University School of Medicine; Dr. David Raimon of the University of California, Los Angeles; Dr. Alan Rogol of the University of Virginia School of Medicine; and myself. You will meet each of the Board members in the early issues of *GROWTH, Genetics & Hormones*.

The editorial content of this quarterly publication was chosen with your interests in mind. This issue, for example, features an article about the incidence of growth hormone deficiency, a review of growth hormone physiology and pathophysiology, and a summary of a recent conference concerning the psychosocial aspects of growth delay. These are scientific, timely, and representative of the topics that *GROWTH, Genetics & Hormones* will address.

Abstracts of pertinent articles and reports will appear in each issue. In the future, the abstracts will serve as "mini-reviews" and integrate multiple reports on a particular topic.

We are pleased to present this inaugural issue to you. We welcome your readership and look forward to hearing from you about *GROWTH, Genetics & Hormones*. We would also appreciate your filling out the enclosed reply card to let us know of your initial interest.

*On behalf of the Editorial Board: Sincerely, Robert M. Blizzard, MD, Professor and Chairman, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville.*

## A Second Letter to Our Readers

March 15, 2000

Dear Colleagues:

Fifteen years have passed since the previous letter was written. Hopefully, *GROWTH, Genetics & Hormones* has served you in accord with the substance of that letter. The Editorial Board is proud of this publication and its accomplishments.

Several members of the initial Editorial Board remain—specifically, Dr. Judith Hall, Dr. Fima Lifshitz, and myself. The other current members are Dr. William Horton, Director of Research, Portland Shriners Hospital; Dr. William Clarke, Professor of Pediatrics, University of Virginia School of Medicine, and Dr. Allen Root, Professor of Pediatrics, University of South Florida. They have served for at least the last 6 years. Other members who served for an extended period in the interim between the writing of these 2 letters are Dr. Jürgen Bierich of Tübingen; Dr. James Tanner of London; Dr. Jean Claude Job of Paris; and Dr. Alan Rogol of Charlottesville.

In the 15 years since the initial publication, there have been more than 100 lead articles, 20 reviews of important international or national meetings, and more than 700 abstracts of pertinent articles from the literature with editorial comments. The Editorial Board expresses its gratitude

to Genentech, Inc. for sponsoring this continuing education publication under an unrestricted educational grant awarded to the University of Virginia. Distribution is to endocrinologists in pediatrics and internal medicine, geneticists, nurses, and other physicians who have requested issues. Distribution to pediatric endocrinologists in Europe was discontinued for budgetary reasons after 13 years. We are pleased to announce that beginning with this issue, members of the European Pediatric Endocrine Society are again receiving their copy, which is now sponsored by Schwarz Pharma AG. Thanks to Schwarz Pharma AG, it is probable that the 3 issues that will be published in 2000 will increase to 4 issues in 2001.

Letters to the Editor are encouraged, as stated in the letter of March 15, 1985. Letters can be sent to the Editor-in-Chief at 1224 West Main Street, Suite 701, Charlottesville, VA 22903.

The Editorial Board wishes to hear from you concerning all aspects of *GGH*. If your issue does not arrive as expected, please write to the Editor-in-Chief at SynerMed, 405 Trimmer Road, Califon, NJ 07830. Please let the Editorial Board hear from you so we can better serve you.

*On behalf of the Editorial Board: Sincerely, Robert M. Blizzard, MD, Professor and Chairman Emeritus, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville.*

# Ethical Issues in Growth Hormone Therapy: Where Are We Now?

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## BACKGROUND

In 1991, a group of pediatric endocrinologists, ethicists, economists, and psychologists convened to address ethical questions arising from expanding use of recombinant human growth hormone (GH).<sup>1</sup> These included: (1) If GH was proven effective at improving the height of children without GH deficiency (GHD), is the diagnosis of GHD morally relevant in determining entitlement to GH treatment? (2) To what extent should the treatment of short stature (SS) be considered a medical problem requiring or justifying medical treatment? Much of the debate at that time centered around the argument put forth by Drs. Allen and Fost in a prior publication that GH responsiveness rather than GHD should guide access to GH.<sup>2</sup> Others at that conference countered that patients with GHD had a *disease* deserving *treatment*, while children with SS from other causes did not have a disease (at least not that of GHD) and to treat them is *enhancement* and not therapy.

The intervening years have not resolved this debate and new, equally important and problematic issues have arisen. The addition of Turner syndrome (TS) and chronic renal insufficiency (CRI) with SS to the list of approved indications for GH treatment validated the notion that GH effectiveness and not the underlying etiology of SS is the relevant variable in determining the appropriateness of treatment. With GH treatment no longer constrained by the treatment/enhancement distinction, numerous other SS conditions appeared as candidates. Further, new knowledge about important non-growth actions of GH and the importance of GH treatment of severe GHD in adulthood spawned interest in possible uses of GH in other types of SS in childhood. As GH utilization continues to expand, concerns and questions have been renewed about excessive expenditures of limited health-care resources. An additional question is, Can increased height measure the value of GH treatment and, if not, what can? To address these issues, 25 endocrinologists, ethicists, psychologists, and insurance representatives convened in October 1999 for a second ethics conference in Madison, Wisconsin. Adhering to the maxim that "good ethics begins with good facts," an initial session focused on current knowledge regarding the benefits and burdens of GH treatment in children with GHD, TS, and Prader-Willi syndrome (PWS), and in adults with GHD. A second session analyzed how decisions guiding access to GH treatment are and should be made from the perspectives

of local insurance providers, planners of the Oregon Health Plan, and individuals considering the national health-care economy. The group then debated the proper endpoint of GH treatment in children: Should it be the maximal attainable height or simply "normal" height, or should psychosocial adaptation, rather than height, be the appropriate endpoint? Finally, conceptual issues raised by the initial conference<sup>1</sup> were revisited: Specifically, is the treatment/enhancement distinction helpful in guiding access to GH treatment, and how should the use of GH treatment be determined in the context of the American health-care system?

## SCIENTIFIC ISSUES

Dr. Margaret MacGillivray, a pediatric endocrinologist, presented current data describing adult heights of hypopituitary children treated recently with GH ( $-1.5$  to  $-0.7$  SDS). The result is a marked improvement in final height compared with former treatment with pituitary-derived GH ( $-4.7$  to  $-2.0$  SDS). She predicted further improvements with earlier treatment and improved pubertal GH replacement.

Dr. David Sandberg, a psychologist, then addressed perhaps the most complex and contentious topic of the conference: What do we know about the quality-of-life (QOL) benefits of GH-increased final height? While acknowledging the daunting methodologic challenges of such research, he reported that his and other studies failed to show a relationship between adult height and QOL, suggesting that maximizing adult height outcomes does not automatically translate into improved QOL outcomes. Such observations, if confirmed, will have clear relevance to questions regarding both termination of GH therapy and GH treatment of non-GHD children for presumed psychosocial benefit.

The successful treatment of TS girls with GH is now regarded as standard practice, but is the benefit of treating these children truly clinically significant? Dr. Ron Rosenfeld, a pediatric endocrinologist, was assigned the "pro" position for this debate. He presented evidence that GH accelerates growth and improves final height in TS, that this outcome can be achieved without excessive delay in pubertal development, and that GH is safe for these patients. Anecdotal experience suggested a beneficial effect of GH therapy on QOL in children and young adults with TS, but studies assessing impact of growth-promoting therapy on psychosocial function are lacking. Conclusive data will be extremely difficult, if not impossible, to obtain in the current environment. Dr. Harvey Guyda, also a pediatric endocrinologist, was assigned the "con" position. He argued that the desired outcome for most patients (achieving

ing a "normal" height) does not occur for the majority of TS girls treated with GH. The median final height achieved by patients in the Canadian randomized controlled study is only  $-2.3$  SDS. While some individuals have shown dramatic responses, only  $\sim 50\%$  of those in the Canadian study can expect a height benefit  $>5$  cm. Further, Guyda emphasized that treatment has not been proven to lead to improved psychosocial functioning. A challenge remains to determine methods to identify the TS patients who are most likely to benefit from prolonged GH treatment.

The determination of a responsible endpoint for GH therapy for growth promotion in all conditions remains problematic. Dr. David Allen, a pediatric endocrinologist, proposed that treatment should be stopped when the height is within the normal adult range. This represents not only a successful therapeutic outcome but also a more reasonable allocation of resources and preservation of a proper goal for the medical profession in the treatment of SS. On the other hand, Allen stated, since many children with unexplained isolated GHD display normal GH secretion after puberty, continuous treatment to maximal height may include years of unnecessary treatment, during which time GH therapy becomes increasingly expensive and, simultaneously, less effective as final height is approached. From an ethical standpoint, Allen stated, promoting additional growth within the normal adult range could be viewed as enhancement and not treatment. Dr. Michael Kappy, a pediatric endocrinologist, countered that using the lower range of adult height as a goal represented a gender-biased definition of handicap, since demands for daily living (eg, reaching for objects on a high shelf) are not gender-specific functions. Instead, the criterion for discontinuing GH should be purely physiologic, ie, how tall the child would have grown if he or she did not have GHD. He argued that this approach was less arbitrary and reduced the risk that the physician would need to make value judgments.

Novel uses of GH treatment add complexity in identifying appropriate recipients and in determining appropriate outcomes for judging the value of such treatment. For example, adults with severe GHD have abnormal body composition, deficient bone mineral density (BMD), lipid abnormalities, and a decrease in QOL. Dr. David Cook, an internist and endocrinologist, emphasized that while several such entities appear amenable to GH replacement therapy, the focus of insurance companies is favorable changes in mortality figures and reduced bone fracture rates, rather than QOL or metabolic issues. However, awareness of these non-growth or metabolic effects of GH has raised interest in the effect of GH therapy on body composition and physical function in disabled children, such as those with PWS. Dr. Aaron Carrel, a pediatric endocrinologist, reviewed data showing that in PWS children GH therapy improves body composition such as reducing body fat and increasing lean body mass, BMD, fat oxidation, and energy expenditure. Most importantly, physical strength and agility are improved. These benefi-

cial effects were viewed by families to be as important or even more important to the well-being of the child than gain in height. The view of many insurance companies may not be in accord with the views of physicians who are treating these children.

## **ETHICAL, SOCIAL, ECONOMIC, AND POLICY ISSUES**

Appropriately, the conference then turned its attention to ethical, social, economic, and policy issues regarding access to GH therapy. The medical directors of 3 Wisconsin-based HMOs presented their organizations' policies on paying for GH treatment and explanations of how such decisions are made. While all 3 HMOs seemed to use similar approaches, their conclusions were disturbingly divergent. One provided full reimbursement for treatment for GHD and TS; one required 50% copayment; and the third paid nothing for either indication. They all claimed to rely on medical necessity as the central criterion for resolving such questions, but the definition of this term was unclear.

Mark Pauly presented an economist's perspective, one based on the assumptions that GH was safe, effective, and available in unlimited supply, and that market conditions were ideal, including that potential purchasers had full knowledge of the facts. In such a system, the most generous insurance packages would probably cover treatment for very short children with GHD, but that this would be less likely for children already in the normal range or for conditions for which treatment produced only minimal increases in height. He thought it likely that there would be public support to subsidize only the most severely affected children.

With regard to accepting private purchase of GH for children who were not severely affected, this appeared compatible with existing notions in the United States of tolerating individual discretion in spending earned income for health matters, particularly if these decisions did not cause severe harm to those who could not afford treatment. Given the apparently modest gains produced by GH treatment in most non-GHD children, Pauly thought it unlikely that there would be severe overall harm. To him the inequality likely to be produced by such private purchases seemed trivial compared with other consequences of inequality of wealth that are currently prevalent.

Philosopher Paul Menzel described the approach of the Oregon Health Plan with regard to access to GH by its Medicaid population. Oregon has identified 743 "treatment/condition pairs," and currently prioritizes funding down to No. 574. Pituitary dwarfism is included (No. 499), as is GH therapy for TS (No. 506). GH treatment is not supported for any other conditions that presumably fall into the generic category, listed at No. 736, which is titled, "Endocrine or metabolic conditions with no effective treatment or where no treatment is necessary." (However,



which criterion was considered relevant in the exclusion is unclear.) Menzel suggested that from the perspective of social justice in access to health care, the apparently marginal benefit of GH treatment of most non-GHD children with regard to the modest increase in height should not cause great alarm. To the contrary, the more important question might be whether the benefits that accrue to patients with TS can be justified in the Oregon plan when one considers other possible use of the funds.

Pediatrician Douglas Diekema questioned the claim that treatment with GH is a net benefit, particularly for children without GHD. He focused on the ambiguity of psychosocial benefit and thought more consideration should be given to the potential psychological harm of treatment, which is supported by some studies. This could occur from an implied message to children that their parents are unaccepting of them. This may be particularly problematic when treatment produces little or no increase in height. He also pointed out that treatment of these children does not make them tall but only less short.

Dr. Norman Fost, a pediatrician and ethicist, reviewed the hazards of trying to resolve questions of access by relying on traditional distinctions made between health and disease and between treatment and enhancement. He argued that some conditions are clearly diseases, such as the persistent vegetative state, and yet might not warrant expensive prolonged treatment. Similarly, some conditions are clearly not diseases, such as pregnancy, and yet attract wide support for treatment to be included in a basic benefits package. He applied similar analyses to treatment versus enhancement distinctions, stating that some clear treatments did not warrant funding and some clear enhancements did. The latter could include bringing "normal" short children into the normal height range.

Philosopher Allen Buchanan discussed GH as a paradigm of "expansive biotechnologies," which refers to technologies that begin as clear medical treatments and then are found to offer benefits that do not clearly belong in the health-care system. Few would dispute that pituitary dwarfism is a medical problem warranting medical treatment, but many of the newer applications of GH, such as producing marginal increments in height or improving strength or slowing the aging process, are not so clearly defined as medical problems. He compared GH with other technologies and drugs, including artificial insemination and Prozac. These began as treatments for medical problems but expanded to much larger markets that involved problems that were less clearly medical. He expressed concern that these expanding uses for very expensive technologies "might erode our society's already shaky commitment to the right of an adequate level of health care for all."

Philosopher Dan Brock concluded the conference with several summary observations. He cited the need for a clear formulation of the ultimate endpoints we seek from

GH treatment. He also cited the lack of data on the degree to which current endpoints are achieved. He stated that height itself cannot be an appropriate endpoint because "it is only instrumental to improvements in patients' quality of life." He urged that future studies focus on QOL gains since coverage by insurance would be difficult to justify without clearly established QOL benefits. He concurred with Dr. Fost that whether SS is a disease, and whether use of GH is characterized as treatment or enhancement, would not resolve the critical questions.

In reviewing Dr. Pauly's presentation, he noted that health-care markets do not function well, and the inequities in income distribution may be difficult to justify, so that leaving access to the market would be difficult to support as a matter of social justice. On the other hand, Dr. Pauly acknowledged the practical and theoretical difficulties in limiting the freedom of those with discretionary money to spend it as they wish. Brock reminded the group of the axiom that one cannot go from an "is" to an "ought," so that the present arrangements in income distribution and health care cannot be presumed to be fair. He acknowledged that we do not yet have an adequate framework for resolving problems of access and rationing.

## CONCLUSION

A decade ago, Allen and Fost asked whether GH therapy would become a panacea or Pandora's box. The question remains unanswered. Viewpoints expressed at this conference suggest that while some issues have been clarified, new questions have arisen. While GH can increase final adult height in some patients, the effect on their QOL remains unclear. While the safety of long-term GH therapy is clearer, the costs continue to be high, and new indications, such as treatment for metabolic reasons, and new questions about who should have access to GH and who should pay remain unanswered. The other questions asked by Drs. Allen and Fost in 1991 also remain unanswered. Continued frequent discussion of these issues by physicians, ethicists, health economists, and others together is essential if responsible and equitable use of GH during the next decade is to occur.

## REFERENCES

1. Allen DB, Fost NC. Access to treatment with human growth hormone: proceedings of a conference. *GROWTH, Genetics, & Hormones* 1992; 8 (supplement).
2. Allen DB, Fost NC. Growth hormone therapy for short stature: panacea or Pandora's box? *J Pediatr* 1990;117:16-21.

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## PUBLICATION NOTE

The complete proceedings of the conference, including presented papers and an edited transcript of the discussions, will be published as a supplement to *Pediatrics*.

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## Effects of Recombinant Leptin Therapy in a Child With Congenital Leptin Deficiency

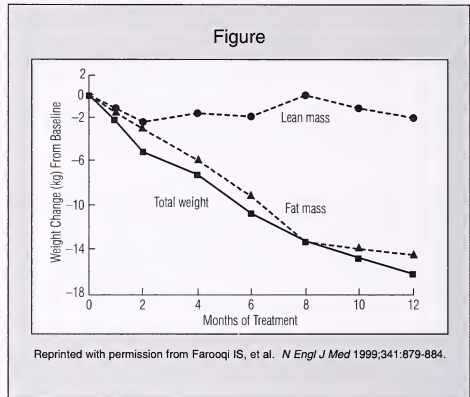
A patient is reported with early onset morbid obesity due to leptin deficiency. The effects of trial therapy with recombinant human leptin also are reported. This patient was normal weight at birth but began growing excessively at 4 months of age (Figure). She had marked hyperphagia and was constantly hungry, demanded food, and was very disruptive when food was denied. She was born in a highly consanguineous family of Pakistani origin. Her nonobese parents were first cousins. She gained excess weight throughout her life, exceeding the 100th percentile and reaching 94.4 kg at age 9 years. Her height was 140 cm (91st percentile). At that time, she was treated with recombinant human leptin, administered subcutaneously daily at 0.028 mg/kg of lean mass for 12 months. The patient lost weight immediately with treatment and her weight decreased 16.4 kg at a rate of 1 to 2 kg/mo. Her height remained at the same percentile throughout the treatment. The body composition measurements revealed a decrease in the body fat by 15.6 kg (95% of the total weight loss). Simultaneously, leptin treatment was associated with a decreased consumption of food and a marked change in eating behavior. The injections were tolerated well.

Farooqi IS, et al. *N Engl J Med* 1999;341:879-884.

**Editor's comment:** This patient with congenital leptin deficiency and morbid obesity is the first one treated with recombinant human leptin. The results were impressive, confirming the importance of this hormone in the regulation of body weight and appetite. This patient and her response to treatment, which replaced the hormone that was lacking, demonstrated

major differences between the human condition from those found in mice with OB and DB mutations leading to leptin deficiencies. The reader is encouraged to review the article as well as the accompanying editorial in this issue by Drs. Michael Rosenbaum and Rudolph Leibel (*N Engl J Med* 1999;341:913-914). I also recommend the review published in GGH (Vol. 14, No. 3, October 1998) of the leptin abnormalities reported to occur in humans that are associated with this type of obesity in children.

Fima Lifshitz, MD

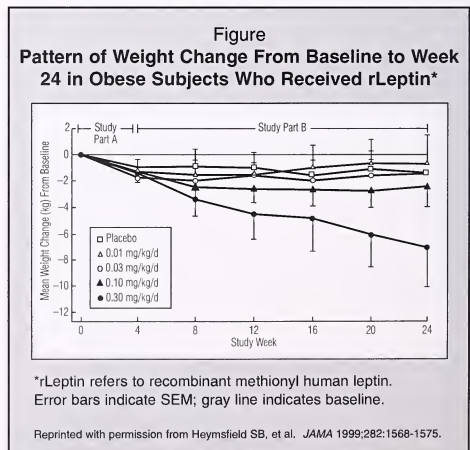


## Recombinant Leptin for Weight Loss in Obese and Lean Adults: A Randomized, Controlled, Dose-Escalation Trial

The protein hormone leptin is produced by adipocytes and regulates the amount of body fat. In animals, leptin also has been found to regulate energy intake. This study reports the effects of different daily doses of leptin in lean and obese individuals. Fifty lean and 73 obese subjects self-injected either 0.01, 0.03, 0.10, or 0.30 mg/kg/d of recombinant human leptin subcutaneously daily for 4 weeks. The obese subjects were given leptin for an additional 20 weeks. All also were placed on an exercise routine and reduced calorie diet. After the first 4 weeks of treatment, both the lean and obese subjects lost weight (-0.4 to -1.9 kg). After 20 additional weeks, the obese group lost additional weight from -1.9 kg, for the 0.10-mg/kg dose, to -7.1 kg for the 0.30-mg/kg dose (Figure). The weight loss was mainly body fat.

Heysfield SB, et al. *JAMA* 1999;282:1568-1575.

**Editor's comment:** This is the first dose-response study showing that increasing body weight loss is associated with increasing doses of leptin. Furthermore, the composition of weight loss was mainly body fat; thus, lean body mass was preserved. These results suggest that leptin may be appropriate for treating obesity in certain individuals. However, the results may not







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be totally attributed to leptin because all subjects were placed on exercise and a reduced calorie diet while being treated with leptin. The investigators should have had a similar treatment group without the corresponding exercise and dietary prescription to clarify the therapeutic value of this hormone in treating obesity. This would have factored out the effects of corresponding treatments on body weight loss. However, from the

results reported leptin should not be considered a panacea for the treatment of obesity. High doses of the hormone were necessary to reduce weight, denoting leptin resistance. I can foresee being forced into treating obesity with leptin as we were for treating short stature with GH therapy.

Fima Lifshitz, MD

## Evidence Supporting an Adipo-Leptin-Growth Hormone Axis in Obesity-Related Hyposomatotropism

Roemmich et al review the evidence that the reduced GH secretion observed in obesity may be related to leptin physiology. The hypothesis is presented in the figure and its legend, which are reproduced here.

In advancing this hypothesis, the authors review the neuroendocrine control of GH secretion; alterations in GH release during childhood, adolescence, and adulthood; the influence of GH secretion on body composition; the altered neuroendocrine control of GH secretion in obesity; leptin physiology; the evidence of an inhibitory role for leptin on GH secretion; and the influence of GH on leptin secretion.

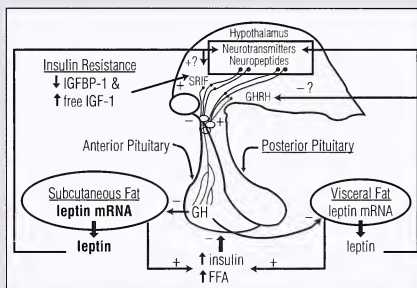
The authors conclude that GH secretion is impaired in obese adults and children but the physiologic mechanisms producing hyposomatotropism remain unclear. Apparently, metabolic signals relay information regarding the body composition and the fat distribution to the hypothalamus and pituitary. Leptin is a logical choice as a messenger of the fat stores because it is secreted directly by the adipocytes and leptin receptors are located in the pituitary and the hypothalamus, including GH-releasing hormone neurons. However, the evidence is not yet convincing enough to conclude that leptin plays a primary role in the modulation of the neuroendocrine GH axis in obesity.

Roemmich JN, et al. *The Endocrinologist* 1999;9:424-430.

**Editor's comment:** This paper is a good, timely review of the physiology of and interaction among GH, leptin, insulin, and GH-releasing hormone. The article is brought to the attention of the readers of GGH as the hypothesis presented is well worth considering. It may be totally true, partially true, or not at all true. Further studies and reflection on these studies are needed. GGH wishes to expose you to concepts as well as facts. Your attention is called to an article published recently in GGH entitled "Molecular Physiology of Leptin and Its Receptor" (Zhang Y, Leibel RL. *GGH* 1998;14:2) that was an excellent review of the facts known as of the date of publication.

Fima Lifshitz, MD

Figure



**Schematic of the hypothesized adipo-leptin-GH axis.** Leptin is predominantly secreted from the subcutaneous fat depot. An accumulation of subcutaneous fat increases serum leptin concentrations that feed back to the hypothalamus and perhaps the pituitary. Leptin receptors have been located in the human hypothalamus and in the rat, but not the human pituitary. Acting through as yet unknown neurotransmitter and neuropeptide mechanisms, leptin could increase somatostatin (SRIF) tone or inhibit GH-releasing hormone (GHRH) tone, resulting in a reduced somatotrophic response at the pituitary. Neuropeptide Y modulates the leptin-induced reduction in GH secretion in fasted rats, but there is no evidence that neuropeptide Y modulates the reduced GH secretion caused by obesity. Leptin also may directly inhibit GHRH secretion because leptin receptors are expressed in GHRH neurons of the rat. However, leptin is not necessary for reducing GH secretion. Both the *ob/ob* mouse and humans with mutated leptin genes are obese, and GH release is reduced in the absence of leptin.

The metabolic hypothesis for obesity-induced reductions in GH secretion is better established. An accumulation of fat in both the subcutaneous and visceral fat depots is associated with an increase in serum insulin and free fatty acid (FFA) concentrations, which act directly at the pituitary to inhibit GH secretion. Obesity (likely through its association with insulin resistance) also reduces IGF-binding protein 1 (IGFBP-1) concentrations, resulting in increased free IGF-1 concentrations, which may feed back to increase somatostatin tone. Regardless of the mechanism, the reduction in GH secretion results in a reduction in GH-mediated lipolysis, further gains in subcutaneous and visceral fat, and further increases in serum leptin, insulin, lipid, and free IGF-1 concentrations.

Reprinted with permission from Roemmich JN, et al. *The Endocrinologist* 1999;9:424-430.

## Blood, Sweat and Tears—or Is It Teeth, Sweat Glands, and Hair?

A very interesting story has unfolded over the past few years regarding the pathogenesis of hypohidrotic ectodermal dysplasia (HED). The gene harboring mutations responsible for the X-linked form of this disorder, which is characterized by abnormal formation of teeth, hair, and eccrine sweat glands, was identified by positional cloning about 3 years ago. Designated *ED1*, it encodes a transmembrane protein, called ectodysplasin (Eda), that contains a collagen-like region in its extracellular domain. This region is thought to mediate formation of trimers as the extracellular portions of the molecules extend from the cell surface.

Mutations in the mouse homologue of human *ED1* have been found in a mouse mutant called tabby (*ta*), which has a murine phenotype equivalent to HED. Since Eda appears to be involved in the induction of ectodermal placodes, which give rise to structures that fail to form in both HED and *ta*, Eda was proposed to function as a membrane-bound ligand, although the mechanism of signal transduction was not known. This speculation led to a search for an Eda receptor. The receptor was predicted to be encoded by an autosomal gene based on the existence of autosomal forms of HED that are clinically indistinguishable from the X-linked form. The search has now ended with the identification of a receptor for Eda.

The latest chapter of the story begins with a mouse mutant named downless (*dl*). The close similarity between the *dl* and *ta* phenotypes suggested the possibility that *dl* encoded the Eda receptor. Positional cloning of *dl* by Headon and Overbeek revealed it to be a novel member of the tumor necrosis factor (TNF) receptor. This argues for its being the Eda receptor since TNF receptors typically bind trimeric ligands, the form proposed for Eda. Moreover, its expression pattern corresponds well to sites in developing skin, where ectodermal placodes form.

Armed with the mouse *dl* cDNA as a probe, Monreal et al quickly cloned the human *DL* gene. They next searched for *DL* mutations in HED patients with suspected autosomal inheritance. They identified *DL* mutations in 3 families with autosomal recessive HED and in 2 families with autosomal dominant HED. Two of the recessive families displayed consanguinity. As expected, the affected members were homozygous for the putative mutations and their parents were heterozygous for the mutations. Affected members in the third recessive family were compound heterozygotes. Mutations in the recessive families probably act through haploinsufficiency.

The mutation in one of the dominant families predicts a truncated protein lacking a key functional domain, the so-called death domain, which lies at the carboxyl terminal of the molecule. Trimerization of the cytoplasmic death domain is required for signal transduction, typically to transmit signals that bring about cell death. Such mutant proteins could participate in interactions of trimeric ligands with trimeric receptor molecules, but they would not transmit signals downstream. Thus, the mutation would act in a dominant negative manner, which would explain why this type of mutation is inherited as a dominant, while the other loss of function mutations are inherited as recessives.

Monreal et al noted that some families with HED do not map to either the *ED1* or the *DL* locus, implying the existence of at least a third HED locus. A mouse mutant named crinkled (*cr*) displays a phenotype very similar to that of tabby and downless. The crinkled gene has not yet been cloned, but it represents a good candidate for the third HED locus.

Another interesting aspect of this story is that although the Eda ligand is membrane bound, it contains an extracellular cleavage site for the secreted metalloprotease, furin. Thus, it is possible that the trimerized ectodomain of Eda is cleaved to produce a ligand that diffuses at least locally in search of its receptor. Such cleavage is characteristic of TNF ligands.

Headon DJ, Overbeek PA. *Nat Genet* 1999;22:370-374.

Monreal AW, et al. *Nat Genet* 1999;22:366-369.

Barsh G. *Nat Genet* 1999;22:315-316.

**Editor's comment:** The evidence to date strongly supports the view that DL in humans (and dl in mice) is a receptor for Eda and that signal transduction involves trimerization of Eda and its receptor as occurs with other TNF:TNF receptor interactions. Membrane-bound Eda may be cleaved from the cell of origin to diffuse to the cells it influences. The evidence further suggests that the downstream signals are required for induction of ectodermal placodes, which give rise to teeth, hair follicles, and sweat glands.

The unfolding of this story provides another good example of how human and mouse genetics are interrelated and how analysis of mutant phenotypes yields insight into normal biology. Also, given the fact that, even in adults, cells in hair follicles recapitulate the developmental program that produces hair in early life, it seems quite possible that this work will lead to advances in the treatment of hair loss and in hair removal.

William A. Horton, MD

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## The Rett Syndrome Gene Silences Many Other Genes

Rett syndrome is a common cause of mental retardation in females, with an incidence of 1 per 10,000. It is characterized by normal development until 6 to 18 months of age when there is regression, including loss of speech, hand-wringing, seizures, autism, ataxia, hyperventilation, and, often, growth retardation. The phenotype is very characteristic; however, most cases are sporadic and it is never observed in males. The hypothesis was that it was an X-linked dominant disorder, lethal in hemizygous males. Familial cases had been localized and linked to the tip of the long arm of the X chromosome in the Xq28 region. Amir et al have reported finding mutations in the *MECP2* gene in 6 sporadic cases and 1 familial case of Rett syndrome. The responsible gene, *MECP2*, encodes a methyl-CpG-binding protein that selectively binds CpG dinucleotides and mediates transcription from a variety of genes by repressing the interaction with histone deacetylases. Thus, the Rett syndrome gene is probably a key player in silencing other genes. In other words, the gene normally plays a role in assembling transcription silencing complexes. However, if these complexes are not working at a specific stage in development, then the genes will continue to make proteins that apparently clog up normal processes and lead to the intellectual degeneration of affected females. The recognition of the *MECP2* gene as being responsible for Rett syndrome is the first time a human disease has been determined to be caused by a defect in a protein that involves DNA methylation and, thus, when the protein is absent or not working, leads to abnormal chromatin packaging and abnormal gene expression. Interestingly, the gene is particularly expressed in the brain, and thus it would appear that the brain is particularly sensitive to an excess of transcribed proteins. Undoubtedly, there will be other such genes but it is a real

breakthrough in understanding abnormalities in developmental time-specific processes.

Willard HF, et al. *Nat Genet* 1999;23:127-128.

Amir RE, et al. *Nat Genet* 1999;23:185-188.

**Editor's comment:** *The Human Genome Project is revealing many genes with no previously described homologies, as well as demonstrating many new, previously unknown processes. It is reassuring to have the gene responsible for a common syndrome defined, but surprising to find it affects many other genes in a specific developmental way. In the process of a child's development, there must be many other episodes of switching on and off. Interestingly, the mouse model for MECP2 deficiency also is X-linked and affected males do not develop at all since it leads to male lethality. Because the human cases are mostly sporadic, the effect of male lethality has not been observed. It seems quite possible that if affected girls could be recognized in the newborn period, some type of therapy could be developed.*

Judith G. Hall, OC, MD

**2nd Editor's comment:** *These articles describe a novel pathogenetic mechanism for genetic disease in humans. If the speculation proves correct, Rett syndrome results from at least partial failure of a global process that keeps transcription in check. As Amir, Willard, and their colleagues emphasize, much more work will be needed to prove the theory, and it will likely turn out to be much more complicated than outlined here. Nevertheless, it is an exciting development in medical genetics.*

William A. Horton, MD

## Chromosome 7p Maternal Duplication With Features of Silver-Russell Syndrome

Maternally uniparental disomy of chromosome 7 is present in about 10% of Silver-Russell syndrome (SRS) individuals, suggesting there is a gene or genes that are imprinted on chromosome 7. Growth-related genes on chromosome 7, including *GRB10* (a growth factor receptor-bound protein), *EGFR* (epidermal growth factor receptor), and *IGFBP1* (insulin-like growth factor-binding protein 1), have all been suggested as candidate genes. However, molecular analysis of a duplication present in both mother and daughter who have SRS shows that it includes *GRB10* and *IGFBP1* (but not *EGFR*), suggesting that one or both of these are the culprits involved in the phenotypic effects.

a result of small duplications or undetected trisomy. Investigations of these possibilities may reveal the nature of the genetic abnormalities underlying this disorder.

Joyce CA, et al. *Hum Genet* 1999;105:273-280.

**Editor's comment:** *SRS is a very common cause of intrauterine growth retardation and subsequent short stature. Its etiology is undoubtedly heterogeneous and is beginning to be unraveled. This article contributes significantly to that task.*

Judith G. Hall, OC, MD

The authors summarize:

We have characterized a duplication of 7p12.1-p13 in a mother and daughter who both show features associated with SRS. It seems likely that a gene or genes contained within this region are responsible for at least some of the SRS features and that, in our patients, duplication of additional contiguous genes has resulted in a slightly atypical SRS phenotype. In contrast to current thinking, which favors the involvement of imprinted genes, we hypothesize that SRS may be caused by the inheritance of an additional copy of chromosome 7 material, either as

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## Growth in Sotos' Syndrome

Persons with Sotos' syndrome have early accelerated growth, advanced bone age (BA), acromegaloïd features, and developmental delay. Typically, the facies is distinguished by frontal bossing, large head circumference, antimongoloid slant of the palpebral fissures, and a prominent jaw. Diagnosis is based on the typical facies together with the large body size for age. Agwu et al report growth data on 40 patients (20 males and 20 females) who achieved their adult height. In addition to measurements of each patient's height and weight, arm span and body segment ratios were determined. Age of menarche was recorded, BA was determined, and target heights were calculated.

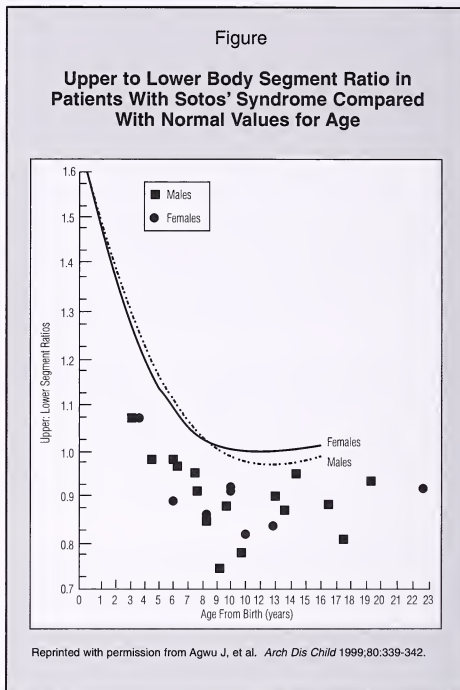
In boys, the mean height SDS in the 2nd and 6th years of life was 3.58 and 3.0, respectively. The mean height SDS for girls was 3.6 and 3.8 in the 2nd and 6th years, respectively. However, the mean height SDS at final height was 1.51 for men and 1.8 for women. These final heights are within the normal range for the British population for women and usually in the normal range for the men. However, the average final height in the men was 11.3 cm greater than their target height, whereas the average final height for women was only 6.2 cm above their target height. Upper to lower body segment ratio was reduced (Figure) and arm span was increased compared with population controls. BAs were advanced above the 97th percentile in those cases for which BAs were available. The mean age of menarche was 12.2 years (range, 8.9 to 15.4 years), which is slightly, but not significantly, earlier than the average for British girls (13 years). The excess arm span and reduced upper to lower body segment ratios suggest that much of the influence on height is a result of increases in limb lengths.

Agwu J, et al. *Arch Dis Child* 1999;80:339-342.

**Editor's comment:** There has been significant concern as to whether linear growth velocity should be reduced in individuals with Sotos' syndrome. Agwu et al demonstrate that the final height of these individuals is not excessive even though it is somewhat above their target height. The article, which presents interesting and important information, would have been strengthened by the inclusion of separate growth curves for girls and boys in the study. Such graphic display of growth velocity at different times during childhood would have enhanced the readers' ability to understand the data. It appears

that children with Sotos' syndrome are born large and remain large throughout infancy and childhood, enter puberty slightly earlier than the normal population, and achieve their final height within the population norm. Thus, the information should be useful to physicians caring for these individuals. Steroid intervention must be individualized since the mean adult heights fall within the normal range, although patients may be tall for their target heights.

William L. Clarke, MD



## Growth Hormone Treatment in Young Children With Down's Syndrome: Effects on Growth and Psychomotor Development

Between the ages of 6 months and 3 years, children with Down syndrome experience a significant reduction in growth velocity, and it also is during that time that a decline in intelligence quotient (IQ) is noted. Thus, Annerén et al treated 15 children (6 boys and 9 girls) with Down syndrome with exogenous GH (0.1 IU/kg/d) for 3 years beginning at 6 to 9 months of age. Height, weight, and head circumference were measured every third month during the first year, every 6 months during the second and third years, and 12 months after therapy. In addition, tests of motor development (motor perceptual tests) and mental development (Griffith's test)

were performed before GH treatment, 1 year into treatment, at the end of treatment, and 1 year after treatment was stopped. Measurements were made of insulin-like growth factor 1 (IGF-1) and serum IGF-binding protein 3 (IGFBP-3). Fifteen age-matched children with Down syndrome served as controls. No child in either the treated or control group had any cardiac malformations.

Two girls dropped out of the GH treatment group during the first year: one because of the development of celiac disease and the other because of an increase in serum aminotransferases. There

were no noted side effects from GH therapy. Specifically, frequent complete blood cell counts were performed to monitor for the risk of acute lymphoblastic leukemia, which is known to occur more frequently in children with Down syndrome. Prior to treatment, the study group had a height SDS of  $-1.8$  and the control group had a height SDS of  $-1.7$ . After 36 months of treatment, the study group SDS rose to  $-0.8$ , whereas the control group SDS decreased to  $-2.2$  (Figure). The study and control groups both had better growth than the average child with Down syndrome. The mean IGF-1 SDS in the study group was  $-1.6$  before treatment,  $0.28$  after 3 years of treatment, and  $-1.11$  one year after the end of treatment. There was a significant difference in head circumference between the 2 groups at the start of therapy, but there was no significant change in head circumference SDS in either group throughout the treatment period. At the end of GH treatment, the mean height of the treated group was significantly above that of the control group, but during the years after treatment these children grew less than the control group. However, there was a slight difference in heights at the age of 6.5 years (3 years after the end of treatment;  $P < 0.05$ ). The mean IQ decreased in the GH-treated group, and no difference between the treated and untreated groups was noted at the age of 3½. These results demonstrate that GH can increase height velocity in children with Down syndrome but has no effect on head circumference or mental performance.

Annerén G, et al. *Arch Dis Child* 1999;80:334-338.

**Editor's comment:** This carefully conducted study provides significant data useful for making decisions about whether to treat children with Down syndrome with GH. Interestingly, the authors concluded that they would not recommend GH treatment of such children unless GH deficiency was proven. Indeed, there was no effect on mental or motor development, and the differences in height at 3 years posttreatment were minimal. Whether such short-term effects of GH in children with Down syndrome would be observed had there been cardiac or other significant congenital malformations remains to be shown. It would appear that should GH treatment be used, it may be necessary to continue therapy until adult height is achieved.

William L. Clarke, MD

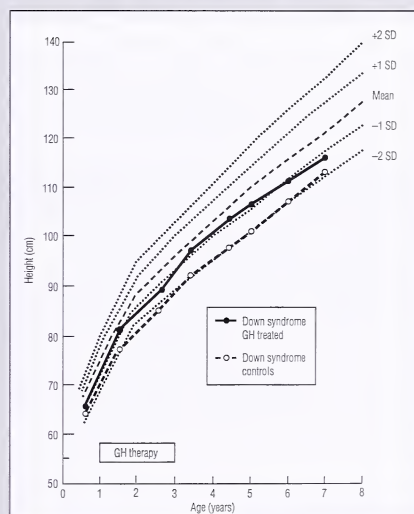
**2nd Editor's comment:** These data demonstrate that administration of GH therapy to infants with trisomy 21 does not prevent the deterioration in mental development that occurs in patients with Down syndrome between 12 and 36 months of age (ie, IQ scores declining from 70 to 40). The effect of GH therapy on the fine

motor skills of these children requires validation and assessment of its significance and impact on the quality of life of these subjects. At present, routine administration of GH therapy to children with trisomy 21 cannot be recommended outside of controlled investigative studies.

Allen W. Root, MD

Figure

**Mean Height of 12 Boys and 3 Girls With Down Syndrome Treated With GH for 3 Years From the Age of 6 to 9 Months (Mean, 7.4 Months), Compared With That of an Untreated Group of 6 Boys and 9 Girls With Down Syndrome**



The results are presented on the Swedish growth chart for normal boys.

Reprinted with permission from Annerén G, et al. *Arch Dis Child* 1999;80:334-338.

## Growth Hormone Improves Body Composition, Fat Utilization, Physical Strength and Agility, and Growth in Prader-Willi Syndrome: A Controlled Study

Fifty-four children, aged 4 to 16 years, with genetically confirmed Prader-Willi syndrome (PWS) were enrolled in this randomized, placebo-controlled study using GH. Subjects continued to pursue habitual energy intake throughout the study, and their diet history was analyzed every 6 months. After a 6-month period, children were randomly assigned to receive either placebo ( $n=19$ ) or  $1 \text{ mg/m}^2/\text{d}$  of GH therapy ( $n=35$ ) for 12 months. Stimulated GH levels (with clonidine) and serum levels of insulin-like growth fac-

tor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3), osteocalcin, type 1 procollagen, fasting and 2-hour glucose and insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acid, triglycerides, free thyroxine ( $T_4$ ) and thyroid-stimulating hormone (TSH) were determined at 0 and 12 months. Height was measured at  $-6, 0, 6$ , and 12 months. The mean baseline growth rate of all children was  $4.8 \pm 1.7 \text{ cm/y}$ . Bone age was determined annually. Body fat (BF), lean body mass (LBM), and bone mineral density

(BMD) were determined by dual energy X-ray absorptiometry (DXA). Resting energy expenditures (REE) and respiratory quotients (RQ) were determined by indirect calorimetry. Physical strength and agility were determined by using a modified Bruininks-Oseretsky test.

After 12 months, GH-treated children had a mean growth rate of 10.1 cm/y, compared with 5 cm/y among controls. Mean IGF-1, IGFBP-3, osteocalcin, and type 1 procollagen levels significantly increased in GH-treated children. Baseline body composition analysis revealed increased BF (45.2%  $\pm$  8.3% vs 18%  $\pm$  3.6%) and lower LBM (50% vs 80%) compared with healthy children without PWS. GH-treated patients experienced an 8% decrease in BF. Their LBM increased compared with controls (Table). Ninety-five percent of PWS children had normal baseline BMD. Femoral BMD increased by 0.09  $\pm$  0.02 g/cm<sup>2</sup>, while lumbar spine and total body BMD remained unchanged during GH treatment. Although baseline REE was low in PWS, it did not increase after GH treatment; however, RQ values decreased during GH treatment. GH treatment increased strength, agility, and respiratory muscle force. GH therapy improved the lipid profile as cholesterol and HDL cholesterol levels fell. Lastly, GH therapy did not significantly affect fasting and 2-hour postprandial insulin levels.

The authors concluded that GH treatment promoted growth and positively affected body composition, physical strength, and agility in children with PWS.

Carrel A, et al. *J Pediatr* 1999;134:215-221.

**Editor's comment:** Obesity, short stature, and hypotonia are some of the most striking physical signs of children with PWS. This interesting, well-designed, prospective study describes the effects of GH treatment—promoting growth while improving body composition and physical capacity—in children with PWS. The data are in agreement with previous studies and clearly showed that GH therapy could represent one of the most important therapeutic approaches to this disorder.

The response of PWS patients to GH treatment resembles the positive effects of GH in hypopituitary patients. In addition, the possibility that PWS patients are GH deficient because of hypothalamic abnormalities needs to be considered, although the GH axis has been difficult to assess because of the obesity of PWS patients. The deleterious effects of GH treatment should be considered as PWS children have an increased incidence of scoliosis,

which could be aggravated by GH treatment. Finally, the chromosomal abnormalities characteristic of PWS may add a risk factor for potential GH-associated malignancies. Therefore, the estimated potential benefit of this kind of treatment should be evaluated on a long-term basis.

Fima Lifshitz, MD

**2nd Editor's comment:** This topic is of much current interest. To supplement your knowledge about the recent literature, you can refer to 2 articles abstracted in GGH previously (1999;15[1]).

1. Thacker MJ, et al. Growth failure in Prader-Willi syndrome is secondary to GH deficiency. *Horm Res* 1998;49:216-220.
2. Lindgren AC, et al. Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favorably. *Acta Paediatr* 1998;87:28-31.

The data and conclusions in these articles are consistent.

Robert M. Blizzard, MD

Table  
Body Composition, Bone Mineral Density,  
and Energy Expenditure

|                                       | Baseline                |                            | 12-Month Values         |                            |
|---------------------------------------|-------------------------|----------------------------|-------------------------|----------------------------|
|                                       | Control Group<br>(n=19) | GH Therapy Group<br>(n=35) | Control Group<br>(n=19) | GH Therapy Group<br>(n=35) |
| Body fat (%)                          | 42.6 $\pm$ 8.1          | 46.3 $\pm$ 8.4             | 45.8 $\pm$ 8.8          | 38.4 $\pm$ 10.7*           |
| Lean mass (kg)                        | 20.5 $\pm$ 5.0          | 20.5 $\pm$ 6.3             | 21.7 $\pm$ 5.0          | 25.6 $\pm$ 4.3*            |
| BMI (kg/m <sup>2</sup> )              | 24.2 $\pm$ 6.5          | 25.0 $\pm$ 6.7             | 25.2 $\pm$ 8.9          | 23.7 $\pm$ 6.3             |
| Femoral neck BMD (g/cm <sup>2</sup> ) | 0.636 $\pm$ 0.09        | 0.656 $\pm$ 0.19           | 0.707 $\pm$ 0.09        | 0.797 $\pm$ 0.09†          |
| Spine BMD (g/cm <sup>2</sup> )        | 0.753 $\pm$ 0.12        | 0.744 $\pm$ 0.14           | 0.793 $\pm$ 0.13        | 0.834 $\pm$ 0.15           |
| REE (kcal/m <sup>2</sup> /h)          | 22.5 $\pm$ 3.4          | 22.4 $\pm$ 4.4             | 25.1 $\pm$ 6.9          | 28.2 $\pm$ 7.4             |
| RQ                                    | 0.83 $\pm$ 0.1          | 0.81 $\pm$ 0.07            | 0.84 $\pm$ 0.04         | 0.77 $\pm$ 0.04*           |

BMI, body mass index; BMD, bone mineral density; REE, resting energy expenditure; RQ, respiratory quotients.

\*P < 0.01 paired t test before and after GH therapy, compared with either baseline values of treated patients or 12-month values of nontreated patients.

†P < 0.05 paired t test before and after GH therapy, compared with either baseline values of treated patients or 12-month values of nontreated patients.

Reprinted with permission from Carrel A, et al. *J Pediatr* 1999;134:215-221

## Growth Hormone Treatment Increases CO<sub>2</sub> Response, Ventilation and Central Inspiratory Drive in Children With Prader-Willi Syndrome

Hypoventilation commonly occurs in subjects with Prader-Willi syndrome (PWS). This has been attributed in large part to obesity and the imposition of a large mechanical load, thereby impairing movement of the thoracic cage. However, there also is a problem with ventilatory control in PWS patients, as evidenced by abnormal responses to hyperoxia, hypoxia, and hypercarbia, as well as decreased peripheral chemoreceptor activity (Menendez AA. *Eur J Pediatr* 1999;158:941-942).

Lindgren et al studied resting ventilatory regulation in 9 prepubertal children with PWS prior to and after 6 to 9 months of therapy with

rhGH (0.23 mg/kg/wk). This therapeutic program did not alter body mass index in this interval, although changes in body composition were not reported. They found that in response to rhGH, minute ventilation (mL/kg/min) increased 126%; the ventilatory response (ie, sensitivity) to 4% CO<sub>2</sub> increased 8-fold; and the central inspiratory drive, a measurement of airway occlusion pressure that is little affected by pulmonary mechanics (determined by the change in airway pressure during the first 0.1 second after beginning an inspiration), increased 170%. In contrast, the response to hyperoxia, measured as an increase of the inspired O<sub>2</sub> concentration from room air to 100%, did not change during rhGH administration.



The investigators suggest that the impairment of respiratory function in PWS may be due in large part to an abnormality in the function of a hypothalamic respiratory regulatory center rather than to excessive weight, that rhGH may have had a direct central stimulatory effect on this structure, and that the hypoventilation of PWS may be amenable to treatment.

Lindgren AC, et al. *Eur J Pediatr* 1999;158:936-940.

**Editor's comment:** Although current data did not explore the possibility that the increased respiratory effort during rhGH treatment of PWS subjects was due, at least in part, to increase in lean body mass and improved muscle strength, these findings support the suggestion that rhGH may be useful in the management of PWS, which is a conclusion that this reviewer has been very reluctant to draw. Lindgren et al (*Acta Paediatr Scand* 1998;87:28-31) also have reported that in a controlled, randomized trial of rhGH in PWS subjects, rhGH led to increases in growth, lean body mass, physical activity, and endurance, and

improvements in behavior. Carrel et al (*J Pediatr* 1999;134:215-221) confirmed these findings and also observed that rhGH improved physical strength, agility, and inspiratory and expiratory muscle strength in PWS. Since chronic respiratory insufficiency often leads to pulmonary hypertension and right ventricular heart failure, and is a major cause of death in PWS, treatment of PWS may be expanded to include diet, exercise, and rhGH. Before rhGH becomes the standard of care for PWS, however, further controlled and, hopefully, double-blind studies must be conducted that confirm these reports and justify the large expenditures that such treatment will engender, as well as demonstrate that the quality of life of rhGH-treated PWS subjects is superior to that achieved by a rigorous residential dietary and exercise program alone.

Several abstracts and editorial comments regarding the treatment of PWS have appeared in GGH in 1999 (see Vol 15, pages 10 and 11).

Allen W. Root, MD

## Postnatal Sex Reversal of the Ovaries in Mice Lacking Estrogen Receptors $\alpha$ and $\beta$

The investigators developed mice in which both estrogen receptors (ERs)  $\alpha$  and  $\beta$  had been disrupted or "knocked out" ( $\alpha\beta$ ERKO) by breeding phenotypically normal mice heterozygous for loss of either one or the other ER.  $\alpha\beta$ ERKO mice were phenotypically intact and survived normally. Adult male mice in which both ERs had been eliminated had normal internal genitalia but slightly smaller testes than control animals, with loss of germinal epithelium and subnormal spermatogenesis; they were infertile, which is consistent with earlier reports of the  $\alpha$ ERKO male—indicating that estrogen and the ER are necessary for complete spermatogenesis. Adult female mice in which both ERs had been disrupted also had normal but hypoplastic internal genitalia, indicating that neither ER is necessary for Müllerian duct differentiation. Having ER $\alpha$  is necessary for uterine response to estrogen. The ovaries of  $\alpha\beta$ ERKO adult female mice contained both healthy (with oocytes) and sex-reversed follicles; the latter were characterized by degeneration of the oocyte and "transdifferentiation" of follicles into seminiferous-like tubules, with Sertoli-like cells that expressed increased amounts of mRNA for *Sox9/MIS*. Similar changes are not observed in adult female mice who lack one or the other ER.

The authors suggest that the ovarian follicles of the  $\alpha\beta$ ERKO adult female mice "redifferentiated" into testes-like structures. Although

sex reversal of fetal rodent ovaries has been accomplished by their transplantation into an adult animal or one in which there is transgenic overexpression of *MIS* or by in vitro exposure to *MIS*, it previously has not been recorded in adult ovaries. The investigators suggest that in the absence of both ERs, the differentiated ovarian follicle is able to form a testes-like structure, possibly because of the loss of estrogen-mediated persistent repression of *MIS* and *Sox9*.

Couse JF, et al. *Science* 1999;286:2328-2331.

**Editor's comment:** This paper documents the need for both ER  $\alpha$  and  $\beta$  and therefore estrogen to maintain ovarian differentiation in the adult mouse. Wnt-4 recently has been shown to be necessary for ovarian and Müllerian duct differentiation and BAX regulates the longevity of the ovarian follicle. Future studies will be directed to elucidating the mechanism(s) of estrogen action and the identification of other factors involved in this complex process. If the reader has not read the lead article in this issue of GGH titled "Estrogen and Growth", he/she will miss a golden opportunity to integrate these 2 presentations regarding the actions of estrogen. Keep in mind, however, that mice and humans may not be identical in all actions of estrogen.

Allen W. Root, MD

## Directed Pharmacological Therapy of Ambiguous Genitalia Due to Androgen Receptor Gene Mutation

The authors report a 46,XY infant with ambiguous genitalia and undescended gonads due to the partial androgen insensitivity syndrome (PAIS). A T $\rightarrow$ C transition resulted in a missense mutation of codon 807 in the ligand binding region of the androgen receptor (AR). The mutated AR had only 15% of the binding capacity for testosterone and 15% of the in vitro transcription activating function as the wild-type AR. Yet this mutated AR bound dihydrotestosterone (DHT) with high efficiency and effective function. Topical periscro-

tal application of a preparation of DHT gel led to raised serum DHT concentrations, rugation of the scrota, descent of the gonads, and enlargement of the phallus. The authors conclude that functional assays as described of a mutated AR may identify subjects who are androgen responsive and thus could be reared in their genetic sex with DHT administration.

Ong YC, et al. *Lancet* 1999;354:1444-1445.



**Editor's comment:** These data demonstrate the direct clinical benefit that may accrue to selected subjects with a mutated AR if the mutated gene's structure and function can be evaluated rapidly after birth. The authors do not state how much time it took to complete the molecular studies. Nordenstrom et al (J Clin Endocrinol Metab 1999;84:1505-1509) suggested that genotyping of neonates with 21-hydroxylase-deficient congenital adrenal hyperplasia detected in neonatal screening programs will identify infants at significant risk for the salt-losing form of this disease and thus permit more specific management.

The case report of Ong et al raises the question of why the genitalia of the reported infant was ambiguous if DHT was biologically effective in utero. It is clear that male fetuses with deficiency of 5 $\alpha$ -reductase are not virilized in utero and, thus, DHT is essential for this process. The investigators demonstrated that the M870T AR did not respond well to lower levels of DHT, although the maximum responses of the mutated and wild-type receptors were similar. Thus, in utero physiologic levels of DHT must have been biologically ineffective. An excellent review of the mutations identified in the human AR patient has been published recently by McPhaul and Griffin (J Clin Endocrinol Metab 1999;84:3425-3441).

Allen W. Root, MD

## **Drosophila S6 Kinase: A Regulator of Cell Size**

Although many of the growth factors and intracellular signaling pathways that regulate cellular multiplication have been deciphered, the mechanisms that determine cell size are not as widely understood. These investigators report that in *Drosophila melanogaster* inactivation of the gene *Drosophila* S6K = *dS6K*, which encodes a physiologic kinase for the ribosomal protein S6, inhibits increase in cell size without affecting the cell number of a given structure. The S6 kinases control the translation of mRNAs that in turn encode ribosomal proteins involved in the translational process. *dS6K* was inactivated by altering the 5' noncoding or transcription-activating region of the gene, or by excising part of the first exon of the gene that encodes a portion of the catalytic domain.

Insects with a homozygous null mutation in *dS6K* were symmetrically dwarfed to half the size of normal flies. Cell numbers in both the eyes and wings of the dwarfed insects were similar to those in control insects but the cell sizes were 30% smaller. Inhibition of cellular growth was present in the larval stage of insects homozygous for inactivation of *dS6K*. The investigators also generated insects that expressed 3 copies of *dS6K* in targeted segments; these insects apparently had larger cell sizes in selected structures. The authors conclude that the kinase encoded by *dS6K* regulates cell size without affecting cell number in *Drosophila melanogaster*.

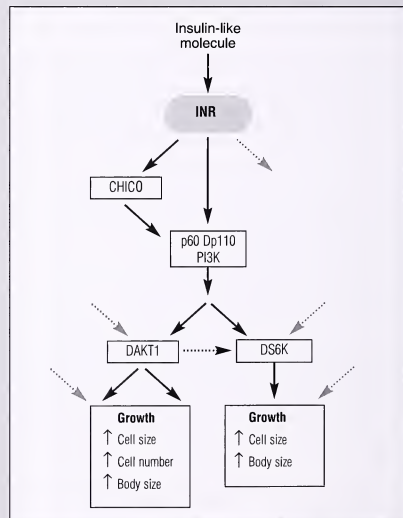
Montagne J, et al. *Science* 1999;285:2126-2129.

**Editor's comment:** Under normal circumstances, organ and body size reflect both cell number and cell size. The 2 processes of cell growth and replication seem to be coupled. Identification of a factor that regulates cell size specifically is an important advance in our understanding of the mechanisms that influence growth. In both insects and mammals, S6 kinases regulate synthesis of ribosomal proteins that are encoded by 5'-terminal oligopyrimidine tract mRNAs. These proteins influence translation (Leever SJ. *Science* 1999;285:2082-2083). S6 kinases are under the control of the insulin signaling pathway involving the insulin receptor, insulin receptor substrates, phosphatidylinositol 3-kinase (PI3K), and its target Akt/PKB or DAKT1 (Figure). *Drosophila* with loss-of-function mutations in the insulin receptor substrate are small and cell size and number are both reduced, whereas loss of the insulin receptor, PI3K, or DAKT1 is lethal. The difference between these experimental models of impaired growth suggests the presence of alternate but interactive intracellular pathways regulating cell growth and cell division. *Drosophila* with loss-of-function mutations in other genes (Minutes) that regulate ribosomal protein

synthesis also have slow rates of cell growth and replication but normal cell size. The application of these data to our patients with primordial disorders of growth seems imminent. To follow the resultant revelations will be exciting.

Allen W. Root, MD

**Figure  
Signaling for Growth**



The likely interactions (solid arrows) between molecules that regulate *Drosophila* growth, based on studies of interactions between their mammalian homologues. Broken arrows indicate where branching is possible and where links with other molecules may occur. INR, the *Drosophila* insulin receptor homologue; CHICO, the *Drosophila* homologue of mammalian IRS1-4; Dp 110, the *Drosophila* class 1A PI3K; p60, its adaptor protein; and DAKT1, the *Drosophila* homologue of Akt/PKB.

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**GROWTH, Genetics, & Hormones Volume 16, Number 1**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

Please note that each question may have more than one correct answer.

1. Which of the following beliefs in the past remain beliefs currently?
  - a. Estradiol turns off GH secretion.
  - b. Children produce more GH than young adults.
  - c. Estradiol is primarily responsible for the adolescent growth spurt in females.
2. Estradiol 800 ng/kg/d has been demonstrated to \_\_\_\_ in patients with TS.
  - a. Increase GH secretion.
  - b. Increase IGF-1 levels.
  - c. Inhibit ulnar growth.
3. Which of the following is/are true?
  - a. In familial male precocious puberty, androgen antagonistic therapy is effective in slowing the growth rate and epiphyseal maturation.
  - b. Estradiol increases the pulse amplitude and frequency of GH.

4. The phenotype of a pubertal-aged female with aromatase deficiency includes which of the following?
  - a. Prominent adrenarche.
  - b. Acne.
  - c. Virilization.
  - d. Advanced bone age.
5. The phenotype of estradiol deficiency in the male includes:
  - a. Tall stature.
  - b. Impaired epiphyseal maturation.
  - c. Eunuchoid habitus.
  - d. Acromegaly features.

Answer Key: 1. c 2. a,b,c 3. a 4. a,b,c 5. a,b,c

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**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

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# GROWTH

## Genetics & Hormones

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## Effects of Glucocorticosteroids on Growth

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### INTRODUCTION

Glucocorticoid therapy in pharmacologic doses is the treatment of choice in various chronic inflammatory and immune-mediated diseases in childhood. Glucocorticoids also are needed for immunosuppression after organ transplantation. Long-term, high-dose glucocorticoid treatment inevitably leads to protein catabolism and growth failure. Recent evidence indicates that these side effects are partially mediated by alterations of the somatotrophic hormone or growth hormone (GH) axis.

This review summarizes our current knowledge of the pathogenesis of glucocorticoid-induced growth failure. It also summarizes available therapeutic options. In particular, it answers the question whether glucocorticoid-induced growth failure can be counterbalanced by concomitant treatment with recombinant human GH (rhGH).

### PATHOGENESIS OF GLUCOCORTICOID-INDUCED GROWTH FAILURE

#### Effects on GH Secretion

While short-term glucocorticoid administration stimulates GH secretion,<sup>1,2</sup> long-term, high-dose treatment diminishes spontaneous GH secretion (Table 1). Several studies performed in man<sup>3</sup> have demonstrated that the inhibitory effect of these steroids on GH secretion in vivo are due to enhancement of hypothalamic somatostatin (SRIF) release. Recent data indicate that arginine infusion

Table 1  
**Interference of Long-Term, High-Dose  
Glucocorticoid Treatment With the Integrity of  
the Somatotrophic Hormone Axis**

| Organ                   | Effect of Glucocorticoids  |
|-------------------------|--|
| Hypothalamus            | Somatostatin tone ↑  |
| Hypophysis              | GH secretion ↓   |
| Liver                   | GH-induced IGF-1 mRNA ↓  |
| Circulation             | IGF-1 levels normal or ↑<br>Induction of IGF-1 inhibitors<br>IGFBP-2 ↑                                     |
| Epiphyseal growth plate | Cell proliferation ↓<br>Matrix production ↓<br>Paracrine IGF-1 secretion ↓<br>GH and type 1 IGF receptor ↓ |

is able to normalize the GH response to GH-releasing hormone by inhibition of the endogenous hypothalamic SRIF tone.<sup>4</sup> In peripubertal children with renal transplants, a significant reverse relationship between the daily dose of glucocorticoids and the peak amplitude or mean levels of GH was noted, whereas the GH pulse frequency was not changed.<sup>5</sup> Similar results were seen in experimental rats treated with methylprednisolone.<sup>6</sup>

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## Effects on GH Receptor Expression

There is experimental evidence that these steroids suppress GH receptor expression, at least in the liver. While adrenalectomy does not appear to affect hepatic GH receptor mRNA and binding or plasma levels of GH-binding proteins (GHBPs) in the rat, these parameters were markedly reduced in a dose-dependent fashion by dexamethasone (DEX) treatment.<sup>7</sup> Estrogens<sup>8</sup> as well as glucose and amino acids<sup>9</sup> interact with DEX to control the expression of GH mRNA in cultured hepatocytes. Under clinical conditions, GH receptor status can be assessed by determination of the high-affinity GHP, which is derived from the extracellular domain of the GH receptor by proteolytic cleavage. We observed a significant reduction of circulating GHP levels compared with age-matched controls.<sup>10</sup>

## Effects on Insulin-Like Growth Factors and Insulin-Like Growth Factor-Binding Proteins

Secondary to impaired GH secretion and GH receptor expression by glucocorticoids, one would expect a decrease of circulating insulin-like growth factor (IGF). However, controversial findings have been reported so far. Under experimental conditions, the GH-induced rise in serum IGF-1 in *hypophysectomized* rats was significantly inhibited by high doses of DEX.<sup>11</sup> Concomitantly, the GH-induced IGF-1 mRNA content in the liver and various other tissues was inhibited by pretreatment with DEX. There also was a reduction in the hepatic IGF-1 mRNA in DEX-treated *intact* rats, which, however, did not result in decreased IGF-1 serum levels. The authors suggested 2 possible explanations: (1) Hepatic IGF-1 mRNA is not translated into protein, or (2) glucocorticoids alter IGF-1 translation, synthesis, and secretion in such a way that IGF-1 mRNA no longer reflects IGF protein synthesis. In patients with chronic endogenous glucocorticoid excess, IGF-1 levels were elevated.<sup>12</sup>

Although glucocorticoids do not consistently reduce circulating immunoreactive IGF-1 levels, they inhibit IGF bioactivity both in children with a variety of disorders, including the nephrotic syndrome,<sup>13</sup> and in adults given a single 16-mg dose of prednisone.<sup>14</sup> Hence, glucocorticoid excess apparently does not impair immunoreactive IGF-1 levels, but rather antagonizes the action of IGF by direct and/or indirect mechanisms, possibly by increased production of IGF inhibitors. IGF inhibitors, which have a molecular weight of 12 to 20 kd and which clearly differ from IGF-binding proteins (IGFBPs), have been identified. In one study, glucocorticoid excess in patients with Cushing's syndrome was associated with a slight increase of IGFBP-3, normal IGFBP-1 levels, and clearly elevated IGFBP-2 levels.<sup>12</sup> In contrast, DEX 5 mg given for 4 days to normal volunteers decreased IGFBP-2 levels while nearly doubling serum IGF-1 concentrations, which was in parallel to an increase of serum IGFBP-3 levels.<sup>15</sup> Further studies to explain these apparent paradoxes are needed.

## Effects of Glucocorticoids on Growth Plate Chondrocytes

These steroids inhibit sulfation of cartilage matrix, mineralization and formation of new bone, and cell proliferation in the growth zone. Contrary to expectations, DEX caused a tissue-specific stimulation of GH receptor mRNA associated with a biphasic dose-response relationship in rabbits.<sup>16</sup> These data suggest that glucocorticoid-induced GH insensitivity cannot be explained by decreased GH receptor mRNA levels. To the contrary, DEX causes a tissue-specific stimulation of GH receptor mRNA levels with a biphasic dose-response relationship. In contrast, investigators using cell culture experiments demonstrated that DEX time dependently decreased DNA synthesis and cell proliferation of epiphyseal chondrocytes through a reduction of GH receptor expression and inhibition of homologous upregulation of both the GH and

### CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Figure 1

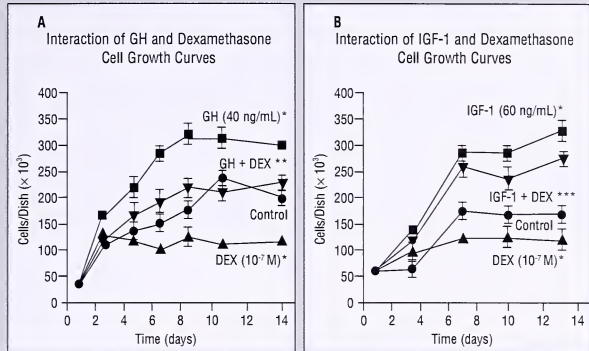
Effect of DEX, GH, and IGF-1 on chondrocyte proliferation in primary cultures (growth curves). The cells were synchronized (starved), and the medium was then changed to one containing 0.2% bovine serum albumin and 0.3% Ultrasol plus hormones and solvent as indicated. Data are mean  $\pm$  SE of 4 parallel dishes per group and day.

\* $P < 0.001$  vs control

\*\* $P < 0.001$  vs DEX and GH

\*\*\* $P < 0.001$  v. control, DEX and IGF-1 alone

Reprinted with permission from Jux C, et al. *Endocrinology* 1998;139:3296-3305.



IGF-1 receptor and through the inhibition of paracrine IGF-1 secretion (Figures 1 and 2).<sup>17</sup> These experiments have been confirmed in primary cultured hepatocytes.<sup>18</sup> Unfortunately, little is known about the precise mechanisms by which glucocorticoids regulate the gene activity that eventually leads to growth disturbance.

Glucocorticoids exert their genomic effects after cytosolic binding to specific receptors. Once conformational changes and translocation to the nucleus have occurred, the complexes bind to DNA at specific consensus sites, termed "glucoid response elements," on the upstream promoter sequence of steroid-responsive genes.<sup>19</sup>

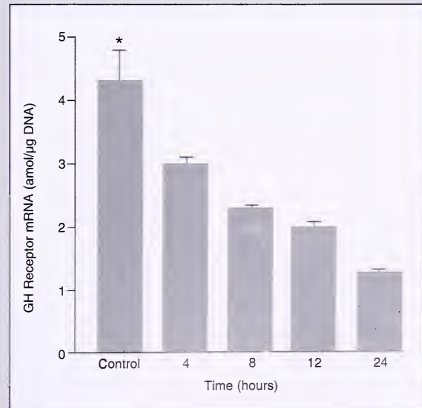
### Clinical Presentation

Only long-term systemic glucocorticoid treatment impairs growth, whereas inhaled or topical glucocorticoids do not significantly reduce growth in most patients. This general statement holds true for patients with bronchial asthma and atopic eczema.<sup>20,21</sup> In patients with either frequently relapsing or steroid-dependent nephrotic syndrome, growth-retarding effects up to temporary growth arrest have been observed. Those patients receiving repeated courses of high-dose steroids or prolonged maintenance therapy were at greatest risk of growth failure. However, when Foote et al<sup>22</sup> examined the heights of 80 patients with frequently relapsing nephrotic syndrome, it became evident that final height was only slightly reduced by  $-0.2$  SD (equivalent to a height deficit of 1.5 cm below average height). Total glucocorticoid dose correlated negatively but only weakly with changes in height SDS.

Persistent hypoalbuminemia seems to be an independent risk factor for growth retardation, as seen in patients with steroid-resistant nephrotic syndrome.<sup>23</sup> In patients with juvenile chronic arthritis or inflammatory bowel disease,

glucocorticoid therapy frequently is needed when non-steroidal drugs fail to control symptoms. These patients frequently have growth retardation. However, the pathogenesis of growth failure in such patients is multifactorial. In chronic inflammatory disease, malnutrition and other factors are contributive and the relative contribution of glucocorticoids is difficult to assess, since systemic treatment usually is needed in the most severe cases. Inflammation

Figure 2



Time-dependent downregulation of GH receptor mRNA by DEX ( $10^{-7}$  M). RNase-protection solution-hybridization assay. \* $P < 0.005$  vs all treatment groups.

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might be so active that growth is possible only with glucocorticoid treatment. Depending on the dose of steroids, the same treatment may permit or inhibit the growth rate.

The situation seems to be more clear in pediatric transplant recipients, in whom glucocorticoid treatment is used for prevention of rejection episodes. According to the North American Pediatric Renal Transplant Cooperative Study, catch-up growth after renal transplantation is unlikely to occur in 75% of renal allograft recipients. It primarily occurs in recipients <6 years of age. Reduced allograft function and glucocorticoid treatment were identified as the main risk factors, and a negative correlation between growth and cumulative glucocorticoid dose is noted after correction for renal function.<sup>24</sup>

## THERAPEUTIC OPTIONS

### Intermittent Corticosteroid Treatment

There is general agreement that alternate-day treatment given in 1 dose significantly improves but does not normalize growth rates. Increased growth rates have been observed in patients with juvenile rheumatoid arthritis<sup>25</sup> and in patients with renal allografts.<sup>26</sup> Alternate-day treatment often is combined with a dose reduction of glucocorticoids. It has not been established to what extent the dose reduction or the intermittent mode of treatment contributes to the improvement in growth. The success of the treatment strategy varies from patient to patient. Therefore, catch-up growth does not consistently occur. One major problem is the individual's sensitivity to glucocorticoids, which currently cannot be satisfactorily measured.<sup>27,28</sup> Another problem is that only stable patients are shifted from daily to intermittent treatment, which skews the results. Clinical studies suggest that third-generation glucocorticoids such as deflazacort have fewer side effects

while maintaining equipotent anti-inflammatory and immunosuppressive activity. Although uncontrolled clinical studies have demonstrated positive results,<sup>29,30</sup> controlled studies are missing, and no gold standard is available with which to compare the immunosuppressive properties of different glucocorticoids.

### Treatment With rhGH

During recent years, concomitant GH treatment has been demonstrated to diminish or even completely counterbalance the growth-depressing effects of glucocorticoid treatment. The success of such a treatment strategy seems to be dependent on the given dose, each individual's sensitivity, and the underlying primary disease. Furthermore, it is very likely that certain threshold doses, which may be different for different individuals, cannot be counterbalanced by rhGH. However, these doses are difficult to define.

### Animal Studies

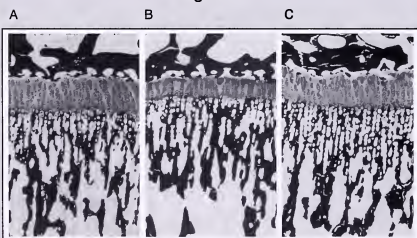
In cell culture studies focusing on growth plate chondrocytes, rhGH counterbalanced the antiproliferative effects of DEX in a dose-dependent manner.<sup>17</sup> In healthy and uremic female rats, rhGH was able to counterbalance prednisone in doses up to 9 mg/kg/d (Figure 3).<sup>6</sup> These results were extended by studies in rats demonstrating that administration of GH in vivo resulted in an increased cortical bone mass of both the vertebrae and the femoral diaphyses.<sup>31</sup> In piglets, DEX treatment resulted in lower plasma osteocalcin, urinary N-telopeptide, and whole-body and femoral mineral density. However, all of these could be prevented by concomitant treatment with GH.<sup>32</sup> In rat experiments from our laboratory,<sup>6</sup> methylprednisolone 2 mg/kg/d decreased and rhGH 0.6 mg/kg/d independently increased weight gain, whereas normal weight gain was observed with concomitant treatment.

Chrysis and Underwood<sup>33</sup> did not see a downregulation of the ubiquitin system in skeletal muscle with rhGH 3 mg/kg/d in catabolic rats receiving DEX 5 mg/kg/d, whereas downregulation was noted with IGF-1 treatment. This is consistent with the notion that GH affects protein synthesis but not proteolysis.

### Studies in Man

Horber and Haymond<sup>34</sup> demonstrated that rhGH 0.1 mg/kg/d prevented the protein catabolic side effects of prednisone 0.8 mg/kg/d in 32 healthy adult volunteers. An extension of these studies by Bennet and Haymond<sup>35</sup> demonstrated that the anabolic effects were observed in subjects receiving long-term treatment with one quarter of the dose of glucocorticoids and one eighth of the dose of GH. Leucine kinetic data showed that the negative protein balance during prednisone treatment was due to increased proteolysis, whereas GH had no effect on proteolysis but increased whole-body protein synthesis. As

Figure 3



Light photomicrographs of proximal tibia growth plate. Sections stained with toluidine blue. Sham-operated control animals were fed ad libitum receiving either solvent (A), methylprednisolone (B), or methylprednisolone and GH (C). Methylprednisolone-induced growth retardation can be prevented by concomitant GH treatment.

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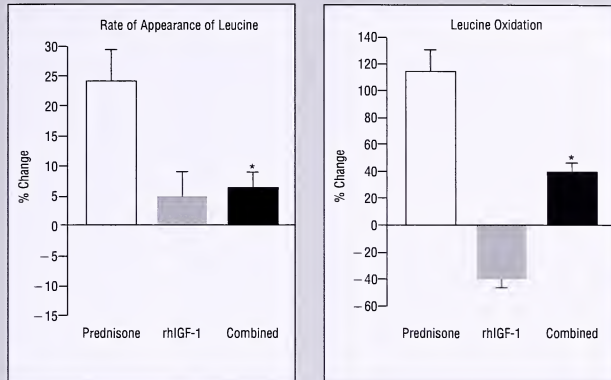


Figure 4

In healthy volunteers, oral prednisone (60 mg/d) increased both leucine turnover and leucine oxidation. The relative increase in protein turnover and oxidation was significantly decreased with concomitant rhIGF-1 treatment (100 µg/kg subcutaneously twice daily).

\*  $P < 0.01$  vs prednisone-treated group

Reprinted with permission from Mauras N, Haymond MW. *Pediatr Nephrol* 1996;10:318-323.



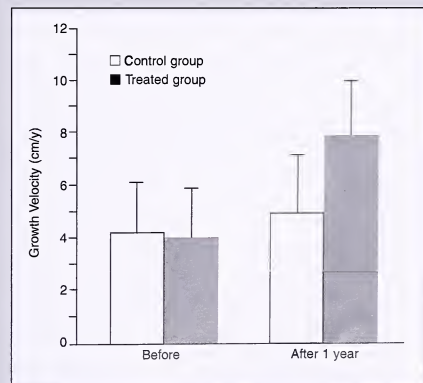
prednisone and GH had differential effects on fuel metabolism and insulin antagonism, the authors assumed independent mechanisms were involved in which GH and prednisone may reciprocally regulate the oxidation of protein and fat while decreasing the efficiency of glucose disposal. Mauras and Haymond<sup>36</sup> examined the question of whether similar anabolic effects can be obtained with IGF-1 in prednisone-treated subjects without a reduction in carbohydrate tolerance. Like GH, IGF-1 in low doses increases protein synthesis, which is in contrast to the marked suppression of proteolysis seen with high doses. It also ameliorates the steroid-induced protein catabolism with no rise in plasma glucose and with a significant reduction in circulating insulin levels (Figure 4).

In early studies, no significant metabolic response to exogenous GH in glucocorticoid-treated children was noted.<sup>37</sup> In contrast, Davies et al<sup>38</sup> subsequently reported positive effects of rhGH on growth in growth-retarded children with rheumatoid arthritis. In a recent study, rhGH 1.4 IU/kg (0.5 mg/kg) per week was administered to 14 patients with rheumatoid arthritis. All patients showed an increase in growth velocity with a mean increase from 1.9 to 4.5 cm/y. This effect of pharmacologic doses of GH prevented a further decrease in height SDS, but it did not result in catch-up growth. All patients were severely stunted (mean height SDS, -4), which was the consequence of the primary disease and to a minor extent of glucocorticoid treatment.<sup>39</sup>

Allen and Goldberg<sup>40</sup> studied the effects of GH treatment (0.3 mg/kg/wk for 6 to 21 months) in 7 slowly growing children with various diseases treated with glucocorticoids. The mean height velocity increased from 3.4 to 6.7 cm/y. In the National Cooperative Growth Study,<sup>41</sup> 83 patients with various diseases who were receiving glucocorticoids and concomitant rhGH were identified.

The mean height SDS was  $-3.7 \pm 1.2$  SD and the mean growth velocity was  $3.0 \pm 2.5$  cm/y. After 12 months of rhGH therapy, the mean growth rate increased to  $6.3 \pm 2.6$  cm/y and the mean height SDS improved by  $0.21 \pm 0.4$  cm/y ( $P < 0.01$ ). Responsiveness to rhGH was negatively correlated with the dose of glucocorticoids (Figure 5).

Figure 5

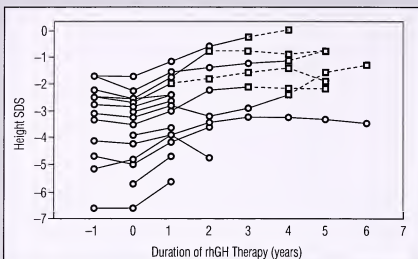


Growth velocity after 1 year of rhGH treatment in 85 children with a renal transplant and glucocorticoid treatment. The patients were randomized for rhGH treatment and controls. rhGH significantly increased growth velocity.

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Figure 6



Height SDS related to chronologic age in 14 children before and during 1 to 6 years of combined treatment with methylprednisolone and rhGH. Patients who entered puberty are indicated by ---□--- (O.M. and B.T., personal observations).

In glucocorticoid-treated children with renal allografts, the positive effect of GH treatment has been documented in a number of prospective, open-labeled studies (Figure 6).<sup>42,43</sup> Height velocity during the first treatment year in these children *prepubertally* can often be doubled by pharmacologic doses of rhGH. Promising results also have been obtained in growth-retarded *pubertal* children posttransplantation. Eighteen adolescents demonstrated an impressive growth response to rhGH. After 2 years of rhGH, they achieved an increase in height with a mean of  $15.7 \pm 5.1$  cm, compared with  $5.8 \pm 3.4$  cm in retrospectively matched controls.<sup>44</sup> This growth response occurred whether rhGH 4 or 8 IU ( $1.3$  or  $2.6$  mg/m<sup>2</sup>/d) was given. In a large randomized study involving 90 children treated with rhGH 30 IU ( $10$  mg/m<sup>2</sup>/wk), growth velocity was significantly increased from  $4.1$  to  $7.7$  cm/y; in the control group, no significant increase ( $4.2$  to  $4.6$  cm) was noted. Four independent factors predicted the response to therapy: (1) slow growth velocity prior to GH treatment (negative); (2) low glomerular filtration rate (negative); (3) the mode of corticosteroid administration; and (4) a significant degree of insulin resistance (negative). However, overall the height velocity remained above baseline during 4 years of observations, and the mean height SDS increased from  $-3.5$  to  $-2.5$  within 3 years of treatment.<sup>45</sup>

The risk of GH treatment in children with renal transplantation must be considered with respect to possible deleterious effects on the survival of the transplant. Theoretically, there is concern that the immunostimulatory effects of GH might reduce the effectiveness of immunosuppression by glucocorticoids. Although *preliminary data* of the French randomized study showed a 35% increase in the rejection rate in high-risk patients who already had experienced more than 1 rejection episode before initiation of GH therapy,<sup>46</sup> in the *final*

*analysis*,<sup>47</sup> biopsy-proven acute rejection episodes were *not* significantly more frequent in the group of patients who received rhGH. During the first year, 9 rejection episodes occurred in 44 rhGH-treated patients and 4 occurred in 46 control patients. Nevertheless, in all future studies in which rhGH is given concomitantly with glucocorticoids, careful analysis must be done to assess the potential negative effects of rhGH on immunosuppression.

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## ERRATUM

Regarding question 3 of the post-test for *GGH* Volume 16, Number 1, neither statement is true. In familial male precocious puberty, aromatase inhibition slows growth rate and epiphyseal maturation.

## Is Short Stature a Handicap? A Comparison of the Psychosocial Functioning of Referred and Non-Referred Children With Normal Short Stature and Children With Normal Stature

Heretofore, most reports that short stature conferred significant academic and social handicaps have utilized subjects who were referred for pediatric endocrinologic evaluation. The present authors evaluated the psychosocial status of 2 populations of children with normal short stature (NSS, or ISS; height below the National Center for Health Statistics [NCHS] 5th percentile not associated with illness, hormonal deficiency, or dysmorphic syndrome) in comparison to that of a third control group of children of average height. In 27 children with NSS referred for pediatric endocrinologic evaluation (group 1), mean height SDS was  $-2.7$  (range,  $-4.5$  to  $-1.3$ ). In 34 nonreferred children with NSS (group 2) who were identified through a public school screening program, the mean height SDS was  $-1.7$  (range,  $-3.2$  to  $-1.3$ ). For the 29 in the third group, the mean height SDS was  $0.06$  (range,  $-0.7$  to  $+0.7$ ). Tests of verbal and nonverbal intelligence (Kaufman Brief Intelligence Test [K-BIT]) and educational achievement (Kaufman Test of Educational Achievement [KTEA]) were administered. Family coherence and adaptability were assessed using the Family Adaptability and Cohesion Evaluation Scales (FACES II), as were the adaptive and problem behaviors (by the Behavior Assessment System for Children [BASC]).

No relationship was found between the height SDS and psychosocial functioning. The composite IQs of all 3 groups were similar (K-BIT). Composite and spelling achievement (KTEA) were similar in all groups, but the individuals in group 3 were significantly advantaged in mathematics and reading achievement over those in groups 1 and 2. As assessed by parents, the NSS subjects in group 1 had higher aggressivity, hyperactivity, conduct problems, and attention deficit scores, and lower social skill scores (BASC) than did nonreferred NSS or normal-statured students. Nonreferred ISS and normal height children (groups 2 and 3) had similar behavioral profiles. Teachers discerned no differences in adaptive or problem behaviors among the 3 groups. There were no intragroup differences in parental cohesion and adaptability (FACES II).

The investigators concluded that the results are consistent with the *hypothesis* that "discrepancies between earlier and more recent research on the psychosocial functioning of children with SS [short stature] may be explained, at least partly, by referral bias." These results also provide further evidence indicating that SS per se is not a handicapping condition.

Kranzler JH, et al. *J Pediatr* 2000;36:96-102.

**Editor's comment:** This report confirms those of other investigators that NSS is not a "handicapping condition." The reason why average height children were more adept in mathematics and reading than NSS children in this study is not apparent. Could this reflect a gene or gene-associated phenomenon linking these 2 skills?

Voss and Saenger (*J Pediatr* 2000;136:103-110) debate the usefulness of treatment of NSS with GH. Voss argues that treatment with GH is not justified on the basis of auxologic findings (short stature, slow growth rate) because "short-term

growth data . . . cannot reliably distinguish between normal and abnormal growth" and because "there is no correlation between successive annual height velocities, so that height velocity neither predicts the future nor reports the past." Voss continues that treatment does not appear justified on the basis of either psychological or learning disabilities. Voss discusses in his presentation the definition of "normality," and concludes that "differences are tolerable; deviance demands action."

Saenger attempts to argue for treatment of NSS and to defer final judgment regarding treatment until more data have been accumulated on the auxologic and psychologic efficacy of therapy, a position that Voss effectively rebuts.

In a different article (*Arch Dis Child* 2000;82:10-15), Hall discusses the utility of growth screening in schools. He points out the many methodologic problems associated with measurements of height and suggests a height less than the 0.4 percentile ( $-2.67$  SD) as the cutoff measurement below which it is reasonable to do further evaluation. He states that in a group of 400 children with height less than this percentile, as many as 30 children with isolated GH deficiency and 12 with Turner syndrome, and an additional group consisting of undiagnosed illnesses producing SS, will be identified.

Allen W. Root, MD

**2nd Editor's comment:** Readers are referred to GGH 2000;16:1-5 to read the lead article, "Ethical Issues in Growth Hormone Therapy: Where Are We Now?" This article is based on a seminar workshop held at the University of Wisconsin in October 1999.

Robert M. Blizzard, MD

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## Short Stature in Carriers of Recessive Mutation Causing Familial Isolated Growth Hormone Deficiency

The phenotype in patients with isolated GH deficiency (IGHD) type 1B is identical to patients with IGHD type 1A, which results from homozygous absence of the *GH-1* gene. Patients with type 1B have a loss of function mutation in the *GH-1* gene. Both 1A and 1B patients initially respond favorably to rhGH. However, they differ in that type 1A patients develop GH antibodies that then inhibit growth. Patients with type 1B do not develop GH antibodies and continue to respond.

These investigators report that family members who were heterozygous for the loss-of-function gene are frequently shorter than their homozygous normal relatives for the *GH-1* gene.

The authors studied an extended, interrelated Bedouin family with a G to C transversion at the 5th base in intron IV of *GH-1*, leading to loss of a splice site and utilization of a cryptic splice site in exon IV that resulted in loss of 73 bp and a nonfunctional 196 amino acid product. Among a sample of 50 first- and second-degree relatives of the 9 homozygous patients, 33 were found to be heterozygous for the *GH-1* mutation and 17 to be homozygous normals. The heterozygous subjects were significantly smaller than the normal individuals ( $-1.67$  vs  $-0.40$  SDS;  $P>0.05$ ) without relation to sex or age. In 33% of the heterozygous group, heights were  $\geq 2$  SDS below the mean (Tables 1 and 2). Stimulated secretion of GH was normal in the heterozygous subjects tested.

The authors *hypothesized* that this mutation impaired transport of the product to the secretory granules and that there was subnormal spontaneous GH secretion. They concluded that the described mutation manifested itself as short stature in heterozygous subjects and suggested that this or similar mutations in *GH-1* in the heterozygous state might account for some of the phenotypic variability in population heights and for some of the patients with normal short stature encountered in the clinic.

Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.

Table 1  
Mean Standard Deviation Scores for Height  
in Heterozygotes and Normal Homozygotes  
According to Sex

|         | Heterozygotes<br>Mean SDS ( $\pm$ SE) | Normal Homozygotes<br>Mean SDS ( $\pm$ SE) | P       |
|---------|---------------------------------------|--|---------|
| Males   | $-1.22 (\pm 0.28)$<br>$n = 13$        | $-0.10 (\pm 0.19)$<br>$n = 11$             | NS      |
| Females | $-1.97 (\pm 0.29)$<br>$n = 20$        | $-0.95 (\pm 0.31)$<br>$n = 6$              | NS      |
| Total   | $-1.67 (\pm 0.21)$<br>$n = 33$        | $-0.40 (\pm 0.19)$<br>$n = 17$             | $<0.05$ |

Reprinted with permission from Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.

**Editor's comment:** Limited clinical manifestations of rather severe autosomal recessive disorders are being recognized with increasing frequency. Some heterozygous relatives of patients with loss-of-function mutations of the GH receptor or of the GH-releasing hormone receptor may be inappropriately small, and occasional adult females who are heterozygous for loss-of-function mutations of CYP21B may manifest evidence of mild hyperandrogenism. The findings here may be of great significance in explaining some of the variation of stature that is seen in families.

Allen W. Root, MD

Table 2  
Mean Height (cm) and Mean Standard Deviation Scores (MSDS) for Height in Heterozygotes  
(H) Compare With Normal Homozygotes (N) According to Age Groups

|             | Adults         |                | Adolescents    |                | Children       |                |
|-------------|----------------|----------------|----------------|----------------|----------------|----------------|
|             | H<br>$n = 19$  | N<br>$n = 6$   | H<br>$n = 5$   | N<br>$n = 5$   | H<br>$n = 9$   | N<br>$n = 6$   |
| Height      | 158.6          | 169.3          | 143.4          | 157.4          | 105.2          | 126.5          |
| ( $\pm$ SE) | ( $\pm 0.57$ ) | ( $\pm 1.51$ ) | ( $\pm 2.41$ ) | ( $\pm 2.63$ ) | ( $\pm 2.53$ ) | ( $\pm 2.45$ ) |
| MSDS        | -1.43          | -0.21          | -2.22          | -0.90          | -1.88          | -0.18          |
| ( $\pm$ SE) | ( $\pm 0.23$ ) | ( $\pm 0.24$ ) | ( $\pm 0.67$ ) | ( $\pm 0.38$ ) | ( $\pm 0.51$ ) | ( $\pm 0.32$ ) |

Reprinted with permission from Leiberman E, et al. *Am J Med Genet* 2000;90:188-192.



## Comparison of the Growth-Promoting Effects of Insulin-Like Growth Factor 1 and Growth Hormone in the Early Years of Life

The authors report that administration of rhGH to 4 young children with isolated GH deficiency (IGHD) due to deletion of *GH-1* increased linear growth rate to a greater extent than did administration of recombinant human insulin-like growth factor 1 (rhIGF-1) to 3 children with GH insensitivity (GHI). The mean birth length (Table) in the 4 children with IGHG was 46.5 cm ( $-3.5$  SDS); in 5 GHI neonates, mean birth length was 46.8 cm ( $-3.3$  SDS). During the first 2 years of life, length of untreated IGHG infants declined to  $-5.7$  SDS, that of GHI infants from  $-3.5$  to  $-6.5$  SDS.

Treatment was initiated in all 4 IGHG patients and 3 of the 5 GHI patients between 1 and 4 years of age. With replacement rhGH treatment, the heights of IGHG children increased between 1.2 and 2.4 SDS over 3 years. In the 3 GHI children treated with rhIGF-1, height increased between 0.5 and 1.4 SDS over 3 years. The patient treated at the earliest age grew the least. By 2 years of age, head circumferences of all subjects were  $< -2.5$  SDS; during administration of rhGH or rhIGF-1, head circumferences increased. The authors conclude that the linear growth response to rhGH is greater in young children with IGHG than the linear growth response to rhIGF-1 in subjects with GHI. This implies that both GH and IGF-1 are necessary for optimal linear growth during early childhood.

Laron Z, Klingler B. *Acta Paediatr* 2000;89:38-41.

**Editor's comment:** Normal cartilage growth requires both GH and IGF-1. GH is thought to cause chondrocyte progenitor cells to differentiate and to increase local production of IGF-1; this growth factor then stimulates clonal expansion of proliferating and hypertrophic chondrocytes. Although rhIGF-1 markedly increases linear growth rates in prepubertal children with GHI, current observations suggest that both GH and IGF-1

are necessary for maximal growth in height, particularly in young children.

Allen W. Root, MD

**2nd Editor's comment:** The authors have presented data on only 7 patients receiving rhGH or, alternatively, rhIGF-1. There were 2 additional patients (GHI) who did not receive treatment. The study was worth doing, but possibly the results were overinterpreted, as the number of patients in each group was small (4 vs 3 vs 2 patients in each group), the doses of rhGH and IGF-1 were not proven to be biochemically equivalent, and patient ages at treatment were not paired for the group. The value of the article to me is the confirmation that birth weights and lengths are pathologically small in all patients reported, as were head sizes; that growth rates increase significantly in IGHG patients receiving rhGH and in GHI patients receiving rhIGF-1; and that individual variation of response, as exemplified by the observation that the poorest response to rhIGF-1 occurred in the youngest patient to receive the hormone at the largest dose, makes comparison of response between groups of such limited number difficult.

As an incidental comment, the head circumferences of IGHG and GHI patients are significantly small. While head circumference does increase with treatment, there are no data to my knowledge suggesting that the increase in head size (presumably brain size) affects intellectual capability. Also of great interest to me is that the heights of the parents, who undoubtedly are heterozygotes for IGHG or GHI, are in the negative SDS range (Table). The question of heterozygosity for certain genes affecting stature is addressed in the previous abstract.

Robert M. Blizzard, MD

Table  
Pertinent Clinical Data at Referral of 4 Patients With Congenital Isolated Growth Hormone Deficiency (IGHD) and 5 Patients With Laron Syndrome (LS)

| No.         | Sex | Diagnosis | CA (y) | BA (y) | Birth Length |                  | Parents' Height (SDS) |        |
|-------------|-----|-----------|--------|--------|--------------|------------------|-----------------------|--------|
|             |     |           |        |        | (cm)         | SDS <sup>†</sup> | Mother                | Father |
| 1           | F   | IGHD      | 3.9    | 2.5    | 46           | -3.5             | -1.45                 | -1.13  |
| 2           | F   | IGHD      | 3.2    | 0.7    | 45           | -4.0             | -1.61                 | -1.53  |
| 3*          | M   | IGHD      | 1.2    |        | 46           | -4.0             | -3.45                 | -2.66  |
| 4* Siblings | M   | IGHD      | 0.9    | 0.2    | 49           | -2.5             | -3.45                 | -2.66  |
| 5           | F   | LS        | 3.6    | 2.5    | 48           | -2.5             | -0.30                 | -0.59  |
| 6           | M   | LS        | 0.6    | 0.2    | 45           | -4.5             | -0.65                 | -1.16  |
| 7 Siblings  | M   | LS        | 3.4    | 1.5    | 45           | -4.5             | -0.65                 | -1.16  |
| 8           | F   | LS        | 2.8    | 1.0    | 49           | -2.0             | -0.70                 | -1.61  |
| 9 Siblings  | M   | LS        | 1.0    | 0.5    | 47           | -3.0             | -0.70                 | -1.61  |

\*Mother also is IGHG. <sup>†</sup>According to Tanner et al. CA, chronologic age; BA, bone (skeletal) age.



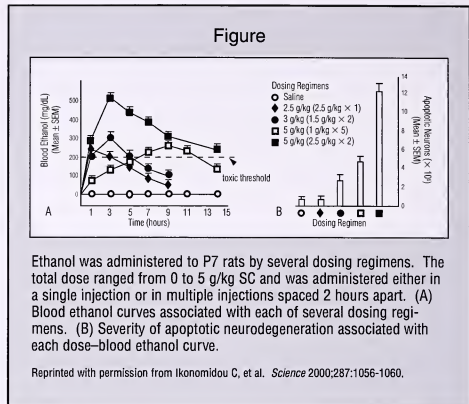
## Fetal Alcohol Syndrome and Brain Receptors

Intrauterine exposure of the human fetus to ethanol damages the developing brain, producing fetal alcohol syndrome (FAS) or fetal alcohol effects (FAEs), depending on severity. The primary manifestations are neurobehavioral disturbances, ranging from hyperactivity and learning disabilities to depression and psychosis. Patients severely affected also exhibit characteristic facies and growth deficiency. It long has been suspected that sensitivity to ethanol correlates with the time when synapses form, which is greatest during the last trimester of gestation for humans. A study headed by John Olney and colleagues provides an explanation for this correlation and identifies a probable mechanism that contributes to FAS/FAEs.

This work was done in rats, in whom the period of synaptogenesis occurs postnatally. Ethanol exposure of 1-week-old rats leads to a generalized loss of brain mass and a specific loss of cerebellar and hippocampal neurons. The authors had previously observed that transient blockade of *N*-methyl-D-aspartate (NMDA) glutamate receptors during the period of synaptogenesis causes widespread apoptosis of neurons in the infant rat brain. Since ethanol is a known NMDA antagonist, Olney and colleagues explored the possibility that apoptosis is the mechanism by which ethanol causes neuronal loss (Figure).

Examination of brains 1 day after exposure to a control injection of saline revealed a low level of apoptosis consistent with the normal process by which biologically redundant neurons are deleted during brain development. However, after ethanol exposure, apoptosis was extensive. When quantitated by neuronal density, degenerating neurons comprised 0.13% to 1.55% of the total neurons in controls compared with 5% to 30% in ethanol-exposed brains. The extent of apoptotic degeneration varied by region. Dosing experiments revealed a threshold for apoptotic changes; blood ethanol concentration had to remain above 200 mg/dL for 4 hours to induce apoptosis. Exposures beyond this threshold led to progressively more severe apoptotic degeneration. They also found a time window from near the end of gestation to 2 weeks of age during which neurons in the forebrain showed transient sensitivity to ethanol. The period of vulnerability varied slightly among different populations of neurons, but coincided with the time when synapses were being formed.

The authors also screened for other drugs that could induce apoptosis of neurons. They found that drugs that block the NMDA receptor for glutamate, which is an excitatory neurotransmitter, or those that activate receptors for the inhibitory neurotransmitter,  $\gamma$ -aminobutyric acid (GABA), trigger apoptosis of neurons during the time of synaptogenesis. The most relevant drugs in this category were benzodiazepines and barbiturates. The authors



caution that even though the window of greatest sensitivity in humans to ethanol and other drugs that block NMDA glutamate receptors or activate GABA receptors is the last trimester of pregnancy, synapses continue to form for several years after birth. They point out that prolonged use of these drugs as anticonvulsants in infants could pose a risk to the developing brain.

Ikonomidou C, et al. *Science* 2000;287:1056-1060.

Barinaga M. *Science* 2000;287:947-948. News.

**Editor's comment:** This paper provides new insight into the mechanism by which ethanol harms the developing fetus. It offers potential explanations for why binge drinking, with its sustained high levels of ethanol, as well as drinking in late pregnancy, after organogenesis is largely completed, can have such severe consequences on the developing brain. A potential danger of misinterpretation in this article is to conclude that drinking small amounts of ethanol in the early and middle stages of pregnancy is not harmful. This is unwarranted given the many aspects of the mechanism uncovered here that remain poorly understood, and the substantial differences in nervous system development between rats and humans. Knowing how ethanol disturbs neuronal development provides the first step to devising ways to prevent or minimize its harmful effects on the unborn.

William A. Horton, MD

## A Novel Subtype of Type 1 Diabetes Mellitus Characterized by a Rapid Onset and an Absence of Diabetes-Related Antibodies

Type 1 diabetes mellitus is classified as type 1A (autoimmune) or idiopathic (type 1B). The second is less frequent, and little is known concerning the entity. This article deals with type 1B, which in turn may be 2 different diseases, as elucidated by these investigators. Imagawa et al classified 56 consecutive Japanese

adults with type 1 diabetes according to the presence or absence of glutamic acid decarboxylase (GAD) antibodies as a marker of autoimmunity. Thirty-six of 56 patients had GAD antibodies, indicative of type 1A diabetes; 20 patients did not. On the basis of elevated versus low glycosylated hemoglobin values, the 20

patients with type 1B were divided into 2 groups: 11 with low values and 9 with high values (Figure). Among the 56 consecutive Japanese patients, 11 were identified with a subtype of diabetes differing from autoimmune diabetes (type 1A) in 3 respects. No autoimmune features were detected, and no diabetes-related serum antibodies such as islet cell, GAD, or insulin antibodies—as occur in type 1A—were detected. Also, neither insulinitis nor hyperexpression of MHC class I molecules was found in the islets when pancreatic biopsies were performed in 3 patients.

These patients differed from the usual patients with type 1B with respect to the low glycosylated hemoglobin values and the rate of clinical onset, which was rapid. Diabetic ketoacidosis occurred less than a week after the onset of hyperglycemic symptoms, while patients in the other 2 groups (type 1A and usual type 1B) had symptoms several weeks before ketoacidosis appeared. The normal glycosylated hemoglobin values in the 11 type 1B subgroup patients probably reflected the short duration of hyperglycemia. Insulin secretory capacity estimated on the basis of urinary C-peptide excretion was significantly lower in these than in the other patients, and the metabolic derangement at the onset was severe. These 11 patients also differed in a third way. The serum pancreatic enzyme concentrations were markedly elevated, which was in accord with lymphocytic infiltration of the *exocrine* pancreas seen in the biopsy specimens obtained. Patients in the other 2 groups had normal serum pancreatic enzyme concentrations and apparent insulinitis (as determined by the limited number of biopsies performed), but did not have the lymphocytic infiltrates in the *exocrine* pancreas found in the 3 patients in this subgroup of 11. The edema, necrosis, hemorrhage, suppuration, cyst formation, and fibrosis that characterize classic acute or chronic pancreatitis were not present.

On the basis of these findings, the investigators believe that diabetes characterized by the absence of GAD antibodies and the presence of low glycosylated hemoglobin values should be classified as nonautoimmune, fulminant type 1 diabetes, a subtype of idiopathic type 1B diabetes.

The investigators state that the precise mechanism of beta-cell destruction in this subtype of diabetes is unknown. However, they suggest a viral cause because of the abrupt onset of diabetes, the presence of lymphocytic infiltrates in the exocrine pancreas, and the affinity of several viruses for exocrine pancreatic tissue. Further studies with younger patients and other ethnic groups may provide a better understanding of this subtype of type 1B diabetes.

Imagawa A, et al. *N Engl J Med* 2000;342:301-307.

**Editor's comment:** This very interesting article clearly defined a new subgroup of type 1 diabetes mellitus in adults of Japanese origin. It remains to be determined whether this subtype is common in whites or blacks. In the article by Imagawa et al, 64% of patients with adult-onset diabetes had type 1A (36 of 56 patients), and approximately half of the remaining 20 patients had different types of what is now called type 1B diabetes. The precise mechanism of beta-cell destruction in the 2 subtypes of type 1B remains undetermined, and very likely will be different for these 2 subgroups. With respect to a viral infection in preliminary studies by Imagawa et al, no viral antibodies were found,

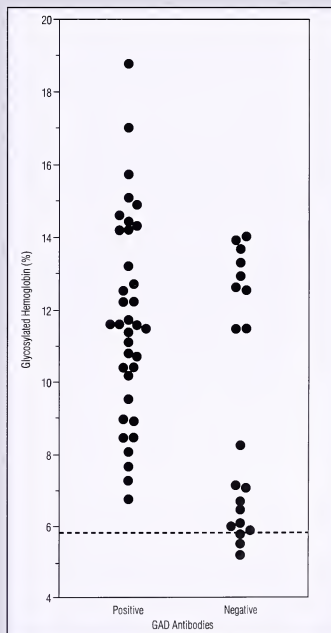
but these were preliminary studies. Fulminant type 1 diabetes is very rare in white, Anglo-Saxon individuals.

The reader interested in diabetes as a clinical entity, especially its origin and its genetics, is encouraged to read this article in its entirety. The accompanying editorial by Dr. Hake Lernmark in the New England Journal of Medicine offers additional insightful comments.

Fima Lifshitz, MD

Figure

**Glycosylated Hemoglobin Values at the Time of the Diagnosis of Diabetes in 56 Patients, According to Whether the Test for Glutamic Acid Decarboxylase (GAD) Antibodies Was Positive or Negative**



The values for glycosylated hemoglobin in the patients with positive antibody tests are scattered, whereas the values in the patients with negative antibody tests are clearly divided into 2 groups: those below 8.5% and those above 11.5%. The broken line indicates the upper limit of the normal range for glycosylated hemoglobin.

Reprinted with permission from Imagawa A, et al. *N Engl J Med* 2000;342:301-307.

## Transient Neonatal Diabetes Mellitus

Neonatal diabetes mellitus (NDM) occurs in both a transient (TNDM) and permanent (PNDM) form. Some cases of TNDM occur because of *paternal* uniparental isodisomy (UPD) of chromosome 6. Such UPD has not been demonstrated in PNDM.

Hermann et al studied 6 patients with NDM: 3 with TNDM and 3 with PNDM. Microsatellite markers and human leukocyte antigen alleles were examined using polymerase chain reaction and DNA fragment electrophoresis. Humoral markers of islet cell autoantibodies also were studied. Of the 6 patients with NDM, 1 of the 3 with TNDM and macroglossia carried UPD of chromosome 6. No maternal chromosome 6 sequences were present.

In the 3 patients with PNDM and the other 2 patients with TNDM, no evidence for UPD could be found. None of the 6 had the high-risk type 1 diabetes human leukocyte antigen alleles. Only 1 patient had islet-specific autoantibodies, but did not have glutamic acid decarboxylase antibodies, which are the antibodies most indicative of autoimmune diabetes mellitus. The conclusion by Hermann et al was that patients with transient and permanent forms of NDM have different genetic backgrounds and represent different disease entities. TNDM is often associated with UPD of chromosome 6, suggesting that an imprinted gene on chromosome 6 is responsible for this phenotype. It seems that 2 copies of the paternal allele are necessary for the development of TNDM in the cases with paternal UPD; therefore, it is likely that overexpression of a putative gene located on chromosome 6 alters pancreatic beta-cell maturation and insulin secretion.

The article by Christian et al reports 2 cases of NDM: 1 with PNDM and 1 with TNDM. The latter had macroglossia, which has been reported in some of the 7 previously published cases. These authors suggest macroglossia in the presence of NDM is an unequivocal indicator to search for UPD of chromosome 6.

Hermann R, et al. *Pediatrics* 2000;105(1):49-52.

Christian SL, et al. *J Pediatr* 1999;134(1):42-46.

**Editor's comment:** NDM is a rare disorder, with an estimated incidence of 1 in 400,000 live births. The 3 patients with PNDM had normal biparental inheritance of chromosome 6, and 1 of the 3 with TNDM had demonstrable UPD of chromosome 6. Cases of TNDM without UPD of chromosome 6 may have mutations of a parental gene on chromosome 6, or some other explanation may exist. Genes on chromosome 6 appear to be involved with the development of beta-cell differentiation and/or maturation of the pancreas. Studies for UPD of chromosome 6 should be performed in all cases of NDM.

Fima Lifshitz, MD

**2nd Editor's comment:** Numerous reports now exist distinguishing NDM from other forms of diabetes. Because of the good prognosis, it is suggested that it is worth screening for paternal UPD of chromosome 6 in all cases of NDM. I certainly am in accord with this recommendation.

The mechanism by which insulin is controlled is obviously very complex. Insulin maps to chromosome 11. In the yolk sac, only the paternal insulin gene is expressed in mice. During embryonic development, there is usually biparental expression. However, something on chromosome 6 has to do with control of insulin expression at the time of birth, since UPD can lead to lack of expression from both insulin genes (ie, both the maternal and paternal genes on chromosome 11). Between 6 months and 3 years of age, a different mechanism must control insulin expression, since children outgrow their transient neonatal lack of insulin. This is what happens in patients with TNDM. Normally, in adults there is biparental expression of insulin in the pancreas.

It is of interest that the case with UPD reported by Hermann and colleagues also had macroglossia, which of course occurs in Beckwith-Wiedemann syndrome. Interestingly, in that syndrome there is overgrowth and hyperinsulinemia associated with the macroglossia and paternal UPD for chromosome 11. In the patients with NDM, birth weights are low or low normal.

Judith G. Hall, OC, MD

## Incidence of Diabetes Mellitus and Impaired Glucose Tolerance in Children and Adolescents Receiving Growth Hormone Treatment

Cutfield and colleagues investigated 85 cases of diabetes mellitus, abnormal glucose tolerance, and hyperglycemia reported to the Pharmacia and Upjohn International Growth Study (IGS) database between 1987 and 1997. The IGS database is an international pharmacologic survey of the safety and efficacy of GH therapy in children and adolescents. The database includes more than 23,000 children. The information regarding date of diagnosis, presenting symptoms, family history, measurements of antibodies, oral glucose tolerance testing, and risk factors for diabetes was recorded. Data were categorized using the American Diabetes Association (ADA) Expert Committee recommendations for the definition of diabetes. The observed incidence of type 1 diabetes was compared with information available in 12 of the different countries from which the

GH data were extracted. The incidence of type 2 diabetes was matched by age to data from recently reported studies of type 2 diabetes in children from Cincinnati, Ohio, and Japan.

Using the ADA Expert Committee criteria, 42 of the 85 cases reported with abnormal glucose tolerance had to be excluded. Of the 43 remaining cases, 11 were diagnosed with type 1 diabetes, 18 with type 2 diabetes, and 14 with glucose intolerance. Three of the type 1 patients had ketosis, 3 had islet cell antibodies, and 3 had low secretion of C-peptide. In the 18 children who developed type 2 diabetes, 7 had at least 1 risk factor for diabetes. All had persistent diabetes after GH therapy was stopped. The incidence and age at diagnosis of children treated with GH were not different from expected values. However, the



incidence of type 2 diabetes was significantly higher in the adolescents aged 10 to 19 years: 46.3 per 100,000 years of GH treatment, versus 7.2 per 100,000 years of GH treatment in 10- to 19-year-old adolescents from Cincinnati. The children aged 6 to 14 years with type 2 diabetes had an incidence of 27 per 100,000 years of GH treatment, which was greater than that found among Japanese children in the same age range (4.6 per 100,000 years of GH treatment).

The authors express concern that the incidence of type 2 diabetes was 6-fold higher in children treated with GH compared with published controls. They point out that the treatment database used for this study lacks a prospectively acquired control population. They also point out that their data cannot be compared with that from the National Cooperative Growth Study (NCGS) of children treated with GH in the United States, since the published reports from that study did not distinguish between different types of diabetes. They warn, however, that since they used the very strict diagnostic criteria of the ADA Expert Committee, they excluded the diagnosis in almost half of their original 85 subjects.

Cuttliff W, et al. *Lancet* 2000;355:610-613.

**Editor's comment:** Clearly, this report from the KIGS database presents information that differs from that of the NCGS database (*J Clin Endocrinol Metab* 1996;81:1704-1710). The reasons for these differences may be attributed to the larger number of countries

from which the data are being collected in the current report and the use of more recent ADA Expert Committee diagnostic criteria for types 1 and 2 diabetes and impaired glucose tolerance. It is important to point out, as the authors did, that patients with some of the underlying diagnoses, such as Turner syndrome, intrauterine growth retardation, and Prader-Willi syndrome, are already at high risk of developing type 2 diabetes. The authors do not separate out these individuals in their results. Since there is a well-recognized increasing incidence of type 2 diabetes among children and adolescents, utilizing retrospective and country-specific controls may not be appropriate. Regardless, a 6-fold higher increased incidence is highly significant.

An accompanying editorial by William Jeffcoat, Nottingham, utilizes the data to caution physicians about treating adults with GH. By virtue of age and other risk factors, these adults may already be at significant risk for type 2 diabetes.

These data will have worldwide significance. It is important that pediatric endocrinologists become familiar with these results and their implications. This will lead to better and longer observation of children treated with GH. These data cannot be dismissed and, clearly, the tacit implication is that children undergoing therapy with rhGH should be screened periodically for glucose intolerance both during therapy and after therapy if therapy is stopped when patients reach adulthood.

William L. Clarke, MD

## Birth Weight and the Insulin Resistance Syndrome: Association of Low Birth Weight With Truncal Obesity and Raised Plasminogen Activator Inhibitor-1 but Not With Abdominal Obesity or Plasma Lipid Disturbances

The insulin resistance syndrome was defined as the combination of hypertension, insulin resistance, and dyslipidemia. Clusters of physiologic factors, including hypertension, impaired glucose tolerance, insulin resistance, lipid disturbances, and impaired fibrinolytic activity, were studied intermittently from birth to 70 years of age in males. These factors are related to birth weight among a large cohort of adult men studied in the Uppsala, Sweden Longitudinal Study of Adult Men. This study included all men born between 1920 and 1924 and still living in Uppsala. These men were studied between 1970 and 1973, at an age of approximately 50 years, and again in 1991, at an age of approximately 70 years. The investigators were able to trace birth records of more than 1,300 of the participants in the original study ( $n=24,841$ ), and selected cutoffs for birth weight for their studies ( $<3.25$  to  $3.75$ ,  $3.75$  to  $4.25$ ,  $>4.25$  kg). The phenomena investigated at age 50 ( $n=1,268$ ) included height, weight, skinfold measurements, blood pressure, intravenous glucose tolerance tests, and blood lipids. At age 70 ( $n=734$ ), in addition to an oral glucose tolerance test, a euglycemic hyperinsulinemic clamp study was performed and plasminogen activator inhibitor-1 (PAI-1) activity was assessed. The latter is a marker of impaired fibrinolytic activity. Information on smoking and socioeconomic factors was recorded.

Of the men studied at age 50, type 2 diabetes occurred in 2%, and 26% were hypertensive. At age 70, 14% had type 2 diabetes, and 67% were hypertensive. At age 50, triceps skinfold

thickness was positively associated with birth weight. Inverse relationships between birth weight, fasting insulin, and insulin resistance previously have been published, as have positive associations with bone mineral density at age 50 and insulin sensitivity at age 70. When adjusted for body mass index, birth weight was inversely related to waist-hip ratio, PAI-1 activity, and insulin and proinsulin concentrations. Serum triglyceride concentrations and high-density lipoprotein cholesterol levels were not significantly associated with birth weight. Socioeconomic factors and smoking history did not change the relationship of birth weight with the insulin resistance factor.

The authors point out that they have demonstrated strong associations between reduced size at birth, adult hypertension, insulin resistance, glucose intolerance, cardiovascular mortality, and high PAI-1 activity. A strong relationship has been demonstrated between birth weight and insulin resistance syndrome in British men, aged 64 years, and US men at age 31.5 years. The participants in those studies were more obese than those in the current study, and the authors state that this could mediate the stronger effect of birth weight on the insulin resistance syndrome in those groups. Finally, the authors question why low birth weight predicts only some components of the insulin resistance syndrome and not others, and what the physiologic pathways linking these disturbances might be.

Byberg L, et al. *Diabetologia* 2000;43:54-60.



**Editor's comment:** Pediatricians, generalists, and internists need to be aware of the deleterious effects of low birth weight on the subsequent morbidity and mortality of adults. These carefully collected and analyzed epidemiologic data of adult men are exceedingly important. Although socioeconomic factors did not influence the incidence of the insulin resistance syndrome, it remains unclear whether personal psychological factors such as the desire to feed and/or overfeed a small baby and/or the need to restrict caloric intake in the large infant might have a bearing on subsequent outcomes.

The findings that smoking and socioeconomic status did not

influence the results are of particular importance. As the insulin resistance syndrome and type 2 diabetes, in particular, have become more prevalent both in children and adults, it is important that significant effort be placed into determining factors that contribute to the onset and persistence of these adversities. A better understanding of the relationships between the variables presented in this article is needed, and perhaps the nutritional principles taught to pediatric residents need to be carefully reviewed if a significant impact is going to be made in the reduction of the near-epidemic disorder of insulin resistance in children.

William L. Clarke, MD

## Mosaicism Is a Likely Explanation for the Variability Observed in Androgen Insensitivity Syndrome

Holterhus et al report on 5 patients with somatic mosaicism for abnormalities of the androgen receptor. In all 5 patients, there was a lack of family history; and in all 5 clitoromegaly or micropenis with scrotalization of the labia was present. Each of the 5 patients had a different mutation that had arisen during postzygotic development. It appears that somatic de novo mutations of the androgen receptor occur at a particularly high rate. Thus, somatic mosaicism should be considered when there is more masculinization than expected from a particular mutation.

Holterhus P-M, et al. *Pediatr Res* 1999;46:684-690.

It is desirable to study both blood leukocytes and tissue fibroblasts to determine whether an individual is mosaic. Individuals with androgen insensitivity mosaicism may need to undergo early gonadectomy in order to avoid further masculinization. Variable expression of wild-type gene products, based on somatic mosaicism, is probably the mechanism for much of the variability that is seen in androgen insensitivity syndrome.

**Editor's comment:** The more we study, the more we learn. Somatic mosaicism appears to be quite common in a number of disorders, but seems to be variable depending on the particular gene. It is important to be aware that the androgen receptor gene seems to be particularly mutable during the course of development and, thus, we can explain the variability seen as related to a specific mutation. Geneticists like to think that there can be genotype-phenotype correlations that are straightforward, but somatic mosaicism leads to confounding situations. Keep an eye out for unexpected variations and consider somatic mosaicism as a possible explanation.

Judith G. Hall, OC, MD

## Perceptions of the Outcome of Orthopedic Surgery in Patients With Chondrodysplasias

Hunter has taken the time to carefully interview 197 individuals with disproportionate short stature. Seventy-four of the 197 had undergone a total of 221 surgical procedures. In general, individuals felt they had improved outcomes. However, the attitude very much depended on the particular disorder. The percentage of individuals undergoing surgery ranged from 8.3% for hypochondroplasia to 87.5% for diastrophic dysplasia. The worst outcomes were for foramen magnum-cervical surgery and the best for thoracolumbar procedures to release nerve compression.

Most of the leg lengthening experience in disproportionate short stature is related to hypochondroplasia and achondroplasia (ACH). The procedure can add an extra 4 to 6 inches, which can make an enormous difference in the daily life of individuals whose height is approximately 4 ft. It is terribly important that data continue to be accumulated and combined since each type of disproportionate short stature is relatively rare. Collaborative studies on an international basis are really needed.

Judith G. Hall, OC, MD

Gross points out in his editorial that leg lengthening has been revolutionized by the Ilizarov technique. The complication rate has dropped dramatically as experience has increased, decreasing to only 7% for patients with leg lengthening related to leg length discrepancy or short stature.

**2nd Editor's comment:** Physicians dealing with short stature need to be aware of these 2 articles and the lead article by Dr. Deborah F. Stanitski, "Limb Lengthening in the Skeletal Dysplasias and Short Stature Conditions: State of the Art in 1997," that appeared in *GGH* (1997;13[2]:17-22). You are invited to read again the excellent presentation by Dr. Stanitski.

Hunter AGW. *Clin Genet* 1999;56:434-440.  
Gross R. *Lancet* 1999;354:1574-1575. Editorial.

**Editor's comment:** Quality-of-life issues have become very important in healthcare outcomes analysis. Clearly, many patients reported by Hunter indicated that they experienced major improvements from orthopedic procedures. However, the perceptions related to specific disorders. The outcomes and natural history also must be related to specific disorders.

The article by Hunter is a general article, reporting levels of patient satisfaction for procedures performed for 2 different types of chondrodysplasias. The information will be of use to you in helping patients who are contemplating surgery, whether of the spine or extremities. However, it will not tell you the information you need to advise the patient.

The commentary by Gross in *Lancet* may be more helpful to

pediatric endocrinologists and geneticists than the article by Hunter, as it deals with limb lengthening. He presents the historical aspects and then presents a concise summary of Ilizarov's contributions in the 1980s. The Verona surgeons have now described their results in 230 tibial lengthenings by monolateral fixation performed between 1990 and 1995; 58 were in ACH patients. Using these data, he points out that 40 days in a fixator was required for 1 cm of lengthening, or 200 days for 5 cm. The complication rate is now much less than previously, and Aldegheri reports (J Bone Joint Surg [Am] 1999;81:624-634) that only 7% of patients had complications undergoing lengthening with his latest modification in technique. Gross points out that physical and mental scores for adults with ACH do not differ from the general population until

about age 40, when back pain, weakness, and arthritis become disabling. Whether the effect of leg lengthening will speed up or delay this process in ACH, or adversely affect hip anatomy and function, remains unknown. Gross also comments:

If tibial lengthening is successful in a patient with ACH, treatment remains incomplete until the femur and humerus have also been successfully lengthened. The financial and physical costs are substantial and there simply is no follow-up information to justify routine lengthening of several long bones. Thus, despite the gratifying improvements, the results of these procedures still need long-term evaluation and review.

Robert M. Blizzard, MD

## Russell-Silver Syndrome Begins to Be Unraveled

Russell-Silver syndrome is a very common diagnosis associated with intrauterine growth retardation.<sup>1</sup> However, it has become clear that it is a heterogeneous disorder. Approximately 10% of cases have been associated with maternal uniparental disomy for chromosome 7. This observation suggests there are genes on chromosome 7 that are imprinted. *MEST* (also known as *PEG1*) is an imprinted gene expressed only from the paternal allele, which maps to human chromosome 7q.<sup>2</sup> Thus, patients who have maternal uniparental disomy lack paternal activation of the gene.

Lefebvre and coworkers disrupted the murine homologue, *Mest*, by gene targeting in embryonic stem (ES) cells.<sup>2</sup> The targeted gene was imprinted and reversibly silenced by passage through the female germ line. Paternal transmission activated the allele and caused embryonic growth retardation. Interestingly, *Mest*-deficient females showed abnormal maternal behavior, including impaired placentophagia. Thus, in mice, both growth and behavior are affected.

Interestingly, imprinting of *PEG1/MEST* is lost in lymphocytes and transformed lymphoblastoid cell lines. This is not entirely surprising since genomic imprinting is usually regulated in a tissue-specific way. In addition, imprinting may be controlled in a promoter-specific way such that promoters allow expression from a particular parental allele.<sup>3</sup> Imprinting can be governed in an isoform-specific way such that a single transcription unit will encode for different proteins via alternative splicing. Kosaki et al<sup>4</sup> demonstrate that there are different isoforms in lymphoblastoid tissue where isoform 1 is expressed only from the paternal allele while there is biallelic expression of isoform 2. Interestingly, there may be differences in mouse and human expression of isoforms, again in a tissue-specific way.

In addition to the paternally expressed *PEG1/MEST* gene in the 7q32 region, there also is a *g2-COP* gene<sup>5</sup> with biallelic expression in fetal brain and liver and in adult peripheral blood, and monoallelically paternal expression in other fetal tissues, including the skeleton, muscle, skin, kidney, adrenal glands, placenta, intestine, lung, chorionic plate, and amnion. Absence of paternal *g2-COP* transmission during embryonic development may contribute to the Russell-Silver phenotype. It may well be that other imprinted genes are present in this chromosome region. However, the expression is clearly under tight

control in terms of tissue specificity and time of expression in development.

Duplication of 7p11.2-p13 also has been described as resulting in the Russell-Silver phenotype. The report by Monk et al<sup>1</sup> describes a chromosomal duplication within the region where gene *GRB10* (growth factor receptor-binding protein 10) has been identified. This suggests that Russell-Silver syndrome could result from overexpression of a maternally expressed imprinted gene as well as absence of a paternally expressed gene.

1. Monk D, et al. *Am J Hum Genet* 2000;66:36-46.
2. Lefebvre L, et al. *Mest*. *Nat Genet* 1998;20:163-169.
3. Lefebvre L, et al. *Peg1*. *Hum Mol Genet* 1997;6:1907-1915.
4. Kosaki K, et al. *Am J Hum Genet* 2000;66:309-312.
5. Blagitko N, et al. *Hum Mol Genet* 1999;8:2387-2396.

**Editor's comment:** Imprinted genes seem to lie in regions where there are both maternally and paternally imprinted genes. As the Human Genome Project proceeds, we should be able to identify all genes in a given region. It does seem that many regions are very complex and that each may be under different types of control. Clearly, chromosome 7 has something very important to do with growth and behavior since either deficiency of paternal expression or the duplication of maternal genetic material can lead to important changes in growth and behavior. Other forms of growth retardation very possibly are attributable to the imprinting phenomenon.

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**GROWTH, Genetics, & Hormones Volume 16, Number 2**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit. Please note that each question may have more than one correct answer.

1. rhGH at a dose of 0.1 mg/kg/d in healthy adult volunteers \_\_\_\_\_ the protein catabolic side effects of prednisone.
  - a. did not affect
  - b. enhanced
  - c. diminished
  - d. prevented
2. Recent studies suggest that rhGH can produce positive effects on growth in growth-retarded children receiving glucocorticoids in which of the following:
  - a. rheumatoid arthritis
  - b. prepubertal children with renal allografts
  - c. prepubertal adolescents with renal allografts
3. Independent factors that predicted the response to rhGH therapy in patients with chronic renal failure receiving glucocorticoids are:
  - a. the degree of growth velocity prior to rhGH treatment (negative relationship)
  - b. the degree of growth velocity prior to rhGH treatment (positive relationship)
  - c. low glomerular filtration rate (positive relationship)
  - d. the degree of insulin resistance prior to rhGH treatment (negative relationship)
  - e. other

4. The authors state that \_\_\_\_\_ is known about the gene activity that eventually leads to growth retardation with glucocorticoid treatment.
  - a. much
  - b. a moderate amount
  - c. little
5. Glucocorticoids \_\_\_\_\_ sulfation of cartilage matrix in the growth plate.
  - a. paradoxically stimulate
  - b. inhibit
  - c. have no effect on

**Answer Key:** 1. d 2. a, b, c 3. a, d, e 4. c 5. b

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# GROWTH

## Genetics & Hormones

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## Maturity-Onset Diabetes of the Young (MODY): The Past, Present, and Future

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### INTRODUCTION

The entity maturity-onset diabetes of the young (MODY) was first recognized 4 decades ago among young diabetic patients who had a distinct clinical phenotype and inheritance pattern of diabetes mellitus (DM).<sup>1</sup> Patients with this entity characteristically differed from the usual young patient with type 1 DM in having a slow rather than an abrupt onset of DM, by not requiring insulin, by not having the severe symptoms of type 1 DM, and by having an autosomal dominant inheritance pattern. These observations led to the development of criteria to establish the diagnosis of MODY and distinguish it from type 1 DM. The strict application of these criteria (Table 1) resulted in the characterization of large multigenerational pedigrees that has greatly facilitated genetic studies during the last 5 years. Dramatic progress as a result of positional cloning efforts and candidate gene approaches led to the identification of several of the defects that underlie this early-onset form of type 2 DM. At least 5 forms of MODY have been elucidated to date. Reexamination of populations with common late-onset type 2 DM indicates that mutations in MODY genes may be associated with type 2 DM in general.

Since MODY is almost universally associated with an insulin secretory defect, these observations challenge the widely held belief that insulin resistance determines the expression of the diabetic phenotype. Rather, most of the patients with MODY and a significant portion of those with adult-onset type 2 DM have a genetically programmed impairment in the capacity

Table 1  
MODY Diagnostic Criteria

- Onset <25 years
- Autosomal dominant inheritance (3 generations)
- Not insulin requiring for  $\geq 5$  years after initial presentation

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of the pancreatic  $\beta$  cells to accommodate peripheral tissue insulin requirements.

The purpose of this review is to present MODY as a paradigm for type 2 DM, discuss the role of genetic screening in diagnosing and enhancing treatment of MODY, relate the identification and function of the 5 currently identified MODY genes, and discuss what we may learn about MODY from identification of other MODY genes and their mutations.

### In This Issue

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## MODY As a Paradigm for Type 2 DM

The advances in our understanding of MODY are relevant to our understanding of the genetics and pathophysiology of type 2 DM in general. This is exemplified by the identification of mutations in MODY genes in subjects with late-onset type 2 DM.<sup>2-4</sup> Furthermore, there is often variation within MODY pedigrees with regard to the age of onset such that multiple affected members in some MODY pedigrees may have onset of disease after age 40. While the later age at diagnosis sometimes reflects a delay in ascertainment because of a mild phenotype, it also suggests that other factors, both genetic and environmental, may modify the expression of diabetes due to specific mutations in MODY gene loci. This leads to the consideration that mild mutations or polymorphisms in MODY genes may result in only a slight impairment of protein function and, therefore, may contribute to the expression of diabetes in a polygenic context. A common polymorphism at codon 98 of the *HNF-1 $\alpha$*  gene (*MODY3*), which is not linked to DM in a Mendelian fashion, is nevertheless associated with reduced serum C-peptide and a reduced insulin response to glucose challenge. The prevalent yet incompletely penetrant D76N mutation in *IPF-1* (*MODY4*) is associated with marked impairment in insulin secretion even in normal glucose-tolerant carriers of the mutation. Digenic inheritance in a family with late-onset type 2 DM has been documented in which the severity of the diabetic phenotype appears to relate to the cosegregation of 2 distinct mutations in 2 different pancreatic transcription factor genes, *IPF-1* (*MODY4*) and *IBT*, a transcriptional regulator of *GLUT2* gene expression. Mutations that impair  $\beta$ -cell compensatory mechanisms also could act in concert with genetic defects in insulin action to cause diabetes.

## POSSIBLE ROLE OF GENETIC SCREENING

Genetic screening for specific mutations in diabetes genes may offer several therapeutic advantages.<sup>5</sup> Determination of the genetic mutations in cases of MODY may assist in the determination of prognosis (Table 2), choice of optimal therapy, and early implementation of the appropriate lifestyle to reduce complications. Although all forms of MODY identified so far are characterized by an insulin secretory defect, the precise nature of the defect and the clinical course vary according to the genetic defect(s). For example, *MODY2* is characterized by mild fasting hyperglycemia that is often already evident in early childhood. However, less than half of the patients will progress to overt diabetes, few will develop complications, and most will not require medical intervention, except during pregnancy. These characteristics allow a clinical approach of limited monitoring. As another example, the clinical phenotypes of *MODY1* and *MODY3* are both characterized by progressive deterioration of glucose

Table 2  
Clinical Phenotype of Maturity-Onset Diabetes of the Young (MODY) Subtypes

| Type of MODY | Diabetes | Complications                                  | Therapy                           | Other                        |
|--------------|----------|--|-----------------------------------|------------------------------|
| 1            | Severe   | Similar to late-onset type 2 diabetes mellitus | Oral hypoglycemic agents, insulin | —                            |
| 2            | Mild     | Rare   | Diet, oral hypoglycemic agents    | Low birth weight             |
| 3            | Severe   | Similar to late-onset type 2 diabetes mellitus | Oral hypoglycemic agents, insulin | Low renal glucose threshold? |
| 4            | Moderate | Not determined                                 | Diet, oral hypoglycemic agents    | —                            |
| 5            | Severe   | Nephropathy                                    | Insulin                           | Renal dysfunction and cysts  |

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homeostasis, with some subjects remaining well controlled on diet or sulfonylureas and others progressing to insulin therapy. The incidence of complications for *MODY1* and *MODY3* resemble those of late-onset type 2 DM. *MODY5* appears to be particularly associated with renal complications and cysts. Genetic screening can be an important predictor of both quality and quantity of life (Table 2) and the need for more rigid therapy than in *MODY2* patients. Therefore, childhood diabetes is a specific instance in which genetic screening can be helpful. Several studies have now attributed previously diagnosed type 1 DM (usually autoimmune) to mutations in *HNF-1 $\alpha$*  (*MODY3*).<sup>6,7</sup> These subjects were not characterized by the expression of autoimmune markers but were given the diagnosis of type 1 DM because of the early age of onset. This is significant, as the future clinical course is distinctly different for type 1 DM and type 2 MODY. Screening and diagnosis of MODY provides a more measured approach in terms of clinical therapy, especially involving insulin. Routine screening in the clinical setting, however, will require ongoing and future technological advances in mutation detection before it can become practical and economically feasible.

## FUNCTION AND IDENTIFICATION OF MODY GENES

A brief review of glucose metabolism, glycolysis, and insulin secretion provides a foundation for comprehending the function and identification of MODY genes.<sup>2,3</sup> The islet  $\beta$  cell is uniquely equipped to sense blood glucose concentrations and to secrete insulin in a precise

fashion to maintain glucose in a narrow physiologic range.<sup>8</sup> In the pancreatic  $\beta$  cells and in hepatocytes, uptake of glucose is mediated by the high  $K_m$  glucose transporter GLUT2. Glucose metabolism must be initiated to stimulate insulin secretion. Glycolysis is the first step in glucose metabolism and occurs in both the pancreas and liver. Glucokinase, the low  $K_m$  rate-limiting hexokinase that phosphorylates glucose to glucose-6-phosphate, is the catalyst. Glycolysis in the  $\beta$  cell results in the generation of adenosine triphosphate (ATP), which causes the closure of ATP-sensitive potassium channels. This depolarizes the  $\beta$  cell, causing calcium channels to open. Calcium then flows into the cells, triggering secretion of insulin.

The first MODY locus to be identified (*MODY2*) was found to encode glucokinase, the key regulatory enzyme in glucose metabolism (Table 3).<sup>9,10</sup> Interestingly, mutations in the glucokinase (*MODY2*) gene not only produce DM but also can produce reduced birth weight in the fetus.<sup>11</sup> Most mutations in the glucokinase gene decrease insulin secretion and appear to cause diabetes through a gene-dosage effect. Interestingly, there is at least 1 activating mutation that causes hyperinsulinemia and hypoglycemia,<sup>12</sup> further establishing the critical function of glucokinase in sensing blood glucose levels and in maintaining a normal glucose-induced secretory insulin response. This may reflect decreased fetal insulin, which functions as a growth factor in utero. Glucokinase mutations in the fetus may impair fetal insulin secretion in response to normal maternal glucose levels. If, however, the mother also is heterozygous for the glucokinase mutation, the higher maternal glucose levels will provoke greater fetal insulin secretion, leading to *MODY2* infants with normal birth weights. Other characteristic features of *MODY2* include the mildness of the disease compared with *MODY1*, *MODY3*, and *MODY5*; the multiple variants of mutations found (~50); and the rarity of complications.

All of the MODY genes<sup>1,3,4</sup> except that for *MODY2* encode transcription factors localized to the pancreas and other tissues such as liver and kidney (Table 3). Two approaches, positional cloning and screening of candidate genes, resulted in the identification of specific transcription factor mutations. Positional cloning using the previously characterized large MODY pedigrees led to the identification of *MODY1* and *MODY3* as the genes encoding hepatocyte nuclear factors *HNF-4 $\alpha$*  and *HNF-1 $\alpha$* , respectively. The HNFs were originally discovered as a heterogeneous family of transcription factors that control liver-specific gene expression. Subsequently, HNFs were identified in other tissues, including pancreatic islets.<sup>13</sup> The HNFs form a network of cross-regulatory transcription factors that regulate expression of genes involved in a wide range of cellular processes in

metabolism, but often are equally important in differentiation and development.

The most extensive and well-characterized MODY pedigree is the RW pedigree, which has been followed prospectively since 1958; it now consists of 455 members in 7 generations and includes 74 diabetics.<sup>14</sup> Diabetes in the RW pedigree was linked to a DNA polymorphism on chromosome 20 in 1991.<sup>15</sup> Once *HNF-1 $\alpha$*  was determined to be the *MODY3* gene, a scan of known genes in the *MODY1* interval raised *HNF-4 $\alpha$*  as an intriguing candidate, since *HNF-4 $\alpha$*  is a known upstream regulator of *HNF-1 $\alpha$* . This hypothesis was confirmed with the identification of the Q268X nonsense mutation in the RW pedigree.<sup>2</sup> The relationship between *HNF-1 $\alpha$*  and *HNF-4 $\alpha$*  in diabetes is further underscored by the identification of a MODY mutation in the *HNF-4 $\alpha$*  promoter in an Italian MODY pedigree.<sup>16</sup>

*HNF-4 $\alpha$*  encodes an orphan member of the nuclear hormone receptor superfamily that regulates gene expression required for glucose transport and metabolism. The Q268X mutation results in the synthesis of a truncated protein that does not activate gene transcription.<sup>17</sup> Mutations in *HNF-4 $\alpha$*  remain a relatively rare cause of diabetes as only 6 mutations have been reported. Interestingly, 1 of these mutations (V3931) was identified in a family with late-onset type 2 DM, demonstrating the overlap that different mutations provide for MODY and the adult phenotype of type 2 DM.

Table 3  
MODY Genes

| MODY | Chromo - some | Mutated Gene  | Gene Product  | Distribution                     | Regulatory Function   |
|------|---------------|---|---|----------------------------------|---|
| 1    | 20q           | Hepatocyte nuclear factor ( <i>HNF</i> )-4 $\alpha$ | Transcription factor, nuclear hormone receptor family | Islets, liver, kidney, intestine | Glucose transport and metabolism genes; <i>HNF-1<math>\alpha</math></i> gene expression |
| 2    | 7p            | Glucokinase ( <i>GK</i> )                           | Enzyme  | Islets, liver                    | Glucose phosphorylation   |
| 3    | 12q           | Hepatocyte nuclear factor ( <i>HNF</i> )-1 $\alpha$ | Transcription factor, POU homeodomain                 | Islets, liver, kidney            | Insulin, glucose transport and metabolism genes   |
| 4    | 13q           | Insulin promoter factor-1 ( <i>IPF</i> -1)          | Transcription factor, Antp homeodomain                | Islets, duodenum, stomach        | Pancreas development, $\beta$ -cell gene expression                                     |
| 5    | 17-cenq       | Hepatocyte nuclear factor ( <i>HNF</i> )-1 $\beta$  | Transcription factor, POU homeodomain                 | Islets, liver, kidney            | Dimerization partner for <i>HNF-1<math>\alpha</math></i>                                |

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The identity of the *MODY3* locus also was determined by a positional cloning approach. In 1996, a cytosine insertion was identified in codon 291 of *HNF-1 $\alpha$*  (P291fsdelC). This segregated with DM in a *MODY3* pedigree. This particular mutant of the *HNF-1 $\alpha$*  gene is a frameshifted truncated protein that appears to function in a dominant negative manner.<sup>18</sup> Mutations (57 to date) in *MODY3* are highly prevalent, accounting for 64% of *MODY* in the United Kingdom, 30% of early-onset type 2 DM in Germany, and 15% to 20% of *MODY* in Japan. The P291fsdelC mutation has appeared in at least 9 distinct haplotypes in Germany, Britain, the United States, Sweden, and Japan, implying the existence of a mutational hot spot. *HNF-1 $\alpha$*  appears to regulate transcription of the insulin gene, the *GLUT2* gene, and other genes encoding components of the  $\beta$ -cell glycolytic pathway.

The most recently described *MODY* gene (*MODY5*) encodes HNF-1 $\beta$ , another member of the transcriptional regulatory network that includes HNF-1 $\alpha$  and HNF-4 $\alpha$  (Table 3). The *HNF-1 $\beta$*  gene was screened because it was known that HNF-1 $\beta$  can function as a heterodimerization partner with HNF-1 $\alpha$ . A nonsense mutation in *HNF-1 $\beta$* , R177X, was identified in a small Japanese pedigree with early onset of DM at age 10 and 15, but one member developed DM later, at age 40.<sup>12</sup> All had evidence of diabetic neuropathy. Subsequent screens have identified 2 additional mutations in families with early-onset DM and *MODY* associated with renal failure and renal cysts, and another mutation in late-onset type 2 DM not associated with kidney disease.

The identity of the *MODY4* locus reflects the close relationship between *pancreatic development* and glucose homeostasis. Experimental gene inactivation in mice is uncovering a growing number of transcription factor genes whose normal expression is required for full development of the exocrine and endocrine pancreas (Table 4). These genes are being evaluated as candidate diabetes genes. The first positive example of this approach came from mice with homozygous disruption of the *IPF-1* gene and resultant total pancreatic agenesis. *IPF-1*, a homeodomain transcription factor, is implicated in the transcriptional regulation of key  $\beta$ -cell genes. In humans, the first *MODY4* family was discovered when a rare subject with pancreatic agenesis was found to be homozygous for an inactivating cytosine deletion mutation in the protein coding sequence of *IPF-1* (Pro63fsdelC).<sup>19</sup> Subsequently, the heterozygous mutant allele within both branches of the extended family of the proband was linked to *MODY*.<sup>8</sup> Three members of this pedigree satisfy the strictest criteria for the diagnosis of *MODY*, and 2 additional heterozygous subjects developed diabetes or glucose intolerance by 30 years of age, thus establishing *IPF-1* as the *MODY4* gene. To

Table 4  
Transcription Factor Gene Knockouts  
and Pancreas Development

| Factor       | Knockout Mouse Phenotype                                     |
|--------------|--|
| IPF-1        | Pancreatic agenesis  |
| Pax-4        | Decreased $\beta$ and $\delta$ cell numbers                  |
| Pax-6        | Decreased $\alpha$ cell numbers                              |
| Isl-1        | Dorsal pancreatic agenesis                                   |
| Beta2/NeuroD | Decreased $\beta$ -cell numbers                              |
| Nkx2.2       | Impaired $\beta$ -cell differentiation, no insulin synthesis |
| Nkx6.1       | Decreased $\beta$ -cell numbers                              |
| p48          | Pancreatic agenesis; ectopic islet cells in the spleen       |

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date, at least 7 additional heterozygous *IPF-1* mutations have been discovered in approximately 5% to 6% of familial late-onset type 2 DM patients in France and the United Kingdom and in a small number of additional *MODY* pedigrees.

## THE FUTURE

While the recent advances in *MODY* genetics have been most exciting, there remain additional *MODY* genes to be uncovered. In 2 *MODY* populations in France and England, in which screening for possible mutations in all 5 *MODY* genes has been undertaken, the genetic defect in 16% to 20% of *MODY* pedigrees remains a mystery. An ever-increasing number of genes whose normal function is required for full development of the pancreas are being identified through analysis of the phenotypes of knockout mice (Table 4).<sup>20</sup> Some of these genes probably will turn out to play a role in human type 2 DM. In support of this concept, several mutations in the human *BETA2* gene were recently reported in familial late-onset type 2 DM.<sup>21</sup> Other members of the transcriptional regulatory network of hepatocyte nuclear factors also are logical candidate diabetes genes to consider.

Most *MODY* subjects exhibit decreased insulin secretion and lean body mass. However, there are other *MODY* pedigrees (in which mutations in *MODY1*,



*MODY2*, and *MODY3* have been ruled out) that include diabetics with high circulating insulin levels and also a higher incidence of obesity than their unaffected relatives.<sup>22</sup> This form of *MODY* may be caused by mutations in a distinct class of genes whose function is not specifically involved in the regulation of  $\beta$ -cell development and function.

## CONCLUSION

*MODY* is an autosomal dominant monogenic form of type 2 DM that is characterized by a primary defect in insulin secretion. Four of the 5 *MODY* genes discovered to date encode transcription factors that regulate  $\beta$ -cell development and function. This observation has transformed our concept of diabetes into a disorder of the  $\beta$  cell and has intensified research efforts aimed at improving the function and mass of insulin-producing  $\beta$  cells. Additional *MODY* genes remain to be uncovered. The identification of mutations in *MODY* genes in common late-onset type 2 DM indicates that *MODY* is a useful paradigm for type 2 DM and that a genetically programmed impairment of the  $\beta$  cell may underlie

a greater proportion of type 2 DM than previously suspected.

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# Genetic Biotechnology and Patent Rights

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## INTRODUCTION

As the Human Genome Project (HGP) nears completion, scientists have engaged in a spirited discussion about ownership of genetic information and to what extent it should be patentable. Billions of dollars of private investment have contributed to cloning DNA sequences and protecting biotechnology resulting from this research, such as genetic tests. Many private companies have attempted to broadly protect intellectual property rights by patenting total or partial gene sequences and the products these sequences encode. Many of the patents obtained on gene sequences and related biotechnological inventions have been and remain controversial.

Just as the HGP promises to transform the future practice of medicine, the associated legal developments signal a transformation in medical economics. In this article the historic perspective of this topic is succinctly presented, and the legal requirements for patentability are discussed. This article also reviews recent controversies about patenting genomic sequences, partial gene sequences, and genetic tests, and the patent rights of a

patient when biological inventions are made from his own tissues. The results of several recent patent infringement lawsuits also are summarized. In the conclusion section, public policy issues about patenting genetic technologies are discussed.

## HISTORICAL PERSPECTIVE

The controversy about patents dealing with genetic inventions is only the latest chapter in a long and uncomfortable history of patents in medicine. At the *beginning of the 20th century*, the US Patent Office was reluctant to grant patents on medical inventions because medicine was considered too unscientific for its inventions to deserve the imprimatur of a patent. Conversely, many physicians considered patents unethical because medicine was an altruistic calling that was inconsistent with anything as commercial as a patent.

These attitudes changed in the *mid-20th century* when medicine developed a more scientific basis. During the golden age of pharmacology in the 1940s and 1950s corporations began to spend millions of dollars developing new drugs. Patent protection was needed to prevent competitors from taking unfair advantage of the expensive experimental work of research-based corporations. The attitude of practitioners in organizations such as the American Medical Association (AMA) also evolved, and



### Letter From the Editor

This lead article is different than most in *GROWTH, Genetics, & Hormones*. The relationship of patenting medical devices, drugs, diagnostic procedures, and genes to the practice and economics of medicine is knowledgeably presented by Dr. Noonan, Doctor of Jurisprudence (1980) and Doctor of Medicine, magna cum laude (1994), Resident in Medicine and Ophthalmology, Professor of Law and Medicine, recipient of many outstanding awards as physician and as lawyer, former member of the National Board of Medical Examiners, and a recurrent witness in Washington, DC, and in Portland, Oregon, where he lives and is a patent lawyer.

Robert M. Blizzard, MD

by mid-century the AMA changed its canon of ethics to allow patenting of most medical inventions. Inventors of medical devices and pharmaceuticals began to take advantage of the rewards offered by patent protection, which allowed them to prohibit others from using a patented invention without paying a royalty to the patent owner.

A revolution in patent law occurred in the 1970s, when Boyer and Cohen obtained their basic patents on recombinant DNA techniques. In 1980, the US Supreme Court dramatically expanded the scope of patent law by permitting the patenting of living, genetically modified organisms. The importance of patents in the life sciences also was increased by the Bayh-Dole Act of 1980, which granted patent rights to universities. Universities could hold title to patents developed with federal research grant money. Much of this research was related to medical and biological technologies, and many early biotechnology companies such as Genentech, Inc. were founded using university technology that had been patented.

Following these changes in the law, the US Patent and Trademark Office (PTO) was inundated with hundreds of thousands of patent applications on biological inventions during the 1980s. The PTO began to issue patents on a broad spectrum of such inventions, including (1) recombinant DNA and proteins, (2) transgenic animals, (3) research tools such as the polymerase chain reaction

(PCR), (4) new cell lines, (5) enzymes and probes, (7) gene sequences, and (8) mutations associated with disease, such as polymorphisms seen in cystic fibrosis or muscular dystrophy.

### REQUIREMENTS FOR PATENTABLE INVENTIONS

Genetic patents puzzle some scientists, who wonder how a patent can be issued on something that already exists, such as a naturally occurring DNA sequence or a protein product of a gene. The explanation is outlined in Table 1. Things occurring in nature that are converted into something that does not exist in nature are patentable, if they are sufficiently nonobvious. A DNA sequence is not patented as it occurs in nature, but it can be patented in a purified, isolated, or synthetic form that is useful in a laboratory or that can be introduced into a vector for gene delivery or for in vitro protein production. Since a laboratory form of DNA does not exist in nature, it is "new" in the sense required by the patent law. Moreover, if the sequence of an unknown gene is not predictable in advance, it cannot be said to be obvious in the legal sense and is, therefore, patentable.

### PATIENTS' RIGHTS IN PATENTED TISSUE AND CELLS

The last 2 decades have seen unceasing controversy about genetic and other biological patents. An early case was *Moore vs University of California*, which concerned a patient (Moore) who was treated for hairy cell leukemia at the University of California. Moore's spleen was removed as part of his treatment. The spleen cells were found to produce large amounts of lymphokines, which were potentially commercially valuable. The spleen cells were immortalized, patented, and licensed to a biotechnology company. Moore then found out that his physicians and the university had used his biological tissue for their own profit.

Moore sued, asking to be named as a co-inventor on the patent, asserting that the cells were his property. The Supreme Court of California decided in 1990 that a patient *does not* become a co-inventor by donating tissue, nor does he own the cells once they are taken from his body. However, Moore still won because the Court found his physicians failed to obtain a fully informed consent. Physicians and researchers must tell a patient if tissue taken from their body is to be used for potentially profitable research. Damages can be collected from physicians or researchers who fail to obtain informed consent from a patient whose tissue is used in research.

### PATENTS RELATED TO GENOMIC SEQUENCES AND PARTIAL GENE SEQUENCES

Perhaps the greatest controversy about patents in biotechnology has been the furor over patenting isolated nucleic acids having sequences that are found in the

Table 1  
PATENTING GENES AND PROTEINS

- Must be new, useful, and nonobvious
- Product of nature patentable if converted to a new form
  - Purified or synthetic DNA
  - Coupled to a nonnative promoter
  - Inserted in a vector

human genome. Patents have been issued for many years on purified, synthetic, or isolated DNA sequences that encode proteins of medical or other biological interest. The DNA sequence patents are considered important because they protect the “factories” that produce recombinant proteins. In the absence of strong patent protection at the molecular level, private investment in new technologies is diminished. Patents on genomic DNA sequences also protect techniques of molecular diagnosis, such as the detection of polymorphisms associated with disease. This protection is considered important by companies developing test kits for detecting genetic diseases.

As genomic information has become available from the HGP and elsewhere, isolated or purified nucleic acid molecules containing new genetic sequences have been patented on a large scale. For example, Celera Genomics Corporation has filed patent applications on many thousands of isolated partial gene sequences and expressed sequence tags (ESTs), even though a complete gene sequence or the biological significance of the sequence is unknown. The PTO has issued patents on

such purified or synthetic “new” sequences. These are considered “nonobvious” because the sequences could not be predicted in advance. Purified EST molecules also were considered to satisfy the “useful” requirement for patentability because they could be used as gene probes to find the full gene from which the EST was derived.

Since the PTO has issued patents on multiple ESTs that map to a single gene, many different patents often exist that protect a partial gene sequence before the complete gene is sequenced and before the biological significance of the gene product is determined. This aberration has upset the balance of innovation because anyone who wants to work with the completed gene may have to obtain a patent license from 5 or 6 different patent holders who have staked a patent claim to different portions of the gene sequence. This situation acts as a disincentive to researchers and to companies that do the hard work of fully sequencing a gene and determining its biological function.

The PTO has recently changed its policy about protecting fragments of DNA sequences of unknown function such as ESTs. In March 2000, the PTO issued new guidelines that now require that a patentable DNA molecule have a particular and more substantial use than merely acting as a probe for use in further research. Moreover, possession of a partial DNA sequence such as an EST will only entitle one to patent a molecule that contains the short sequence itself, and not a longer DNA molecule such as a gene or cDNA that includes the shorter EST sequence. Hence, even if a patent is obtained on an EST, anyone who eventually sequences the entire gene and determines its function will not have to obtain a patent license from the EST patent owner to work with the full gene. These policy changes will likely limit some of the more egregious instances of DNA patent abuse.

## CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for internists, pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Drug companies and researchers also are making DNA sequence information available through public databases as quickly as it is known. It then becomes "prior art," which can be used to prevent subsequent patenting of nucleic acids that contain the sequence, or at least prevent others from subsequently obtaining broad patent protection on related gene sequences.

## PATENTS ON GENETIC TESTS

Patents on the development of laboratory tests that permit the detection of a genetic disorder also have been controversial. For example, Myriad Genetics holds patent rights on genetic tests that detect mutations in the gene *BRCA1*. When present, these mutations predispose to the development of breast and ovarian cancer. Some university researchers complained that the \$2,500 fee charged by Myriad for each test was so high that it precluded them from continuing research about the gene and its mutations. Although the test fee was subsequently reduced, such high charges may deter unfettered research and can create obstacles to medical advances.

However, a fair discussion of genetic patents requires that both the benefits and burdens be considered. A benefit is that biotechnology patents have attracted billions of dollars of private research into genetic and molecular biology research, which ultimately advances patient care. Patents are essential tools to biotechnology companies, as evidenced by substantial changes in the companies' stock prices in response to news about their patents. The importance of patents to biotechnology companies was reflected in the collapse of biotechnology stocks in mid-March of this year (2000) after President Bill Clinton and Prime Minister Tony Blair made a joint statement that seemed to question whether DNA molecules should be patentable. President Clinton subsequently clarified that he was questioning only whether raw genetic information should be patentable. However, genetic information per se is not, and has not been, patentable; only purified molecules that include the sequence are patentable subject matter.

Although genetic patents do increase the cost of genetic tests, the billions of dollars of investment capital they have attracted to genetic research promise to transform medicine quickly, and move genetics to a central role in medical practice. Just as pharmaceutical patents provided the impetus for the transformation of medicine in the *mid-20th century*, biotechnology patents will very likely provide the economic incentives for the practice of widespread molecular medicine in the *21st century*. Private companies are already developing high-production genomic techniques for large-scale genetic diagnosis by using sophisticated software technologies and cDNA microarrays. Such technologies should quickly

move genetic practitioners and researchers to an even more important role in medical practice.

## GENETIC PATENT LAWSUITS

Several high-profile patent infringement cases concerning DNA patents illustrate the importance of "genetic" patents. Recently, the University of California, San Francisco sued Genentech, Inc. concerning its patented sequence of cDNA, which included a coding sequence of hGH. Genentech, Inc. allegedly used the hGH sequence to produce rhGH. Genentech, Inc. settled out of court by paying a large amount to UCSF for the alleged violation of UCSF's patent rights.

In another suit, the University of California accused Eli Lilly of infringing its patent on recombinant mammalian insulin. However, the court in this case found the patent invalid. UC scientists had determined the cDNA sequence of the gene for preproinsulin and proinsulin only in *rats*. The court held that the patent as written was invalid because only the *rat* gene sequence was disclosed in the patent application. Since the scientists had determined the sequence only in the rat, the patent was invalid for attempting to also protect all *mammalian* sequences (including the human sequence that had not yet been determined).

## CONCLUSION

Patents concerning biotechnology are controversial but necessary. They are designed to prevent others from making, using, or selling the patented invention without permission. Their purpose is to reward inventors and investors and to encourage private investment in technology development. The broader interests of society also are protected by limiting the effective life of a patent to 20 years from the day the application is filed, after which time the technology is available for all to use. Typically, gene patents are used to cover items listed in Table 2.

Table 2  
Typical Gene Patent Items Covered

- Purified protein (if novel)
- DNA (*ORF*) encoding the protein
- rDNA operably linked to a promoter
- Cell transformed with the nucleic acid
- Transgenic animal into which the transgene is introduced
- Oligonucleotides (probes) of 20, 30, or 50 contiguous nucleotides; antisense oligonucleotides
- DNA that hybridizes to the sequence (includes variants)



Patent rights usually are decided in courts of law. The actual outcomes of court cases concerning biological patents illustrate, in my opinion, that legislative changes are only occasionally necessary to curb perceived problems with patents. Currently, courts construe genetic patents very narrowly and invalidate them if the patent has been written too broadly. The legal stringency courts have applied to patents recently has prompted the PTO to examine biotechnology patent applications much more diligently before issuing a patent.

The public policy challenge of the coming years will be to find a beneficial balance between the need to encourage private investment in molecular medicine with reasonable patent rights, while not unduly limiting the ability of researchers to rapidly advance new discoveries within medical science. Some professional organizations, such as the American College of Medical Genetics (ACMG), have issued policy papers stating that genes and mutations are naturally occurring substances that should not be patented (<http://www.faseb.org/genetics/acmg/pol-34>). If implemented, such an extreme position could undermine the biotechnology industry, drive private capital out of the field, and greatly slow the progress of molecular medicine.

A more moderate position was taken by the American Society of Human Genetics (ASHG) (<http://www.faseb.org/genetics/ashg/policy/pol-08>), which did not oppose the patenting of nucleic acid molecules that were found to code for therapeutic proteins or could be used as disease gene probes for specific diagnostic tests. However, ASHG did oppose patenting EST molecules because they were only tools for further research and therefore lacked "patentable utility." ASHG also was concerned that EST patents would create a morass of competing patents for the same DNA molecule when its full sequence was eventually determined. ASHG subsequently commended the US PTO (<http://www.faseb.org/ashg/policy/pol-39>) for the new guidelines it adopted in March 2000, which will make it much more difficult to patent EST molecules that are only tools for further research.

Recent court decisions and changes in PTO policy have raised the standards for patentability of nucleic acid molecules and have addressed many of the objections to the broad scope of earlier nucleic acid patents. These changes should help overcome many of the more reasonable objections to patents dealing with biotechnology and allow molecular medicine to continue to develop rapidly with the economic protection provided by patents.

#### Abstracts From the Literature

### Gender Assignment and Reassignment in 46,XY Pseudohermaphroditism and Related Conditions: A Commentary

Meyer-Bahlburg discusses the current intense debate in managing patients with intersexuality. Three major issues are the focus of the presentation: (1) gender assignment, (2) indications for genital surgery (particularly in the newborn period), and (3) the disclosure of medical information to the patient. The pertinence of these considerations was precipitated in part by Diamond's and Sigmundson's published guidelines (*J Sex Res* 1997;34:199-211, and *Arch Pediatr Adolesc Med* 1997;151:1046-1050). These guidelines strongly recommend assigning all 46,XY patients who incurred penile loss, micropenis, androgen insensitivity stages 2 and 3, hypospadias, 5 $\alpha$ -reductase deficiency, or 17 $\beta$ -OH steroid dehydrogenase deficiency to the male sex. The Intersex Society of North America, primarily consisting of adults with intersex problems dating to infancy, also has published recommendations that center on the avoidance of genital surgery without the patient's informed consent, unless it is absolutely necessary for the physical health and comfort of the intersex child.

Meyer-Bahlburg appropriately and wisely states, "to evaluate such recommendations we have to place them in historical perspective." He proceeds to do so, and starts with consideration of the gender question and how we have increased our knowledge since the early 20th century so that we now are aware that *nature* (the influence of male hormones in utero on imprinting the brain along male lines) is an important phenomenon, particularly in determining gender role and, to a lesser extent, gender identity. He emphasizes the significant difference in effect, how-

ever, among species (eg, guinea pigs and humans). He also emphasizes that much data support the role that *nurture* plays, meaning the effect of environment resulting from early sex assignment to a newborn who has the genitalia to function in the sex of assignment. He stresses that evidence from long-term follow-up of intersex patients themselves will be the final arbiter of the adequacy of a given management policy, and that this evidence is extremely limited, especially in the case of male pseudohermaphroditism. The very few scattered cases that are discussed in great detail in the media and public arena are inadequately documented in sufficient psychological detail to determine an absolute management recommendation. Meyer-Bahlburg describes in detail his reasons for reservations in the John/Joan case, maintaining that *nature* was proven to be the decision-making factor in a male child with ablated penis who was raised as a girl (Joan) from 21 months and then elected to return to a male role at 14 years (John). Meyer-Bahlburg appropriately states, "To move forward in this difficult area, we must carefully distinguish between conclusions for which we really have good evidence and statements based on interpolation." Meyer-Bahlburg summarized that the evidence available to date permits only tentative policy-relevant conclusions, which are:

- The organizational effects of prenatal androgens are more noticeable in gender role (behavior) than in gender identity.
- Gender identity can develop as female or male over wide variations of gender role (behavior).



- The majority of 46,XY intersex patients seem to develop an identity commensurate with the assigned gender and do not change their gender later.
- Gender identity assigned in childhood usually will continue into adolescence and adulthood, but patient-initiated gender change in intersex patients seems to happen more often at those times. Thus, long-term follow-up into mid-adulthood is essential if one wants to arrive at definitive conclusions concerning the appropriate way to assign intersex patients at birth.
- More female-assigned 46,XY patients initiate gender change to male than male-assigned 46,XY patients initiate gender change to female. There is suggestive but not conclusive evidence that this is more frequent in patients with a history of fully male-typical prenatal androgenization.
- There is, at this stage of research, no unambiguous evidence for or against female gender assignment of 46,XY patients, even in the prenatally most androgenized conditions.
- The number of well-documented cases, especially regarding prevalence rates of gender change, is uncomfortably small to draw definitive conclusions, and psychological details and assessment measures often leave much to be desired.
- The only way of obtaining a sufficient empirical basis for an intersex management policy is to conduct sophisticated comprehensive psychological follow-up studies with reasonable sample sizes. This will require collaboration among clinics.

Meyer-Bahlburg HFL. *J Clin Endocrinol Metab* 1999;84:3455-3458.

**Editor's comment:** The issue of the appropriate assignment of a child with a 46,XY karyotype and ambiguous genitalia remains difficult, as evidenced by the controversies and accusations made by individuals and by societies. Meyer-Bahlburg brings a much needed calm, nonemotional scientific approach for consideration.

*The nature and nurture effects on gender identity and gender role are both important and can be compared with the inclusive roles of the gene versus environment on behavior that were in controversial consideration in the early and mid 20th century. The approach of a recently organized North American Task Force on Intersexuality (E-mail: perkos@muscc.edu), which consists of a group of pediatric urologists, endocrinologists, other physicians, psychologists, and others, may produce a scientific forum to enhance our data-gathering capability regarding the outcomes of sex assignments made in the past to children who now are adults. Gathering the data will enhance our capability to make the humane decisions that we all wish to do for our patients. Importantly, in medicine we must remember that there usually are those who suffer as a result of any specific untried medical approach to save lives even though the treatment ultimately proves beneficial. Examples are kidney, heart, and lung transplants, the administration of human GH, and the administration of blood at surgery to prevent death. All of these in the early stages of their use were associated at times with life-threatening hepatitis or AIDS viruses. We as physicians and concerned humans have great empathy for those who suffer as a consequence, but that does not mean that great benefit will not come to many as a result of deliberately pursuing the truth. Personally, I thank Dr. Meyer-Bahlburg and others like him who are pursuing a calm scientific perspective to solve a complex problem.*

Robert M. Blizzard, MD

## What Causes Low Rates of Childbearing in Congenital Adrenal Hyperplasia (CAH): A Commentary

Much has been written concerning this topic over many years. In the present article Meyer-Bahlburg has thoughtfully reviewed the etiologic considerations. The purpose was to revisit the issue, review the status of the empirical evidence—especially the role of behavioral determinants—and suggest additional hormone-related psychological factors that may contribute to the low fertility rates of women with congenital adrenal hyperplasia (CAH). The possible anatomic and psychological factors contributing to the overall reduction of fertility in women with classic CAH are considered initially. A consistent observation has been the predominant occurrence of low birth rates in women with salt-wasting (SW) CAH compared with those with simple virilizing (SV) CAH. Reduced heterosexual activity certainly is a contributing factor, which stems from several causes. Meyer-Bahlburg has reviewed all of these eloquently. However, the limitations of studies pursued in these areas are many. Despite these, the evidence clearly indicates that the reduced fertility of women with classic CAH has a variety of other reasons. Nonoptimal hormonal control remains a major reason. Ovulatory failure secondary to steroid excess is an important barrier to conception in many CAH women. Considerable evidence exists

suggesting that the steroid excess is not just an outcome of corticotropin oversecretion. Other contributing factors appear to be (1) a mild degree of corticotropin hyperresponsiveness to corticotropin-releasing hormone; (2) altered enzyme kinetics, including reduced catalytic efficiency of the mutated 21-hydroxylase enzyme with resulting increases in the precursor hormones progesterone (P) and 17-hydroxyprogesterone (17OH-P) even in the presence of excess glucocorticoid administration; (3) overactivation of the renin-angiotensin-aldosterone axis with ensuing stimulation of adrenocortical biosynthesis; and (4) alterations of the hypothalamic-pituitary-ovarian axis, as indicated by abnormal gonadotropin dynamics, polycystic ovaries, and excessive ovarian production of P, 17OH-P, and androgens. Consequently, new combinations of treatments that go beyond mere corticotropin suppression are being developed to improve the overall quality of hormonal control in CAH.

Meyer-Bahlburg also discusses the possible role that dexamethasone administration to mothers pregnant with CAH female infants might play in enhancing the number of births in these CAH females when they become adults, as such



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## ***GROWTH, Genetics, & Hormones***

**Volume 16, Number 3**

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- |  |   |   |   |   |   |
|--|---|---|---|---|---|
| 1. Educational value of newsletter                         | 5 | 4 | 3 | 2 | 1 |
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| 3. Newsletter style/format                                 | 5 | 4 | 3 | 2 | 1 |
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treatment has the potential to minimize the physical and mental alterations so often seen in virilized CAH female newborns.

Meyer-Bahlburg HFL. *J Clin Endocrinol Metab* 1999;84:1844-1847.

**Editor's comment:** The lead article in the December 1999 issue of GGH (15:3:33-41) was entitled Adult Consequences of Pediatric Endocrine Disease, I: Congenital Adrenal Hyperplasia. The section entitled Pregnancy in CAH Females reads as follows:

*The incidence and prevalence of pregnancy in SWCAH females is very low compared with that in SVCAH (simple virilizing CAH) patients. Mulaikal et al reported on 80 adult female CAH patients (40 with SWCAH and 40 with SVCAH). Twenty-five pregnancies were reported among 15 women with SVCAH, but only 1 pregnancy was recorded in 40 patients with SWCAH. In the Cardiff experience of 16 patients (11 SWCAH, 5 SVCAH), a 40% ovulation rate, as measured by salivary progesterone, was found. Three of 5 patients with SWCAH and 2 of 3 patients with SVCAH who had both an adequate introitus and were sexually active produced 8 pregnancies. This study highlights the potential for improved fertility in compliant patients who are treated early, who have adequately reconstructed genitalia, and who are followed closely during pregnancy for progesterone, 17 $\alpha$ -hydroxyprogesterone, and testosterone levels. A 1998 review by Garner (Semin Perinatol 1998;22:446-456), entitled "CAH in Pregnancy," provides an overview of CAH in both mothers with and without CAH and in potential CAH*

*fetuses in utero. More information on the frequency of pregnancy in SWCAH is very much needed, but the pregnancy rate certainly is low from all data presented to date.*

*What are the possible causes of the difference in pregnancy rates in SVCAH and SWCAH? The first possibility is that therapeutic noncompliance is greater among SWCAH patients. The second possibility is that there is a higher frequency of menstrual irregularity among SWCAH patients, reflecting less ovulation in this group. Third, there is a lower incidence of marriage in the SWCAH patients than in the SVCAH patients and, therefore, there is less opportunity for pregnancy. Fourth, the vaginal introitus is more frequently inadequate in the SWCAH group compared with the SVCAH group (53% vs 18%). Another possibility is that SWCAH patients engage in heterosexual activity less frequently.*

*The necessity for cesarean section in patients with SVCAH is very high. Of the 15 females with SVCAH experiencing 25 pregnancies, 13 carried to term. Nine of these 13 required cesarean sections because of pelvic disproportion. This is not surprising because of the constrictive anatomy that often is present postoperatively.*

Meyer-Bahlburg has expanded on these items and considered other factors in this article. Readers with interest in this topic are encouraged to read in full the articles by Meyer-Bahlburg abstracted in this issue of GGH by myself and/or the article in GGH 1999;15:3.

Robert M. Blizzard, MD

## X Inactivation: The Lyon Repeat Hypothesis

X chromosome inactivation is used by mammals to compensate for females having 2 X chromosomes while males have only 1. As established originally by Mary Lyon nearly 40 years ago, 1 of the 2 X chromosomes in females becomes transcriptionally inactive in every cell of the early embryo and remains so in somatic cells throughout life. This highly unusual form of gene regulation, in which almost a whole chromosome is silenced, has remained poorly understood until recently. Advances in the recent past include recognition that X chromosome inactivation starts at an X inactivation center and spreads for long distances to cover most of the X chromosome. Inactivation may spread to autosomes in instances of X:autosome translocation, although distances are shorter and inactivation is less efficient. A gene has been found (*Xist* in mice, *XIST* in humans) to reside at the inactivation center that is expressed only on the inactive X chromosome. It encodes a nontranslated RNA that after initiation of inactivation spreads to coat the entire inactive X chromosome and portions of autosomes in X:autosome translocations.

How *Xist* RNA spreads and how it silences genes has remained a mystery. It was suggested about a decade ago that "way stations" or "boosters" exist along the X chromosome that promote spreading. Lyon recently suggested that these boosters

might be LINE-1 (L1) elements, for which there was evidence of their presence in higher abundance on the X chromosome than on autosomes in mice and humans. The term LINE refers to long interspersed repeat elements that are mammal-specific, autonomous mobile DNA sequences. According to a recent review by Kazazian and Moran, the human genome is comprised of roughly 15% L1 elements that over time have been inserted into the genome through reiterative rounds of reverse transcription. These events have expanded our genome in both size and complexity.

Bailey and colleagues have new evidence to support the role of L1s in X chromosome inactivation. They carefully examined DNA sequence data from GenBank from the human chromosomes X, 6, 7, 20, 21, and 22 for evidence of interspersed repeat elements. At the time of analysis, in late 1999, the X chromosome sequence was 34% complete. The other chromosomes, which served as autosomal controls, were 19%, 43%, 22%, 39%, and 69% complete, respectively. Using software that recognized repeat sequences, they identified 43% of total available human DNA sequence as interspersed repetitive sequence. It fell primarily into LINE, SINE (short interspersed repetitive element), LTR (long terminal repeat), and DNA repeat element categories of repetitive sequence. The X chromosome had a significantly higher con-

tent of interspersed repeats than other chromosomes, 52% vs 40%. More dramatic was the disparity of L1 elements, which accounted for most of the difference between the X chromosome and autosomes. The genome average for L1 elements was 16%, close to the 15% mentioned earlier. However, the L1 element content of the X chromosome was 27% compared with 13% for the autosomes. In other words, the abundance of non-L1 repetitive elements is comparable between the X chromosome and autosomes, but L1 elements are about twice as abundant on the X chromosome.

Further analysis revealed that the L1 elements cluster at Xq13, which in humans is where the X inactivation center and the *XIST* locus reside. Finally, they observed that the L1 content of Xp22, which contains genes that escape X inactivation, was lower than other regions of the X chromosome and similar to that of the autosomes.

Both Bailey et al and Lyon argue that these observations strongly support the possibility that the L1 elements serve as boosters to propagate the spreading of *Xist* RNA during X chromosome inactivation. One scenario suggests that clusters of L1 elements serve as binding sites for *Xist* or *Xist*/protein complexes that promote packaging of dense (transcriptionally inactive) heterochromatin. Both concede the possibility that insertion of L1s is a consequence of rather than a causative factor for X chromosome inactivation.

The evolutionary aspect of these findings is very interesting. Bailey et al point out that there are subfamilies of L1 elements and that enrichment of L1 elements on the human X chromosome is from younger elements, in particular, those active 60 to 100 million years ago at the time when placental mammals diverged from marsupial mammals. The authors raise the possibility that accumulation of L1 elements was co-opted by placental mammals to construct an efficient X inactivation mechanism. If so, it would mean that repetitive material, often dismissed as junk, acquired a fundamental role in genetic regulation of the mammalian genome.

Bailey JA, et al. *Proc Natl Acad Sci USA* 2000;97:6634-6639.

Kazanian HH, Moran JV. *Nat Genet* 1998;19:19-24.

Lyon MF. *Proc Natl Acad Sci USA* 2000;97:6248-6249.

**Editor's comment:** These papers and accompanying editorials nicely summarize the recent advances in understanding the mechanisms that contribute to X inactivation. The possibility that ancient mobile DNAs were co-opted during evolution to construct a complex mechanism to silence genes over long distances is fascinating. Those readers interested in the phenomena described here will be very interested in the following abstract.

William A. Horton, MD

## Phenotype Associated With a Ring (X) Relationship to *XIST* Locus

Small ring (X) chromosomes lacking the *XIST* gene at Xq13.2 have been associated with a severe phenotype that includes mental retardation, facial dysmorphism, and congenital abnormalities. It has been hypothesized that the loss of *XIST* results in functional disomy for the sequences contained in the ring. The investigators studied 47 females with a 45,X/46,r(X) karyotype and found 7 to have an *XIST*-negative ring. Only 1 of the 7 patients had the severe phenotype. The remaining 6 patients had physical phenotypes consistent with Turner syndrome. The rings were characterized cytogenetically and molecularly.

The severe phenotype in 1 patient can be explained by the absence of *XIST* expression, the relatively large amount of Xp material in the ring, and, possibly, the concomitant maternal uniparental isodisomy. The investigators propose 3 explanations for the unexpectedly mild phenotypes in the remaining 6 patients: (1) The rings contained limited amounts of X chromosome material, and sequences that when functionally disomic

result in a severe phenotype were absent; (2) mosaicism resulted in the absence of the ring from tissues such as the brain that are important in the severe phenotype; and (3) an inactive X was present in some tissues at some time, as exemplified by the demonstration of *XIST* expression in 1 patient.

Turner C, et al. *Hum Genet* 2000;106:93-100.

**Editor's comment:** The presence of the severe phenotype in Turner syndrome was nicely explained previously by the possibility of functional disomy of some parts of the X chromosome. However, this report suggests the situation is more complicated and that each patient needs to be individually studied. The most likely explanation seems to be related to the amount of functional X chromosome DNA that is not inactivated. However, because every tissue in affected individuals is not usually studied, and since these tissues are not studied at various stages of development, all the answers are not in. In the past we have considered an exceptional patient as "weird." Now there seems to be an opportunity to answer many very basic questions. In the past, most of the reported patients were probably selected because of the severe phenotype. The actual mechanism producing the Turner phenotype may come to light by the study of such unusual patients. It is important to look for rings and evaluate these in relation to tissue locations and clinical phenotype.

Judith G. Hall, OC, MD

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## Maternal Uniparental Disomy 7 (Syndrome): Review and Further Delineation of the Phenotype

Uniparental disomy (UPD) is defined as the inheritance of both homologous chromosomes from only 1 parent. So far, maternal UPD 7 has been described in 28 cases. Here, the authors report 4 new cases, present clinical information on 5 cases previously reported by the authors, and review the clinical and molecular findings of all 32 cases. The authors found a phenotype characterized by prenatal and postnatal growth retardation, occipitofrontal head circumference in the lower normal range, a triangular face, and retarded bone maturation. Findings of the facial gestalt included a high and broad forehead and a pointed chin. A broad mouth with downturned corners, prominent ears, café-au-lait spots, hemihypotrophy, or clinodactyly were rarely present. Psychomotor development was delayed in 6 cases. The clinical findings strikingly resemble the phenotype of the heterogeneous Russell-Silver syndrome (RSS). Other anomalies were found less frequently than in RSS. Molecular investigations revealed 11 cases with isodisomy and 17 cases with heterodisomy. In 4 cases this information was not available. From the allelic distribution of the microsatellites investigated, 9 cases might be the consequence of an error at maternal meiosis I, and 6 cases might be due to nondisjunction at maternal meiosis II. Three of the 17 heterodisomic cases had trisomy 7 in chorionic villi. In the remaining cases no prenatal diagnosis through chorionic villus sampling was reported.

Kotzot D, et al. *Eur J Pediatr Res* 2000;159:247-256.

**Editor's comment:** *Kotzot et al's paper emphasizes in the Table the clinical features of the maternal UPD 7 syndrome, which often has characteristics of RSS.*

*The head circumference (OFC) of maternal UPD 7 individuals was around the 50th percentile for gestational age. Height and weight remained below the 3rd percentile whereas OFC usually adjusts to about the 10th percentile over time. It is not clear whether psychomotor retardation is seen as a regular feature of maternal UPD 7 since several cases had complicated pregnancies and/or deliveries that could have affected the intellect. Six of the 32 cases had hemihypotrophy.*

*Final adult height is not known for maternal UPD 7, although it appears that it is below the 3rd percentile. Clinodactyly is common, and a triangular face with a high forehead and pointed chin also are frequently seen. Precocious puberty, simian creases, teeth anomalies, and a squeaky voice were not seen in the maternal UPD 7 cases. The long-term prognosis is not yet known.*

Table  
Frequency of Clinical Findings in Cases With  
Maternal UPD 7 and Russell-Silver Syndrome  
(RSS) According to Wollmann et al

|                         | Frequency in<br>Maternal UPD 7 | Frequency<br>in RSS |
|-------------------------|--------------------------------|---------------------|
| Birth                   |                                |                     |
| Length (<-1 SD)         | 13/15 (87%)                    | 99%                 |
| Weight (<-1 SD)         | 15/20 (75%)                    | 94%                 |
| OFC (>-1 SD)            | 05/10 (50%)                    | 64%                 |
| Last examination        |                                |                     |
| Height (<-1 SD)         | 21/22 (95%)                    | 99%                 |
| Weight (<-1 SD)         | 15/15 (100%)                   | 100%                |
| OFC (>-1 SD)            | 12/16 (75%)                    | 64%                 |
| Retarded bone age       | 10/10 (100%)                   | 100%                |
| Hemihypotrophy          | 06/06 (100%)                   | 51%                 |
| Psychomotor retardation | 06/18 (33%)                    | 37%                 |
| Triangular face         | 16/16 (100%)                   | 79%                 |

OFC, occipitofrontal [head] circumference.

Reprinted with permission from Kotzot D, et al. *Eur J Pediatr* 2000;159:247-256.

*Mothers of isodisomic cases had an average age of 27.1 years, whereas the mean maternal age for heterodisomic cases was 37.1 years, suggesting those in the latter group are likely to be derived from cases of trisomy. Trisomy 7 is a common finding in chorionic villi sampling. Trisomy 7 prenatally diagnosed cases should be investigated for maternal UPD 7. Since most maternal UPD 7 cases probably derive from trisomy 7, the possibility of mosaicism explaining variation also must be considered.*

*RSS is a very common cause of intrauterine growth retardation. The heterogeneity that must exist under the RSS label is evident since approximately 10% of RSS cases appear to have maternal UPD 7 syndrome. All cases of suspected RSS need to be investigated for maternal UPD 7.*

Judith G. Hall, OC, MD

## Preliminary Study of Growth Hormone Therapy for Crohn's Disease

The results of treating 37 adults aged 20 to 55 years who had moderate to severe, active Crohn's disease with daily GH injections for at least 2 years were reported. A combination of radiologic and histologic criteria was used to confirm the diagnosis. In this double-blind, placebo-controlled study, patients were treated by their usual physicians and received other medications at their physicians' discretion. A loading dose of GH 5 mg/d SC was given the first week, followed by 1.5 mg/d

for the remaining 16 weeks of study. The 18 subjects in the control group received an equal volume of diluent.

The primary endpoint of the study was improvement in the Crohn's Disease Activity Index, which monitors the severity of the disease based on 8 clinical variables:

- number of liquid or soft stools per day\*
- severity of abdominal pain\*



- general well-being\*
- presence or absence of abdominal mass
- weight
- use of antidiarrhea drugs
- presence or absence of intestinal manifestations
- hematocrit.

Subjects were assessed at baseline, 1 to 2 weeks after initiation of the study, and monthly thereafter. Laboratory studies were extensive. All subjects were instructed to increase their protein intake by at least 2 g/kg/d, which was monitored with 3-day food diaries.

At 30 days, the subjects treated with GH had a significantly greater reduction in the Crohn's Disease Activity Index than the placebo group ( $P=.02$ ), with further decreases during the next 3 months. The 3 variables that most significantly improved were those marked with an asterisk in the above list. The change in the Crohn's Disease Activity Index scores are seen in the Table. In addition, at the end of 4 months the subjects in the GH group reduced their other drug requirements by 56%, compared with a 4% increase in the placebo group. Insulin-like growth factor 1 increased significantly in the GH group, but no other significant differences were observed between the groups in any of the other biomedical studies measured. The most frequent side effect in the GH group was edema, which occurred in 10 of 19, patients and headache which occurred in 5 of 19. These symptoms occurred only during the first 2 weeks of the study. Two subjects in the GH group had tumors detected during the study (renal tumor, benign schwannoma), as did 1 subject in the placebo group (precancerous cells of the esophagus and a benign polyp of the stomach).

Slonim AE, et al. *N Engl J Med* 2000;342:1633-1637.

**Editor's comment:** This is an intriguing and potentially very important study. As pointed out in an accompanying editorial by R. Balfour Sartor of the University of North Carolina, Chapel Hill, the article by Slonim et al is provocative. There are a number of clinical questions about the optimal dose of GH, the frequency of administration, and the length of therapy that need to be considered. Also, whether intestinal fibrosis with possible resultant intestinal strictures might occur is not known. The mechanism of action resulting in improvement also is not known.

## Risk of Persistent Growth Impairment After Alternate-Day Prednisone Treatment in Children With Cystic Fibrosis

Lai and coworkers report growth data on children with cystic fibrosis who, at 6 to 14 years of age, participated in a trial of alternate-day prednisone (1 to 2 mg/kg body weight) and were followed for approximately 6 to 7 years. Their growth data were obtained from the Cystic Fibrosis Patient Registry. Of the 224 subjects, 151 received prednisone and 73 received placebo. All had mild to moderate lung disease when the trial began. Four years after its initiation, the clinical trial was discontinued when it was determined that the side effects of prednisone outweighed its potential benefits.

Sixty-eight percent of the subjects who were reevaluated were 18 years of age or older. Results were reported 10

Table  
Changes From Baseline in the Crohn's Disease Activity Index Scores During 4 Months of Treatment With Growth Hormone or Placebo\*

| Month        | Placebo         |         |                      | Growth Hormone  |         |                      | P Value† |
|--------------|-----------------|---------|----------------------|-----------------|---------|----------------------|----------|
|              | No. of Patients | Score   | Change From Baseline | No. of Patients | Score   | Change From Baseline |          |
| 0 (Baseline) | 15              | 206±126 | —                    | 19              | 287±134 | —                    |          |
| 1            | 15              | 202±115 | −5±76                | 19              | 186±107 | −100±135             | 0.02     |
| 2            | 15              | 235±109 | 29±77                | 18              | 172±110 | −116±139             | 0.001    |
| 3            | 15              | 204±140 | −3±91                | 17              | 148±123 | −139±159             | 0.006    |
| 4            | 15              | 187±163 | −19±63               | 17              | 145±124 | −143±144             | 0.004    |

\*Plus-minus values are means ± SD. Only the 15 patients in the placebo group for whom follow-up data were available were included in the analysis. Higher scores on the Crohn's Disease Activity Index indicate more disease activity.

†P values are for the comparison of the changes in scores between the 2 groups.

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Obviously, additional studies in both adults and children are desirable. A review of the literature as of July 2000 reveals only 1 report (Henker J. *Eur J Pediatr* 1996;155:1066-1067) of children with Crohn's disease being studied with GH administration. Three adolescents possibly benefited from GH therapy. Studies such as these are difficult to do but, hopefully, are being pursued.

William L. Clarke, MD

years after the trial began. Their Z scores for height declined during prednisone therapy but catch-up growth began 2 years after treatment was discontinued. The mean height for boys 18 years or older was 4 cm years less than that in the placebo group (or 13 percentile points). However, in girls the difference in height between the placebo and treatment groups was no longer present 2 to 3 years after the discontinuation of prednisone (Figure on next page).

The effect of alternate-day prednisone therapy varied depending on the age at which treatment was given. Specifically, boys who started prednisone every other day at 6 to 8 years of age had declines in height Z scores that last-

ed for 10 years. Boys beginning prednisone at 8 to 12 years had catch-up gains beginning about 2 years after stopping therapy. Boys who started prednisone during adolescence (12 to 14 years of age) maintained their baseline Z scores. When indices of pulmonary status were controlled for, the negative association between the use of prednisone and the Z score for height remained strong ( $P<0.001$ ) in boys after prednisone was discontinued, and none of the 3 indices of pulmonary function correlated significantly with Z scores for height.

The authors point out that it is well known that long-term treatment with pharmacologic doses of prednisone correlates with significant reductions in final height. The differences between the sexes and the degrees of growth suppression seen in this study also have been seen in other studies, including those of children with asthma. They speculate that this may be due to the more pronounced deceleration of normal growth rate in boys prior to puberty, which might make them more susceptible to additional slowing of growth, or perhaps the higher secretion of GH in girls prepubertally. They *conclude* that the benefits of prednisone therapy in terms of pulmonary function are not prolonged once therapy is discontinued and do not outweigh the deleterious effect on final growth.

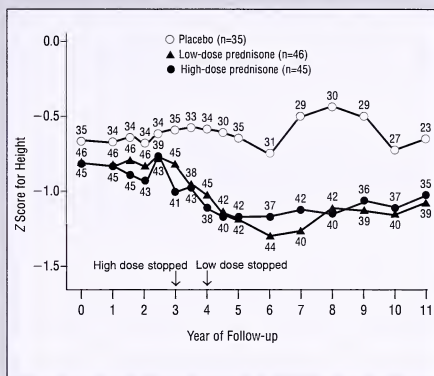
The authors summarized the results as follows: The growth impairment caused by prolonged alternate-day therapy with prednisone in prepubertal boys with CF persisted posttreatment and significantly reduced adult height. Although children gained substantial weight with treatment, this weight did not persist posttherapy. Because of these findings and the failure of therapy to benefit CF symptoms long term, one must conclude that *prolonged therapy is not beneficial* in CF children. If used in the treatment of any disease, glucocorticoid therapy must be monitored and individualized carefully to achieve the lowest effective dose and the shortest duration of therapy possible in order to minimize the risk of permanent growth impairment, particularly in boys.

Lai H, et al. *N Engl J Med* 2000;342:851-888.

**Editor's comment:** This important study demonstrates the significant negative impact of glucocorticoids on linear growth even when prescribed only every other day. It also is important because it stratifies and analyzes the response with regard to different ages at the onset of treatment. Of utmost importance, treatment caused diabetes and cataracts at a rate sufficiently high enough to warrant stopping this trial in 1991, 4 years after its initiation. Thus, the effects of glucocorticoids on other systems is potentially more significant medically than just its effect on height.

Pamela Davis and Carolyn Kercsmar from Cleveland have an excellent editorial in the same issue of the *New England Journal of Medicine* (2000;342:887-888), entitled "Growth in Children With Chronic Lung Disease." Readers are encouraged to review the thoughtful and useful comments pertaining to the causes of growth retardation in CF and the alterna-

Figure  
Relation of Z Scores for Height to Years of Follow-up in Boys With Cystic Fibrosis Who Received Placebo, Low-Dose Prednisone, or High-Dose Prednisone



The low dose of prednisone was 1 mg/kg, and the high dose was 2 mg/kg. The number of subjects at each point of follow-up is indicated. Among the boys, Z scores for height remained significantly lower after 10 years in those who received prednisone than in those who received placebo ( $P=0.03$ ). A Z score of zero corresponds to the 50th percentile of the reference population. A Z score of  $-1.0$  indicates 1 SD below the mean, which corresponds approximately to the 15th percentile.

Reprinted with permission from Lai H, et al. *N Engl J Med* 2000;342:851-888.

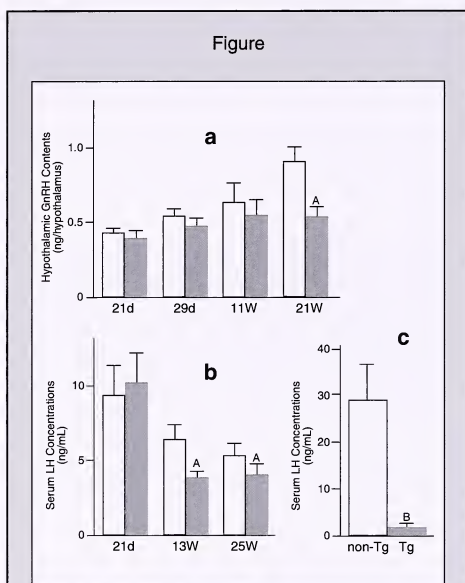
tives for therapy. The authors emphasize that there are multiple reasons for growth failure in CF patients, that glucocorticoids have multiple toxic effects beyond those reported in the article by Lai et al, and that ibuprofen is a less toxic and more proficient anti-inflammatory agent than glucocorticoids. They add that there is a dearth of evidence concerning the efficacy or adverse effects of inhaled glucocorticoids, although 12% of patients with CF in the United States are treated with these. Davis and Kercsmar recommend that with the increased risk of diabetes, cataracts, osteoporosis, and the reduction in height, the price may be too high to pay to use glucocorticoids in CF, especially since the benefits of anti-inflammatory therapy can be achieved in other ways in children with this disease.

The attention of readers interested in this topic is called to the lead article in *GGH* (2000;16[2]:21-26) written by Drs. O. Mehls and B. Tönshoff of Heidelberg. The title is "Effects of Glucocorticoids on Growth."

William L. Clarke, MD

## Accelerated Puberty and Late-Onset Hypothalamic Hypogonadism in Female Transgenic Skinny Mice Over-Expressing Leptin

Transgenic skinny mice were generated by causing overexpression of leptin under the regulation of a liver-specific promoter (human serum amyloid P component). In these animals there is chronic hyperleptinemia (81 ng/mL) compared with nontransgenic litter mates (NTLM; 9 ng/mL). Hypophagia is present, white and brown adipose tissue disappears, and insulin sensitivity and glucose metabolism increase (Ogawa Y, et al. *Diabetes* 1999;48:1822-1829).



Hormonal profile of transgenic (Tg) skinny mice overexpressing leptin (filled columns) and their nontransgenic (non-Tg) littermates (open columns). (a) Hypothalamic gonadotropin hormone-releasing hormone (GnRH) contents. (b) Serum luteinizing hormone (LH) concentrations 15 minutes after intraperitoneal administrations of GnRH. Procedures were performed on day 21 (21d) (filled columns,  $n = 10$ ; open columns,  $n = 8$ ); on the diestrus day at 13 weeks (13W) (filled columns,  $n = 6$ ; open columns,  $n = 4$ ); and on the diestrus day at 25 weeks (25W) (filled and open columns,  $n = 10$ ) of age. (c) Serum LH concentrations at 2,000 hours on the proestrus day between 13 and 18 weeks of age (filled columns,  $n = 6$ ; open columns,  $n = 4$ ). <sup>A</sup> $P < 0.05$  compared with nontransgenic littermates by ANOVA with Fisher's least significance difference test. <sup>B</sup> $P < 0.005$  by Student's test.

Reprinted with permission from Yura S, et al. *J Clin Invest* 2000;105:749-754.

In the present study by Yura et al, heterozygous males and females with 30 copies of the leptin transgene were mated. In the female offspring generated for this study, vaginal opening occurred earlier in the transgenic skinny mice (27.3 vs 29.4 days) than in NTLM ( $P < 0.05$ ). The transgenic animals had larger ovarian follicles but comparable ovarian weights. Uterine weights were significantly increased (22.3 g vs 13.3 g in NTLM;  $P < 0.005$ ). The skinny and NTLM females were comparably fertile at 8 weeks, but not at 22 weeks. At that time, the skinny animals were markedly subfertile with markedly reduced ovarian weights, follicular atrophy, decreased basal and gonadotropin hormone-releasing hormone (GnRH)-stimulated serum luteinizing hormone concentrations, and reduced hypothalamic GnRH values compared with NTLM. Gonadotropin administration restored ovarian size and morphology to those of NTLM. In contrast, in males there was no significant difference in fertility, testicular weights or morphology, or hypothalamic GnRH content between transgenic mice and NTLM.

The investigators concluded that transgenic female mice with hyperleptinemia undergo earlier pubertal maturation than do NTLM and have comparable fertility at younger ages despite no apparent adipose tissue; when older, however, they develop hypogonadotropism due to decreased GnRH production. The mechanism of the latter effects was attributed to downregulation of "hypothalamic leptin signaling." They suggest that the hypothalamic effects of leptin on feeding and reproduction traverse separate and distinct pathways, and that there also is a gender difference in leptin responsiveness.

Yura S, et al. *J Clin Invest* 2000;105:749-754.

**Editor's comment:** According to the "critical weight" hypothesis and clinical experience, body fat is extremely important in promoting normal linear growth and sexual maturation in both males and females, but particularly in females. These investigators have developed an animal model in which sexual maturation is normal/accelerated in males and females but that cannot be maintained in older females, probably due to a decrease in hypothalamic GnRH production. The data confirm the significant role that leptin plays in the regulation of the early maturation of the reproductive endocrine system. The data also complement previous studies in which leptin has been administered to normal or leptin-deficient (but responsive) animals. One wonders if the hyperleptinemia of the obese teenage male may sometimes paradoxically delay the onset of puberty. On the other hand, the hypothalamic hypogonadism that may occur in some obese adult women, but seldom in obese adult males, also may reflect an effect of chronic hyperleptinemia.

Allen W. Root, MD



## Long-Term Outcome of Classical 21-Hydroxylase Deficiency: Diagnosis, Complications and Quality of Life

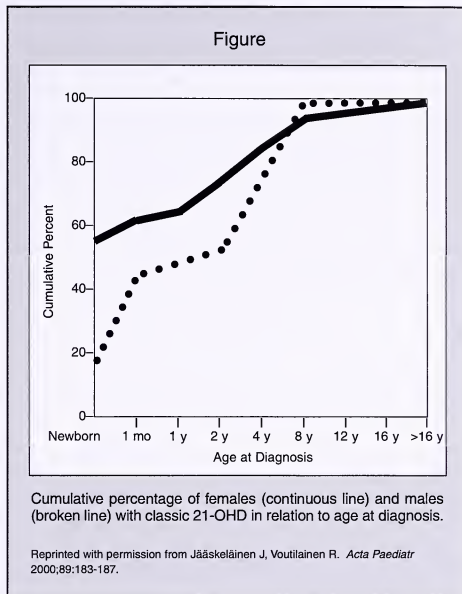
A nationwide search for patients with classic 21-hydroxylase deficiency (21-OHD) was undertaken in Finland to determine the long-term outcome of the disease. One hundred and eight patients were found. Fifty-four (31 females, 23 males), or 50%, had the salt-wasting form of congenital adrenal hyperplasia (CAH). Another 54 (29 females, 25 males), or 50%, had the simple virilizing form from 21-OHD. The age at diagnosis was delayed in males compared with females (Figure). A significant number of severe complications suggestive of glucocorticoid deficiency was found. There were 5 deaths possibly connected with cortisol deficiency (4.6% of all patients). Ten additional patients (9.3%) had been acutely admitted to the hospital 14 times due to symptoms of glucocorticoid deficiency. These symptoms included sudden loss of consciousness, convulsions, and severe fatigue. Afterwards, permanent neurologic defects were detected in 2 of these patients.

Finally, a cross-sectional study was carried out to establish an estimate of the long-term outcome of the disease. Thirty-two, or 55%, of the 58 patients  $\geq 16$  years of age participated in this study. The patient group did not differ from the general Finnish population in terms of education. Three of the patients (5%) had retired prematurely. Surprisingly, the patients felt that their health-related quality of life, as reported in the RAND-36 questionnaire, was better than that of the general Finnish population ( $P=0.023$ ). However, since a significant number of all qualifying patients did not participate in this study, the quality-of-life evaluation results must be interpreted with caution.

The authors conclude that a significant number of complications was found among patients treated for classic 21-OHD. Nevertheless, the disease has a favorable outcome in terms of quality of life.

Jääskeläinen J, Voutilainen R. *Acta Paediatr* 2000;89:183-187.

**Editor's comment:** This report complements the recently published review of the long-term consequences of CAH by Blizzard in GGH (2000;15[3]:33-41) and detailed appraisal of the pathophysiology of this disorder by White and Speiser (*Endocr Rev* 2000;21[3]:245-291). The larger number of females than males with classic CAH is consistent with the probabilities that some males were not identified and that



others perished without diagnosis. Conspicuously absent in this report are clear data on independence and interpersonal relationships, marriage, parenting, and other intimate details that bear on the reproductive function of adults with CAH. However, it is encouraging to learn that the general quality of life related to health in many adults with CAH is good; this may reflect the greater patient-physician contact required by those with CAH, although this point was not investigated. The fact that patients with CAH still die of relatively minor illnesses or become acutely ill because of insufficient glucocorticoid replacement is a sober reminder of this hazard.

Allen W. Root, MD

## A Study of Chromosome Aberrations After rhGH Treatment

Because of the suggestion that rhGH therapy might be associated with certain forms of leukemia, Slyper et al undertook to evaluate patients before and after rhGH therapy. They also looked for carriers of conditions that might increase the levels of spontaneous and induced chromosomal aberrations, and thereby potentially increase susceptibility to neoplasm when treatment with GH is used.

The data collected are not exactly comparable to previous studies. In this study children with the conditions ordinarily treated with rhGH were studied. Metaphase cells were examined for sponta-

neous chromosomal and chromatid aberrations before and after 6 months of treatment with rhGH. In addition, cells from these individuals were exposed to radiation to assess chromosome fragility. Dicentric and reciprocal translocations were specifically sought. They excluded patients with preexisting malignancy, those who had previous radiotherapy or chemotherapy, and those with syndromes that were known to be at risk for malignancy. The investigators studied only metaphase cells that were at first mitotic division after mitogenic stimulation. Chromatid-type aberrations in which there were deletions or exchanges such as triradials and quadraradials were sought. Five hundred cells were



examined from each individual for spontaneous aberrations. Two hundred cells from each person were examined for radiation-induced aberrations. In order to be certain that cells were examined at first mitotic division, the investigators used 5-bromo-2'-deoxyuridine (BrdUrd) and phytohemagglutinin (PHA).

No patient showed a significant increase in aberrant cells with treatment. However, the mean frequency of chromatid-type aberrations was significantly higher after treatment on a per cell basis. Because 2 patients contributed inordinately to this increase, they repeated the studies on these 2 patients. No remarkable changes occurred with time. There also was a low frequency of ring chromosomes in the 6-month samples.

Although these data are not totally comparable to other studies, no real cause for concern about the risk that GH therapy predisposes to leukemia was generated.

Slyper AH, et al. *Pediatr Res* 2000;47:634-639.

**Editor's comment:** Clearly, if there is a risk from GH therapy, it needs to be identified so that it can be weighed against the benefits. The present study does not seem to suggest that there is a major risk. However, it suggests there may be a subpopulation of individuals who would be at risk or contribute to any increase in chromosomal aberrations. The unusual increase in observed leukemia in the Japanese population receiving rhGH certainly deserves further evaluation, and whether there are some subgroups receiving rhGH who might be at risk must be further evaluated.

Your attention is called to a previous paper by Dr. Slyper entitled "How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?" (GGH 1998;14[1]:4-7) Dr. Slyper and his colleagues are providing recommendations and much-needed data to utilize in our considerations of rhGH as a therapeutic tool.

Judith G. Hall, OC, MD

## Inhaled Corticosteroid Use and Bone Mineral Density in Patients With Asthma

The investigators report the results of a cross-sectional survey of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) in a basically healthy, young adult population (20 to 40 years of age; females 119, males 77) who because of mild asthma had received inhaled glucocorticoids (primarily beclomethasone, median dose=876 mg; few to no doses of oral, parenteral, or dermal preparations) for a median period of 6 years. An inverse relationship between the cumulative dose of glucocorticoids and the BMD of the lumbar spine (L2-L4), left femoral neck, trochanter, and Ward's triangle was found. No relationship was found between the daily dose of glucocorticoids and BMD at any site, nor did any subject have a vertebral fracture. Although mean BMD measurements were normal at all sites, doubling of the cumulative dose of inhaled agents resulted in a "decline" in BMD of approximately -0.03 SD at all sites (approximately -0.020 g/cm<sup>2</sup> at L2-L4). The total duration of inhaled corticosteroid intake also was inversely related to BMD at each site. The authors estimated that if a patient received a cumulative dose of 5,100 mg of inhaled corticosteroids over 7 years, the L2-L4 BMD would fall 1 SD; if continued over longer periods, the patient could be at substantial risk for osteopenia and fracture.

Wong CA, et al. *Lancet* 2000;355:1399-1403.

**Editor's comment:** Although these data were accumulated in young adults, they have clear implications for children, many of whom receive prolonged courses of inhaled glucocorticoids for treatment of asthma. Inhaled glucocorticoids have been associated with impairment of growth and adrenal function in children.<sup>1</sup> Glucocorticoids adversely affect chondrocyte proliferation and skeletal mineralization; they depress bone formation by suppressing osteoblastogenesis and hastening osteoblast apoptosis, enhance bone resorption, decrease intestinal absorption of calcium, and increase urinary excretion of calcium.<sup>2,3</sup> Records quantitating the cumulative dose of inhaled glucocorticoids should be maintained on all subjects receiving them. It has been suggested that BMD be determined in the young adult after

he/she has received 5,000 mg of these agents and consideration be given to administration of a bisphosphonate in order to prevent glucocorticoid-induced bone loss.<sup>4</sup> Careful study of mineral metabolism and BMD in children receiving these medications is warranted and necessary. The readers may be interested in the lead article in GGH (2000;16[2]:21-26) entitled "Effects of Glucocorticosteroids on Growth," by Drs. O. Mehlis and B. Tönshoff of Heidelberg.

Allen W. Root, MD

1. Allen DB. *Acta Paediatr* 1998;87:123-129.
2. Manelli F, Giustina A. *Trends Endocrinol Metab* 2000;11:79-85.
3. Manolagas SC, Weinstein RS. *J Bone Miner Res* 1999;14:1061-1066.
4. Sambrook PN. *Lancet* 2000;355:1385.

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## Intrauterine Growth Retardation Associated With Maternal Uniparental Disomy for Chromosome 6 Unmasked by Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is caused by steroid 21-hydroxylase deficiency. The gene (*CYP21*) for this enzyme is located on the short arm of chromosome 6 (6p21.3).

This enzyme deficiency leads to reduced conversion of 17-hydroxy progesterone to 11-deoxycortisol, resulting in a deficiency of cortisol and overproduction of androgens. In female newborns this disorder is associated with ambiguous genitalia. Untreated children show rapid growth, phallic enlargement, precocious pubarche, early epiphyseal closure, and short stature.

In this report, 1 female newborn with intrauterine growth retardation (IUGR) and CAH was found to be homozygous for a rare exon 4 mutation 1172N. The patient showed transient delayed mental development, evidence of early puberty, increased bone age, and accelerated growth. Genetic analysis found that only the mother was heterozygous for this mutation. Further DNA microsatellite analysis confirmed the diagnosis of uniparental disomy.

Spino RP, et al. *Pediatr Res* 1999;46:510-513.

**Editor's comment:** Uniparental disomy is a condition in which both copies of a chromosome segment are inherited from a single parent. There is only 1 other report of a patient with uniparental disomy of the same segment of 6p (van den Berg-Loonen EM, et al. *Hum Immunol* 1996;45:46-51). This patient had IUGR at birth. Clinical symptoms appear to be due to genetic imprinting or expression of recessive traits from the affected chromosome segment and not directly associated with uniparental disomy. Similar reports of uniparental disomy involving the long arm of chromosome 6 have been associated with neonatal diabetes but not IUGR. These data are suggestive that fetal growth gene(s) are located on the short arm of chromosome 6 and that genetic mutations in this particular area (6p21.3) will cause IUGR. This case study shows that a rare underlying genetic mutation can cause multiple clinical manifestations. However, the risk of recurrence of these mutations is negligible in families.

Fima Lifshitz, MD

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**GROWTH, Genetics, & Hormones Volume 16, Number 3**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit. Please note that each question may have more than one correct answer.

1. MODY differs from type 1 diabetes in which of the following ways?
  - a. having an autosomal dominant inheritance pattern
  - b. by not requiring insulin for at least 5 years after the onset of DM
  - c. having no complications in later life
  - d. usually responds initially to oral hypoglycemic agents
2. MODY currently is believed to be attributable by most authorities to:
  - a. insulin resistance
  - b. growth hormone excess
  - c. an insulin secretory defect
3. Currently:
  - a. there are 5 types of MODY
  - b. there are 3 types of MODY associated with severe diabetes
  - c. low birth weight is thought to occur in MODY type 2
  - d. renal dysfunction is often found in MODY type 5
  - e. the data are persuasive that MODY type 1 is similar to type 2 diabetes in respect to the occurrence of diabetic complications

4. Which of the following qualify the issuance of a patent on a biological material?
  - a. the product must be new, useful, and nonobvious
  - b. a product of nature converted to a new form; eg, a purified or synthetic DNA compound, a product coupled to a non-native promoter, or a product inserted in a vector

**Answer Key:** 1. a, b, d 2. c 3. a, b, c, d, e 4. a, b

**CME Accreditation Statement**

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**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Stoffers, Noonan, Clarke, Hall, Horton, and Lifshitz report no conflicts. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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## The Adult Consequences of Pediatric Endocrine Disease, II: Turner Syndrome

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### INTRODUCTION

In Europe, the syndrome we know as Turner syndrome (TS) is known as Ullrich syndrome. This is because Ullrich described the syndrome in 1930, 8 years before Henry Turner described the entity in the United States. The patients were similar, but their ages were not. Therefore, the signs and characteristics differed. Ullrich described a group of *preadolescent* female children with proportionate short stature and various congenital anomalies, including pterygium colli and a wide carrying angle at the elbow, among others. Turner described *adolescent* girls with the same phenotype who remained sexually undeveloped.

In the late 1950s, when chromosomal karyotyping became available, the etiology was clarified. TS was demonstrated to be a genetic disease resulting from the complete or partial absence of 1 of the sex chromosomes. The initially described patients had a total of 45 chromosomes and only one X chromosome. Subsequently, patients were identified who exhibited mosaicism (for example, 45,X/46,XX karyotypes). Others had a partial deletion of 1 of the arms of the second X chromosome, usually with duplication of the remaining arm (p or q), and a resultant second X chromosome with a double gene dose of the remaining arm and zero dose of the absent arm. TS patients with the so-called isochromosome form are designated karyotypically as 46,X,I(Xq), as usually the short arm is absent and the long arm is duplicated. Partial deletions also occur in some patients on the tips of or part of the p and q arms of 1 of the X chromosomes. Sometimes the sticky remaining end portions adhere, so that these

chromosomes are shaped like a ring. The karyotype designation of patients with this gene phenotype is 46,X,r(X). Less frequently there are other associated chromosome and gene anomalies, including the presence of a partial Y chromosome as the second chromosome component.<sup>1</sup>

Considering the types and extent of chromosome and gene anomalies with the association of various somatic anomalies and diseases is important. For example, TS individuals with the 45,X karyotype usually have some or all of the congenital somatic anomalies associated with the syndrome, while many of those with mosaicism, isochromosomes, partial deletions, or ring X chromosomes have fewer somatic anomalies apart from short stature and sexual infantilism. The pertinence of this concept of genetic and somatic association will be more evident as we consider the adult consequences of this pediatric endocrine disease.

With this orientation, a discussion of the *mortality* and *morbidity* of TS patients in adulthood is presented. The object in this presentation is to (1) help physicians caring for adult TS patients understand the need to work closely with these patients to prevent, diagnose, and treat adverse consequences; and (2) help the pediatrician in facilitating the transfer of TS patients to internists, gynecologists, family practitioners, and other appropriate physicians.

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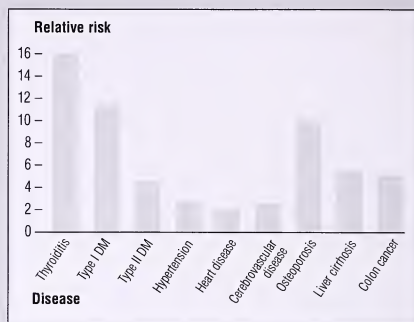
## OVERALL MORTALITY AND MORBIDITY

Relatively little was known about the natural history of women with TS for many years because they were lost to follow-up. In 1986, Price et al<sup>2</sup> brought to the attention of interested persons the *mortality* statistics, *mortality* ratios, life expectancy data, and causes of death for 156 TS patients in Edinburgh who had survived infancy and had been followed an average of 17 years. The data had been collected over 25 years for the Abnormal Karyotype Register in Edinburgh, which was established in 1959. The *reduction in life expectancy* was 12.5 years at age 1, 11 years at age 20, and 10 years at age 40. Fifteen deaths had occurred among the 156 patients observed over the 25 years, a considerable reduction in life expectancy compared with normal newborns. The fraction of TS patients alive at age 60 years was 68%, in contrast to 88% of the general UK population. Eight deaths resulted from diseases of the circulatory system, which will be discussed further in the section about the cardiovascular consequences of TS. The other 7 deaths were due to a broad spectrum of diseases, also discussed later.

Several groups of investigators, primarily in Europe, where medical care is more often closely regulated, have recently reported on the *morbidity* in TS. Gravholt et al<sup>3</sup> undertook a 10-year study (1984 through 1993) of all 594 TS women known to be living in Denmark during this period. The focus was on the primary diagnosis (1<sup>o</sup> Dx) of all hospitalizations. The observed number of admissions for a specific 1<sup>o</sup> Dx versus that expected in the general population is the relative risk for a specific admission and is an indicator of the presence of associated diseases in TS. These investigators found that while most of the associated diseases manifested themselves in childhood or adolescence, many went undetected; *thus, emphasis needs to be placed on screening repeatedly for subtle manifestations of associated diseases in adulthood*. Figure 1<sup>3</sup> is based on some of these data, as published in a review by Elsheikh et al,<sup>4</sup> concerning the relative risks of some of the associated diseases. Gravholt et al also reported a slightly increased risk for colon cancer for all TS patients and a risk for gonadoblastomas in TS patients with a Y chromosome. Therefore, removal of the gonads is recommended for individuals having a Y chromosome or chromosomal fragment containing the testis determinant *SRY* gene.

Garden et al<sup>5</sup> previously (1996) published data from Liverpool regarding undiagnosed *morbidity* in adult women with TS. The morbidities uncovered were those possible to identify by lipid assessment, evaluation of thyroid function, testing of gonadal status, measuring routine biochemical profiles, and determining bone mineral density (BMD) in patients previously diagnosed with and treated for TS. The patients were referred for their continuing care to a clinic where the studies were routine for first visits. Serum cholesterol levels >5.2 mmol/L were detected in a surprising 50%, and 29% had low-density lipoprotein

Figure 1  
Relative Risk of Disease in Turner Syndrome



Women with Turner syndrome have a greatly increased risk of developing autoimmune thyroid disease, ischemic heart disease, cerebrovascular disease, hypertension, as well as type I and II diabetes mellitus (DM). They are also at risk of osteoporosis fractures, cancer of the colon, and liver cirrhosis.

Reprinted with permission from Elsheikh M, et al. *Ann Med* 1999;31:99-105.

(LDL) values >4.0 mmol/L. Twenty-eight percent had at least 1 abnormality of thyroid function. Two had hypothyroidism and 6 had compensated hypothyroidism (normal thyroxine [ $T_4$ ] with elevated thyrotropin). Lumbar vertebral areal BMD (aBMD) was <100% of the age-matched reference range in 84%. Femoral aBMD was similarly depressed. Ninety-five percent of these women allegedly were still receiving estrogen replacement. The authors emphatically and logically concluded that *TS patients leaving the care of pediatricians need appropriate evaluation and assignment to a physician or physicians experienced with the consequences of TS*.

The associated diseases in TS patients discussed in this section, as well as other associated or component diseases of TS, are reviewed in detail in the remaining sections and are presented approximately in the priority of their occurrence in the TS population.

## ENDOCRINE-ASSOCIATED DISEASES AND/OR SIGNS AND SYMPTOMS PRODUCING CONSEQUENCES IN TS ADULTS

*Short stature* (SS) is a major physical characteristic of TS that is generally apparent in early childhood but that may not be evident with respect to the patient's stature falling below the 3rd percentile until 5 or 6 years of age. A normal adolescent growth spurt does not occur even with estrogen supplementation. There is strong suspicion that a chondrodystrophic etiology is the basic cause of the SS. A candidate gene<sup>6</sup> on the X chromosome, *SHOX*, has

been implicated in the SS and Madelung wrist deformity associated with TS.

The 50th percentile for adult TS women on the TS growth chart is 143 cm (56.3 inches).<sup>7</sup> The 95th percentile is 154 cm (60.5 inches), and the 3rd percentile is 132 cm (52.0 inches). The 50th percentile for normally growing girls is 164 cm (64.5 inches). A 21-cm difference is present between normal adult women on the 50th percentile of the normal growth curve and TS adult women who fall on the 50th percentile. The adult height of TS women is proportional to the parents' heights. The mean parental height is a good guide as to the ultimate extent of the SS.

Although the basic growth disturbance is most likely not a result of hormonal deficiencies, the possibility of associated growth hormone (GH), T<sub>4</sub>, or triiodothyronine (T<sub>3</sub>) deficiency should be considered, as should renal acidosis, diabetes mellitus, ulcerative colitis, and Crohn's disease. Thyroid disease is the most frequent contributor to the innate SS.

GH given in pharmacologic doses, although expensive, increases the expected adult height. GH-treated patients in the National Collaborative Growth Study<sup>7</sup> had an 8.4-cm mean increase over their baseline projected adult height; subjects receiving both GH and oxandrolone had a 10.3-cm increase. Currently, studies are in progress in which TS children are begun on GH treatment in early childhood instead of at a minimum of 9 years of age, as in previous studies. It is possible that SS will be less of a consideration in TS patients in the future as a result of early diagnosis and treatment.

*Hypogonadism* is the next most frequent characteristic of TS. It results from involution in utero of the ovarian follicles, although some follicles may not become atretic for ≥10 years. The result is hypergonadotropic hypogonadism. The uterus and vagina develop normally, but the uterus

appears infantile in those adults who have complete lack of endogenous estrogen secretion until appropriate exogenous estrogen is administered. Normal uterine structure results. Gonadotropin levels, particularly follicle-stimulating hormone (FSH) levels, are elevated, even in the presence of spontaneous menses, which occur in 5% to 10% of 45,X individuals and in 20% to 30% of those with mosaicism. Those menstruating tend to develop menstrual irregularity and dysfunctional uterine bleeding associated with premature ovarian failure. Ovulation and fertility are rare. In a review in 1990, Kaneko et al<sup>8</sup> reported a total of 138 pregnancies in 62 women who were recorded in the literature. Eighty-two of the pregnancies produced liveborn infants, 23 of whom had congenital anomalies. Ten of these 23 had chromosome abnormalities. In a study by Sylven et al,<sup>9</sup> 4 (all mosaics) of 49 TS women conceived. All 8 children had normal karyotypes. Since an increased incidence of nondisjunction for both sex chromosomes and autosomes occurs in all types of TS, prenatal screening for chromosomal anomalies is important in any TS woman who is pregnant.

In vitro fertilization using donor oocytes has been successfully utilized multiple times in the last 10 years. Preparation of the uterus requires significant attention, but TS women can be optimistic when using assisted reproductive technologies at centers with excellent records for success. Women who are ovulating can probably enhance their chance of pregnancy by oocyte removal, in vitro fertilization, and implantation. Multiple oocytes and/or embryos sometimes can be organized, preserved, and reintroduced into the TS patient, but only 1 implant should be placed in the uterus per pregnancy because such pregnancies are considered high risk.

The goal of estrogen and progesterone therapy is to correct the sex steroid deficiencies in a manner that optimizes height potential, permits attainment of normal bone mass,

## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

### Letter From the Editors:

The Editorial Board wishes to express its gratitude to Genentech, Inc. who for 16 years has supported publication of *Growth, Genetics & Hormones*, and is again supporting this year's publications through an unrestricted educational grant. This sponsorship has been without exerting influence or opinions about what the Editorial Board selects to publish or elects to comment about in its "Editor's Comments," which follow each abstract.

One year ago, Schwarz Pharma, Inc. of Monheim, Germany, sponsored distribution of *GGH* to pediatric endocrinologists who are members of the European Pediatric Endocrine Society. Schwarz Pharma, Inc. continues such sponsorship in 2001.

The Editorial Board invites all readers to write to the Editorial Board with suggestions for improving the publication. Write in care of Dr. R. M. Blizzard, 1224 West Main Street, Suite 701, Charlottesville, VA 22903. The Board also requests that you express your gratitude to those associated with Genentech, Inc. and Schwarz Pharma, Inc. for their sponsorship of what we hope you believe is an outstanding educational publication.

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and provides feminization with minimal risk of adverse effects. The available data suggest that treatment should be initiated between 12 and 14 years of age. Estrogen is generally started in a dose lower (0.325 mg conjugated estrogens or the equivalent) than necessary for adult replacement, and the dose is advanced every few months until the onset of menses. Cycling with progesterone (eg, oral medroxyprogesterone acetate 10 mg from days 16

through 25) is used to induce normal menstrual cycles and reduce the likelihood of uterine malignancy in the future. A full adult dosage (0.625 mg conjugated estrogens or the equivalent) should be established and daily estrogen given continuously. Determining how rapidly to increase the estrogen dose to achieve full replacement requires some individualizing, taking into consideration the height attainment desired, the patient's psychological need for feminization to occur, and the degree of osteoporosis present.

The duration of sex hormone replacement in adulthood required to minimize the adverse consequences resulting from sex hormone deficiencies is not clearly defined. It is now recognized that estrogen is desirable for prevention of osteoporosis and cardiovascular disease. Converging evidence based on the studies of older women suggests a potential benefit of estrogen on mood and cognitive function, including both verbal and nonverbal memory, of postmenopausal women.<sup>10,11</sup> Since estrogen replacement in adult TS women also normalizes body composition, physical fitness, and liver function,<sup>12</sup> one can make a strong case that all patients with TS should be on long-term steroid therapy (ie, at least until 50 or 60 years of age). Consideration of long-term estrogen therapy necessitates that the risk-to-benefit ratio continues to be analyzed. This is an ongoing process involving the choice of compounds, the route of administration, the dose, the schedule, and the outcome variables to be monitored under research protocols.

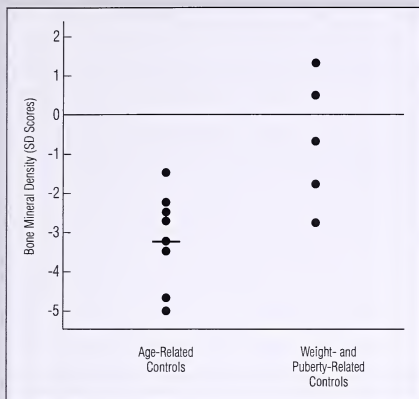
Questions concerning possible androgen replacement therapy have been raised since androgen production is decreased in TS as a result of atrophic ovaries. Currently, studies are under way exploring this issue and no specific recommendation can be made at this time.

*Osteoporosis* is reported to be very frequent in TS women. Controversy exists as to the etiology.<sup>13</sup> Among the possibilities are estrogen deficiency, a primary structural defect of bone, a derangement in the GH/insulin-like growth factor (IGF-I) axis, and/or a methodologic error in calculating BMD. aBMD is used in evaluating BMD in childhood instead of volumetric BMD (vBMD) because it is less dependent on the size and shape of the bones and the body.<sup>14</sup> Unequivocally, adult TS women have an increased incidence of various orthopedic problems, including scoliosis and fractures of the spine, metacarpals, and all long bones of the extremities. Ten percent to 15% of TS women require bracing or surgery. The relative risk (RR) for osteoporosis is 10, and that for fractures 2. Interestingly, collapse of vertebrae is an infrequent occurrence.

The effects of GH and estrogen have been evaluated. Lanes et al<sup>13</sup> compared aBMD of the lumbar spine and femoral neck in patients before beginning estrogen therapy at adolescence and after a mean of 6.1 years of significant estrogen treatment. The aBMD, as plotted for age,



Figure 2  
Bone Mineral Density



Bone mineral density of the lumbar spine represented as standard deviation (SD) scores compared with age-, weight-, and puberty-related controls. The mean is shown by the short horizontal bar.

Reprinted with permission from Lanes R, et al. *Fertil Steril* 1999;72:896-9.

weight, and puberty-related controls, was reduced initially (Figure 2)<sup>13</sup> and did not change with treatment. Bertelloni et al<sup>14</sup> studied the vBMD in young TS women treated with estrogen replacement therapy (ERT) or ERT plus GH. In this well-designed study, the investigators reported that TS patients on ERT from adolescence had normal vBMD values in young adulthood and that their bone densities were related to their years on ERT. Higher vBMD values in patients started on ERT before the age of 14 years could not be confirmed. The authors also noted a difference between the ERT group and the ERT plus GH group that suggested that GH combined with ERT enhanced aBMD. However, this apparent increase in aBMD may be related to the increase in height. This would result because of the influence of height on the calculation and not because GH induced a significant true increase in the collection of bone mass. vBMD differences were minimally increased in this study, but Bertelloni et al stated that vBMD differences possibly could be significant if larger numbers of patients were studied. One of the authors' conclusions was that "since these data and those of others show a methodologically related low aBMD but a normal corrected vBMD, it is unlikely there is an intrinsic feature in TS of bone demineralization."<sup>14</sup>

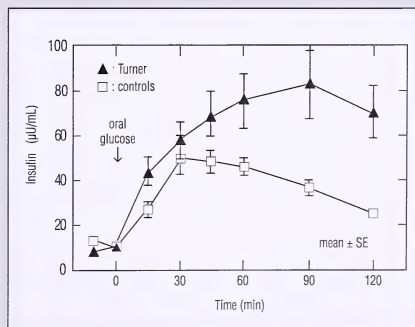
The conflict of data and interpretations will be resolved only with collaborative efforts, that is, by sharing data and rethinking discrepant interpretations. However, from a practical viewpoint, ERT and GH therapy are both desir-

able as both have a long-known positive effect on bone structure, and estrogen prevents the development of postmenopausal osteoporosis. Consideration should be given to use of other antiosteoporotic drugs in adult TS women with previous osteoporosis and/or fractures.

*Diabetes mellitus, hyperinsulinemia, and insulin resistance* have an increased incidence in adult women with TS. Diabetes mellitus type I (insulin-dependent; IDDM) was reported in 9 of 594 TS women in Denmark<sup>3</sup> for an RR of 11.56 as compared with the general population of women. The RR for type II (non-insulin-dependent) diabetes mellitus (NIDDM) was 4.38. Previously, type I diabetes, which by definition requires insulin dependence, reportedly has not been increased, nor have islet cell antibodies, which reflect autoimmune diabetes mellitus, been found in increased incidence in IDDM. The Danish population may differ from most other populations in this respect.

NIDDM, insulin resistance, and hyperinsulinism in the TS population is well recognized (Figure 3).<sup>15</sup> Abnormal glucose tolerance, in association with a 2-fold risk of NIDDM, has been detected in up to 50% of TS women even before ERT.<sup>3,15</sup> A positive correlation between insulin resistance and an increased weight over percent ideal body weight is consistent with the well-known association between increased body mass index (BMI) and insulin resistance.<sup>15</sup> BMI standard deviation (SD) scores are increased starting in adolescence in TS women who have or have not received GH.

Figure 3  
Insulin Resistance

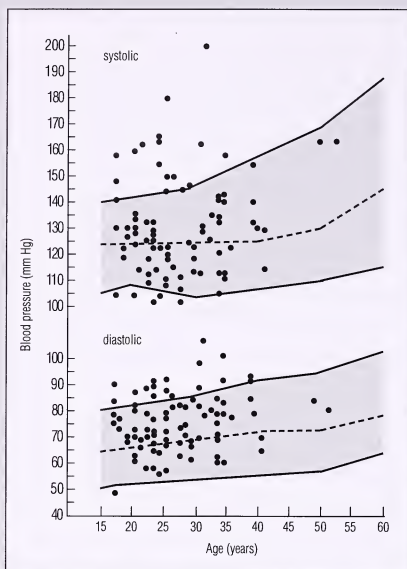


Insulin release following stimulation by oral glucose in 24 adult TS women (filled triangles) and 10 control women (open squares). In the Turner group, insulin release is significantly elevated ( $P < 0.006$ ) and the secretory response delayed peak concentration reached after 90 minutes versus 30 minutes in controls.

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Figure 4  
Distribution of Blood Pressure in TS



Shaded areas 5th to 95th percentile for UK population. Using diastolic BP of 90 mm Hg only 5 (5.5%) would be classified as hypertensive. With age-related percentiles 17 (15.5 %) were hypertensive. A similar underestimate of hypertension is observed with systolic blood pressure.

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TS women are at increased risk for developing *syndrome X* (insulin resistance, hypertension, obesity, and hyperlipidemia). Hyperlipidemia characterized by hypercholesterolemia also starts at adolescence and often precedes estrogen administration. Up to 50% of TS women have been reported to have hyperlipidemia.<sup>15</sup> Hypertriglyceridemia also is frequent and may be a component of syndrome X.

The incidence of *hypertension* is increased (Figure 4).<sup>16</sup> More than 30% of TS women have a systolic and/or diastolic pressure above the 95th percentile.<sup>16</sup> Nathwani et al<sup>17</sup> reported that more than 50% did not have the normal diurnal variation in blood pressure. Hypertension was independent of obesity in one study.<sup>16</sup> The hypertension in TS is generally idiopathic rather than secondary to vascular anomalies, although this should be considered. The idiopathic hypertension is probably related through as yet undefined mechanisms to the BMI and insulin resistance, and it may be underrecognized because of the failure to

compare pressure readings with age-matched normal ranges (Figure 4).<sup>16</sup> Medical intervention with optimal control of elevated blood pressure is essential for prevention of the cardiovascular sequelae of hypertension.

*Autoimmune thyroid disease* is very common in TS but is infrequently associated with other autoimmune endocrine diseases.<sup>4,15</sup> Frequently, antithyroglobulin or antiperoxidase antibodies, or both, are elevated. More than 35% of adult TS women will have clinical or subclinical thyroid disease. Chronic lymphocytic thyroiditis, hypothyroidism, and thyrotoxicosis occur in that order of frequency. Screening with both types of antibody tests and palpation of the thyroid are strongly recommended every 2 years in adult TS women. The presence of goiter with or without thyroid antibodies or abnormalities of thyrotropin or T<sub>4</sub> is an indication to use levothyroxine therapy to prevent subtle hypothyroidism from occurring.

### NONENDOCRINE-ASSOCIATED DISEASES AND/OR SIGNS AND SYMPTOMS PRODUCING CONSEQUENCES IN TS ADULTS

*Cardiovascular diseases, both congenital and acquired*, are important causes of mortality and morbidity in adults. Several review and original references are provided for readers who wish to expand their perspective.<sup>2,5,16,17</sup> Patients with TS have an increased risk of congenital malformations of the cardiovascular system, particularly of the heart. Coarctation of the aorta is the most common anomaly, occurring in 15% to 30% of patients, but often it is not clinically important. A positive correlation has been made between webbing of the neck and aortic coarctation. Patients with the 45,X karyotype are predominantly affected, as is true for other congenital anomalies. Approximately one third of TS patients have bicuspid aortic valves and one quarter have mitral valve prolapse. Cardiac physiologists believe that the bicuspid valve permits a jet of greater than normal pressure against the ascending aorta, and atherosclerosis results. Affected patients should receive prophylactic antibiotics at times of dental or other surgery. All TS patients who were not previously evaluated with cardiac ultrasound should be tested periodically.

Aortic aneurysms occur with and without aortic coarctation. Cystic medial necrosis has been reported in patients with dissection, which suggests that a more generalized congenital vascular dysplasia may exist. This could account, at least in part, for the increased incidence of strokes and cerebrovascular disease. Other contributing factors to vascular disease in various patients are hypertension, obesity, and insulin resistance. The increased risk of heart disease and atherosclerosis in TS is consistent with the recent finding from death certificates "demonstrating that approximately 50% of all deaths were caused by cardiovascular disease occurring 6 to 13 years earlier than expected."<sup>1</sup>

Aortic root dilation occurs with a reported prevalence of between 8% and 28%. Dilation or dissection may occur at any age. *The risk of dissection in the presence of dilation may be as high as 60%. Therefore, regular surveillance of adult patients with TS is recommended.* Magnetic resonance imaging (MRI) should be used if aortic root dilation is detected by ultrasound to assess the severity and provide more precise measurements for follow-up.<sup>4</sup> Coronary heart disease may be twice as likely to occur in TS patients compared with matched controls, according to an extended study reported by Gravholt et al.<sup>3</sup> However, the true incidence of ischemic heart disease in adult TS women remains unknown. These women have several risk factors that make them candidates for premature coronary thrombosis and/or ischemic heart disease. These risk factors include insulin resistance, hyperlipidemia, hypertension, estrogen deficiency, and obesity. For practical purposes, these women should be considered at high risk for coronary thrombosis as well as other vascular diseases.

Less life-threatening vascular malformations that have been reported include hemangiomas, intestinal telangiectasia, venous ectasia, lymphangiectasia, and gastrointestinal lymphangiomas, which can produce protein-losing enteropathy. Lymphedema of the hands and feet, another vascular anomaly, sometimes persists into adulthood and is a site of skin breakdown with superimposed infection, a recurrent aggravation to those who have it. Treatment is difficult.

Partial or extensive *deafness* plagues adult TS women. Sixty-one percent had some hearing loss in one study and 27% required hearing aids, which is probably an underestimate.<sup>9</sup> The hearing loss may be conductive and/or sensorineural. Because of the anomalous eustachian tubes in TS children, multiple ear infections contribute to the deafness in adulthood. The sensorineural loss appears to have other genetic determinants. A characteristic sensorineural dip in the mid-frequency range is frequently identified. Unfortunately, this type of sensorineural hearing deficit results in hearing speech poorly, does not lend itself to improvement with hearing aids, and has been related at times to diminished psychological well-being in TS. Audiologic evaluation and follow-up by an otolaryngologist, as indicated by the initial exam, is highly desirable.

*Renal anomalies (and resultant disease)* are particularly frequent in those with the 45,X karyotype. Between 30% to 60% of TS patients have anomalies such as horseshoe kidneys, double collecting systems, and malrotation. Six percent to 10% of adult TS women may have silent hydronephrosis. Consequently, *ultrasound studies are indicated at least once in adult life and should be repeated 10 years later* even if no silent hydronephrosis was observed the first time. If the collecting system is found to be abnormal, regular screening for urinary tract infection should be initiated. The hypertension observed frequently

in TS is seldom of renal etiology, but this possible cause should always be in the differential diagnosis.

*Skin anomalies* in TS adults include an increased tendency to extensive keloid formation, which must be taken into account when considering cosmetic surgery on the face and/or a webbed neck. Extensive pigmented nevi occur in late adolescence or early adult life, but malignant degeneration does not seem to be a problem. The residual lymphedema mentioned previously can be a difficult problem to treat.

*Hepatic and gastrointestinal disease* need to be considered in adults with TS, although life-threatening complications are believed to be only minimally increased.<sup>9</sup> Cirrhosis, hepatitis, and colon carcinoma are not mentioned in the review by Hall and Gilchrist<sup>1</sup> or the article by Price et al<sup>2</sup> dealing with mortality ratios, life expectancy, and causes of death in patients with TS, although nonspecific inflammatory disease of the bowel had been reported by Price in 1979.<sup>18</sup> Gravholt et al<sup>3</sup> reported an RR of 5.69 for cirrhosis and 2.25 for ulcerative colitis. Elevated liver enzymes have been reported in children and adolescents even before ERT, and in one report there was a conspicuous rise in serum liver enzyme levels occurring with treatment with conjugated estrogens.<sup>19</sup> No essential morphologic equivalent was found in liver sonography or biopsy specimens in a population of tall females receiving a 6-fold larger dose. Wemme et al<sup>19</sup> concluded that since the underlying mechanism is unknown, the possibility of an adverse long-term effect cannot be ruled out. They conjectured that using transdermal estrogen therapy may be preferable to oral or injectable estrogen, as the latter passes in large doses through the portal vein into the liver, while transdermal estrogens do not.

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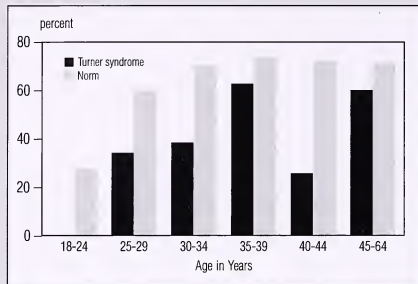
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Figure 5  
Percent of Turner syndrome women married, by age group, as compared with US census (1992) data.



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*Neurodevelopmental characteristics* of TS include impaired visuospatial abilities, leading to poor scores on nonverbal IQ exams and academic difficulty with mathematics and geography. These observations led to reports of frequent mental retardation. However, as Hall and Gilchrist<sup>1</sup> stated in their review, the presence of moderate to severe mental retardation is either not increased or increased only slightly in TS. On full-scale IQ testing, the total IQ is average or above. In 1995, Ross et al<sup>20</sup> reported concerning the neurodevelopmental changes from childhood through adolescence in 56 TS girls. The results demonstrated consistent findings across the age ranges studied (6 through 14 years). Verbal and language skills resembled those of 100 control girls. Performance IQ was relatively depressed, as were tests of visuomotor skills and attention. TS subjects also showed evidence of multifocal or diffuse right cerebral dysfunction. The constellation of neurocognitive deficits observed in TS is most likely multifactorial and relates to a complex interaction between genetic abnormalities, hormonal deficiencies, and other unspecified determinants of cognitive ability.

Several groups followed TS women through adulthood and reported increased social isolation and low self-esteem.<sup>9,21,22</sup> In adulthood, TS women were less likely to date or be married or to be involved in a sexual relationship. Sylven et al,<sup>9</sup> reporting on 49 adult TS women, determined that 31 had been or were married, 6 had become divorced, all had completed elementary school, 8 had university degrees, and 46 were employed. In an excellent study, Pavlidis et al<sup>22</sup> confirmed these data in a series of 80 adult TS women. Thirty-six (45%) reported seeking professional help primarily because of depression or, to a lesser extent, stress/anxiety, independence issues, help in coping with TS, or shyness. Sixty-eight (85%) were

employed with 52% in professional positions. Forty-three (54%) were married (Figure 5). Of the entire group, 45% had not experienced intercourse. *In working with these women, their strengths should be emphasized and their limitations such as visuospatial deficits recognized so that unreasonable expectations are not made by their teachers, employers, parents, and spouses.*

## CONCLUSION

*Women with TS should not be regarded as a group but as individuals with different skills and abilities, and they should be treated according to age and not height. They want to know about TS; and if one does not tell them, they will go to the library or the Internet to find out for themselves, and often obtain incorrect information. Clearly, patients and physicians need to enhance their knowledge about the variety of medical and social problems associated with TS.<sup>9</sup> In conjunction with this approach, this author wishes to encourage the utilization of contact groups such as the Turner's Syndrome Society of the United States (<http://www.turner-syndrome-us.org>).*

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## In Future Issues

**Circadian Rhythms and the Genes and Clinical Conditions Related to These:** Scott A. Rivkees

**Androgens in Puberty:**  
**Role in Metabolism and Growth:** Nelly Maurais

**Endocrine Complications of the Successful Treatment of Neoplastic Diseases in Childhood:** Charles Sklar

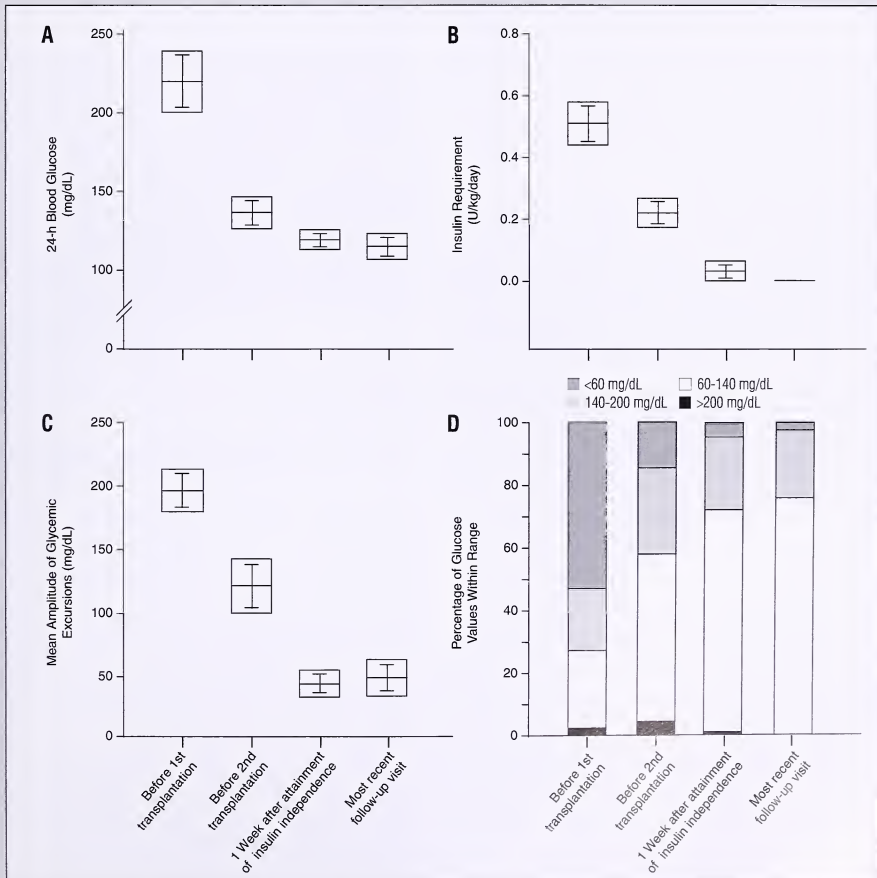


# Islet Transplantation in Seven Patients With Type I Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen

This very important study and report records a significant advancement in islet cell transplantation of tremendous potential. The apparent success of exceeding the usual limited success of islet transplantation is attributed by the authors, first, to transplanting an adequate number of islet cells and, second, to the replacement of glucocorticoids as the immunosuppressive agents with more recently designed nonsteroidal immunosuppressive agents.

The subjects were 7 consecutive patients with type I diabetes of more than 5 years who had essentially no stimulated C-peptide, whose glucose concentrations remained uncontrolled despite insulin therapy, and who had recurrent severe hypoglycemia. The new immunosuppressive agents that were used were *sirolimus* at the usual doses, low-dose *tacrolimus*, and *daclizumab*, which is a monoclonal antibody against the interleukin-2 receptor. The islet cell infusions required 2 sep-

Figure



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arate transplants in 6 patients, and 3 in 1 patient. The percutaneous transhepatic approach was used to gain access to the portal vein into which the islet cells were infused and transported into the liver. The quantity of insulin-producing cells transplanted is approximately double that reported previously.

Not only did the patients have essentially no insulin requirements for the time intervals of follow-up (4.5 to 15.0 months), but they also had no hypoglycemic episodes. The resultant 24-hour blood glucose, insulin requirements, mean amplitudes of glycemic excursions, and percentage of glucose values within normal range are demonstrated in the figure. Toxicity over the short term (up to 15 months) was limited to the requirement for blood transfusions following islet cell infusions (corrected by experience by developing a gel foam pad to be placed with the infusion) and minor superficial ulcerations of the buccal mucosa that resolved after the dose of *sirolimus* was reduced and the capsule formulation of *sirolimus* was substituted for the liquid form. No cytopenia resulting from *sirolimus* was observed. There was effective immunosuppression with no apparent diabetogenic or significant toxic effects, and no evidence of graft rejection, which has been a problem in transplants previously performed utilizing earlier methods and agents.

Shapiro AMJ, et al. *N Engl J Med* 2000;343(4):230-238.

**Editor's comment:** This pilot study undoubtedly will lead to other studies that stand a good chance of confirming these rewarding preliminary results. Patient selection was such that the patients were desperately in need of help to control their diabetic symptomatology but had no significant sec-

ondary complications such as significant renal disease. Hopefully, this procedure will lead to an acceptable and readily available method of treatment for type 1 diabetic patients regardless of various parameters associated with the basic disease. The utilization of an acceptable nonorgan transplant for adolescents and possibly preadolescents stands a good chance of stabilizing the erratic glucose levels that lead to so many problems in adolescent patients, whose self-images deter them from taking insulin on a regular basis. Endocrinologists are inundated when diabetic patients, for many different reasons, fail to adhere to their treatment regimen. The authors point out that availability of cadaver pancreases is greater than one might think. Fewer than one third of such available pancreases are actually transplanted. Therefore, islet cells can be made available to a significant extent.

The article's concluding paragraph is worth noting: "In patients with type 1 diabetes, glycemic control can be achieved with intensive insulin therapy and pancreatic transplantation. Intensive insulin therapy does not normalize glycosylated hemoglobin values and may cause severe hypoglycemia. Pancreatic transplantation provides excellent glycemic control, and although the outcome of the procedure has improved dramatically, it remains an invasive procedure with a substantial risk of morbidity. The findings published here indicate that islet transplantation alone is associated with minimal risk and results in good metabolic control with normalization of glycosylated hemoglobin values, and with sustained freedom from the need for exogenous insulin."

Robert M. Blizzard, MD

## Hypoglycemia: A Complication of Diabetes Therapy in Children

Because of their erratic activity and eating behavior, hypoglycemia in diabetic children is much more difficult to predict and, therefore, to prevent than pediatricians wish to tolerate. The consequences of hypoglycemia are the greatest in this youngest age group, where these problems are paramount. The authors focus on the whys, the wherefores, and the treatment, since hypoglycemia is the most common acute complication in insulin-treated type 1 diabetic patients. The younger the patient, the greater the frequency of both mild and severe hypoglycemia. Tighter glycemic control also is associated with increased frequency of hypoglycemia. Conversely, however, people with poor metabolic control whose glycosylated hemoglobin levels are high also are susceptible to severe hypoglycemia. Does hypoglycemia matter? The authors answer with a resounding yes! Symptoms are uncomfortable and carry the fear of loss of control or unconsciousness. Morbidity occurs frequently, and mortality sometimes occurs. In addition, sometimes the patient's fear of hypoglycemia is greater than the fear of future microvascular complications.

Previous and repeated mild hypoglycemia can induce hypoglycemia unawareness, thereby leading to diminished warning symptoms and impaired hormonal counterregulation. The

authors state that even mild hypoglycemia should be considered as having potentially dangerous consequences.

Following this introduction, they discuss the prevalence of hypoglycemia and begin by establishing definitions they believe should be used for "severe hypoglycemia." Some have defined the entity as an event that causes coma or seizures, while others have defined it as any episode that requires external assistance. The authors recommend that severe clinical hypoglycemia should include only episodes of unconsciousness because these can be ascertained consistently across all age groups, which is not possible with a less intense definition. "Mild chemical hypoglycemia" has been defined by some as glucose values below 54 mg/dL but not

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Volume 17, Number 1

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below 40 mg/dL, whereas others use the cutoff glucose level of 65 mg/dL. The authors argue that 60 to 65 mg/dL (3.3 to 3.6 mmol/L) should be used to define "hypoglycemia" whether the patient is symptomatic or not. Unquestionably, severe hypoglycemia is more frequent in adolescents than in adults, as was demonstrated in the diabetes control and complications trial. This was true whether the patients were in the intensive or conventional treatment groups. Data regarding the number of episodes of coma/seizure and also on moderate hypoglycemia per 100 patient-years were considered. The data are well worth reviewing in the original article, particularly by those who deal with diabetes frequently in their practice. The greatest frequency of severe hypoglycemia was found in children <6 years of age. By the fourth year of the study, this group had 42 events per 100 patient-years. This means that of 100 patients having the disease over a 1-year period, there would be 42 severe hypoglycemic episodes.

The authors consider under the causes errors in treating hypoglycemia, the pharmacokinetic and physiologic differences in

children with diabetes, and hypoglycemic unawareness. Considering the consequences, they discuss symptoms, changes in mental efficiency, and chronic brain dysfunction. In considering prevention, they state the key to prevention of severe hypoglycemia and associated complications is prevention of even mild episodes, which requires regular glucose monitoring, and the development of protective strategies on the part of the diabetic patient and family. Insulin regimens and diet and exercise also are considered in this section.

Becker DJ, Ryan CM. *Trends Endocrinol Metab* 2000;11:198-202.

**Editor's comment:** This article emphasizes the problems of insulin therapy in childhood. It follows the previous abstract (Shapiro et al) because of the potential relationship in future treatment of using islet cell transplants. If the reader has not read this article by Becker and Ryan and is treating children with diabetes, I strongly recommend that he/she do so.

Robert M. Blizzard, MD

## Who Wants to Be a Tissue Engineer?

Tissue engineering is a hot topic and not foreign to *GROWTH, Genetics, & Hormones* since many genetic disorders could potentially benefit from regenerated tissues and since tissue regeneration involves local growth and its hormonal control. Successes have been limited in stimulating regeneration of

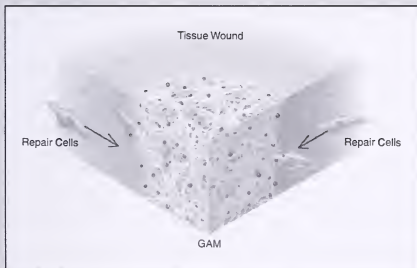
mammalian bone, skin, blood vessel, and spinal cord when bio-material scaffolds, which hopefully might bridge tissues to be regenerated and promote cell migration, proliferation, and differentiation, are used. While attractive, the use of growth factors to enhance regeneration has been hampered by difficulties in selectively delivering potential therapeutic agents at proper concentrations and for extended periods.

Bonadio and coworkers now offer a novel approach to local tissue engineering. The basic concept offered is to introduce plasmid DNA encoding the therapeutic factor into a biodegradable porous scaffold that is implanted into the region where regeneration is desired. The delivery system is called "gene activated matrix," or GAM (Figure 1). As cells grow into the scaffold, they take up the plasmid, express the plasmid DNA, and synthesize the recombinant therapeutic factor. Eventually, the scaffold is degraded as new tissue is formed.

At first glance, this seems too good to be true. However, Bonadio provides evidence that it works. Using wound healing as a model, the group has shown that fibroblasts growing into granulation tissue take up and express recombinant protein for weeks. Referring to earlier work using a canine bone defect model (Figure 2 on page 12), he notes that bone healing is much improved over controls by implantation of GAM-containing plasmids encoding BMP 4 or PTH fragment 1-34, and that the therapeutic effect is enhanced when the 2 plasmids are used together. The results suggest that GAM provides a dose-dependent, reproducible, and safe strategy for stimulating tissue regeneration.

After discussing the rationale for using GAM in wound healing and reviewing experiments with animals, Bonadio turns his attention to how GAM might be used in human medicine. He suggests that the first use of the approach may best be in situ-

Figure 1



The schematic figure shows a GAM implant in a fresh wound site (*inner area*). A GAM at its most basic consists of 2 ingredients: plasmid DNA and a structural matrix carrier. As part of the wound healing response, granulation tissue fibroblasts proliferate and migrate from viable tissue (*outer area*) surrounding the wound into the GAM. Once there, fibroblasts take up and transiently express plasmid DNA. The GAM matrix has 2 functions: it holds plasmid DNA in the wound site (until cells arrive), and it acts as scaffolding that promotes fibroblast ingrowth and accumulation near the DNA. While in the matrix, transfected fibroblasts act as local *in vivo* bioreactors, producing plasmid-encoded proteins that stimulate wound repair.

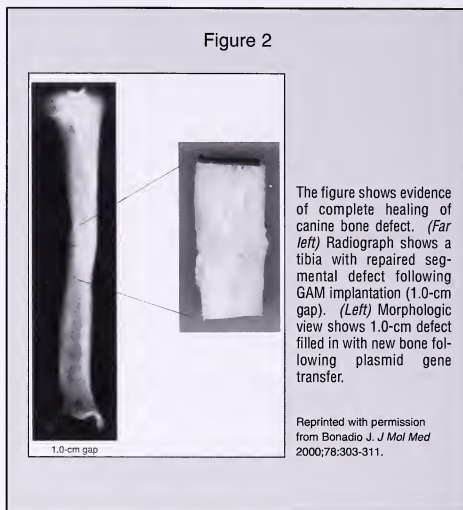
Reprinted with permission from Bonadio J. *J Mol Med* 2000;78:303-311.

ations in which wound healing is inadequate. He describes the rationale and strategy for using GAM containing PTH 1-34 plasmids to treat hip fracture in elderly individuals with osteoporosis—an exciting postulate.

Bonadio J. Tissue engineering via local gene delivery: update and future prospects for enhancing the technology. *J Mol Med* 2000;78:303-311.

**Editor's comment:** It is a long way from elderly osteoporotic patients with hip fractures to children with growth disturbances, but the principles involved in locally delivering plasmids encoding potentially therapeutic genes, as outlined in this article, may be applicable to a variety of disorders of interest to the GGH readership, especially for treatment of localized growth disturbances. The GAM technology is still in its infancy and remains to be proven safe and effective in humans, but the results presented to date are very encouraging. It is important to stress that determining which growth factors or, more likely, which combinations of growth factors are most effective for different clinical situations remains as big a challenge as developing the means to deliver such factors. The concept of being a tissue engineer may have much potential. After you read Bonadio's review you may agree.

William A. Horton, MD



## Long-Term Effect of Bone-Marrow Transplantation for Childhood-Onset Cerebral X-Linked Adrenoleukodystrophy (X-ALD)

The authors report that bone marrow transplantation (BMT) undertaken at the inception of neurologic symptoms in children with X-linked adrenoleukodystrophy (X-ALD) often can halt or reverse the progressive neurologic disease characteristics of this illness. However, the component of primary adrenal failure progresses. Eighteen boys aged 5.3 to 11.8 years with the slowly progressive form of cerebral disease or the advanced form of cerebral disease of X-ALD underwent BMT.

Six transplanted subjects died: 2 of complications of BMT, 2 with advanced cerebral disease, and 2 with slowly progressive cerebral disease that accelerated to advanced cerebral disease after BMT.

Twelve patients survived. Eight patients are in regular school classes; 1 has graduated from high school and attends college. The plasma concentrations of very long chain fatty acids (VLCFAs) decreased in all subjects after BMT. Magnetic resonance imaging (MRI) revealed decreasing myelinization for 1 to 2 years after transplantation; it then stabilized and even increased in 3 patients. Clinically, in 5 patients with mild corticospinal signs, resolution occurred in 3 and remained stable in the other 2. In 2 subjects, seizure control was greatly improved. Vision deteriorated in 3 patients. Verbal IQ (VIQ) scores remained stable after BMT in 10 of 12 subjects. In 5 of 11 patients tested, performance IQ (PIQ) increased by >10 points. In 4 of the 11, PIQ decreased significantly but then stabilized. Language skills, auditory processing, and motor performance increased appropriately over time in most patients. In the majority of a similar population of 13 boys with X-ALD

for whom no compatible marrow donor could be found, 7 have died, 4 are in a vegetative state, and 2 became stable after an initial period of deterioration. The investigators conclude that BMT early in the course of neurologic disease can alter the natural history of X-ALD.

Shapiro E, et al. *Lancet* 2000;356:713-718.

**Editor's comment:** The mutated gene (ALD, OMIM 300100) in boys with X-ALD encodes a peroxisomal membrane ATP-binding transporter protein that, when inactivated, impairs  $\beta$ -oxidation of fatty acids, resulting in accumulation of VLCFAs with 24 to 30 carbons. Esterified to cholesterol in the CNS and adrenal cortex, these compounds prove injurious to these tissues. Present data suggest that bone marrow cells cross the blood-brain barrier and attenuate the process(es) that lead to demyelination and neurologic deterioration in children with X-ALD.

The authors made an additional educational contribution by classifying the severity of X-ALD patients into 4 clinical categories. This classification currently exists in general for X-ALD and goes beyond the characterizations in the 12 patients reported. There is clinical value in this classification, which is repeated here.

1. Patients with no cerebral disease, with or without Addison's disease, in whom MRI and neuropsychological tests are normal. These are not candidates for BMT. About half of this group will develop neurologic signs involving the spinal cord in adulthood.



2. *Patients with slowly progressive cerebral disease, with or without Addison's disease. MRI shows slow progression of demyelination. BMT is to be considered. Disease severity is evaluated by scoring the extent of demyelination on the MRIs and performance on neuropsychological tests. MRIs are scored using a demerit scale ranging from 0 to 34 devised by Loes et al. BMT is recommended for patients whose cognitive abilities exceed a VIQ or PIQ of 80.*
3. *Patients with stable cerebral disease. Included are patients with MRI and neuropsychological abnormalities at diagnosis and in whom follow-up shows no evidence of MRI and neuropsychological deterioration. Close monitoring is required to detect change that may signal decline. (Not stated, but implied, is that those who are declining but whose IQ remains >80 might be candidates for BMT.)*
4. *Patients with advanced cerebral disease. These include patients with rapid progression of disease who decline rapidly to a vegetative state and have marked VIQ or PIQ dysfunction (<80) and neurologic signs. Current methods of BMT are not beneficial.*

The authors also state: "The absence of any correlation between the clinical phenotype and the ALD gene mutation or the biochemical defect, and the effectiveness of BMT ONLY at an early stage of the disease, lead us to recommend careful planning and frequent observation of all boys biochemically identified with X-ALD with normal brain MRI. No biological marker predicting the onset of cerebral demyelination is as yet available. Therefore continued MRI and neuropsychological testing are the only tools allowing the identification of patients who will benefit from BMT. Similarly no existing marker predicts whether or when a patient with a "slowly progressive cerebral disease" will enter into the "advanced cerebral disease" stage. Observations raised the hope that VLCFA could be decreased or even normalized by new pharmacological approaches. BMT, however, remains the only effective therapeutic approach in the cerebral form of X-ALD. The opportunity to recommend BMT at an early stage of cerebral X-ALD should not be missed."

Allen W. Root, MD

Loes DJ, et al. *Am J Neurol* 1994;15:1767-1771.

## Transmission of BSE (Bovine Spongiform Encephalopathy) by Blood Transfusion in Sheep

Houston et al published an early warning report before completion of a study that they were doing to look at cross-species transmission of bovine spongiform encephalopathy (BSE) through blood transfusion. This study was aimed at answering the question of whether there is a concern about blood transfusions transmitting the variant Creutzfeldt-Jakob (vCJD) disease in Britain from anyone living in Britain or who traveled in Britain between 1980 and 1996. Several countries have banned blood donations from people who spent time in Britain during the time of potential exposure to BSE.

Houston et al were engaged in a study to see if it is possible to transmit BSE between sheep by blood transfusion after the blood donor sheep had orally ingested the infecting agent. It turns out that sheep blood types are very complex, so this study was not a simple matter. It had been thought that there was a barrier to cross-species transmission of infectious agents. BSE-infected sheep harbor infection in peripheral tissues (tonsils, for example) prior to becoming symptomatic and thus are similar to humans infected with vCJD. A group of sheep were orally challenged with 5 g of BSE-affected cattle brain. At a later time, their blood was taken and transmitted into scrapie-free sheep. For the most part, whole blood was used for the transfusions and only a single transfusion was made. BSE clinical signs and pathologic changes have occurred in 1 of the sheep who received blood from a BSE-infected animal who was asymptomatic at the time of the transfusion. The donor had been challenged by oral BSE cattle brain 318 days before whole blood was taken. The BSE developed in the recipient animal 629 days after the transfusion. This suggests that the blood was taken from the orally challenged sheep halfway through the incubation period and yet it was nevertheless able to infect the recipient sheep.

This experiment does indicate that BSE can be transmitted between individuals of the same species by whole blood transfu-

sion and thus has implications for the blood transfusion system in general. The United Kingdom has been utilizing leukocyte-depleted blood; however, this may not be sufficient to avoid the problem.

A number of models have been utilized to predict the incidence of vCJD in the United Kingdom. There has been concern that as many as 500,000 individuals could become affected. The models have varying lengths of incubation and various calculations as to the number of people who would become infected and symptomatic after eating meat from an infected cow. The observed number of cases affected in early 2000 was 75 (Table). There appears to be a susceptible prion genotype, which is present in about 40%

Table  
Annual Number of Onsets, Classifications, and  
Deaths From vCJD in the UK

| Year  | Onsets | Classified as vCJD | Deaths |
|-------|--------|--------------------|--------|
| 1994  | 8      | 0                  | 0      |
| 1995  | 10     | 7                  | 3      |
| 1996  | 11     | 8                  | 10     |
| 1997  | 14     | 12                 | 10     |
| 1998  | 16     | 17                 | 18     |
| 1999  | 16     | 17                 | 14     |
| 2000  | 0      | 14                 | 14     |
| Total | 75     | 75                 | 69     |

Based on current classification criteria, applied retrospectively where appropriate.

Reprinted with permission from Andrews NJ, et al. *Lancet* 2000;451:482.



of the population. It is speculated that there are many consumers still at risk, but total vCJD mortality appears to be lower at this time than previously predicted.

Many pathologists have begun to screen tonsil and appendix tissue since they were found to be positive in 1 affected individual 8 months prior to the onset of vCJD symptoms. For practical purposes, no positive specimens have been found when doing population screening (~3,500 cases).

Andrews NJ, et al. Incidence of variant Creutzfeldt-Jakob disease in the UK. *Lancet* 2000;356:481-482.

Brown P. BSE and transmission through blood. *Lancet* 2000;356:955-956.

Dieter RS. Prion protein in tonsil and appendix tissue. *Lancet* 2000;356:505.

Ghani AC, et al. Predicted vCJD mortality in Great Britain. *Nature* 2000;406:583-584.

Houston F, et al. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000;356:999-1000.

Markham D. Prion protein in tonsil and appendix tissue. *Lancet* 2000;356:505-506.

**Editor's comment:** HIV and hepatitis have led to concerns about the safety of the blood transfusion system. This new report about blood transfusion transmission of prion disease in sheep is quite worrisome. There has not been a single documented case of human CJD, such as observed following contaminated GH injection, that could be related to blood transfusion. Nevertheless, it is of great concern from the standpoint of screening and excluding potential donors of blood products. It took Houston et al 3 years to produce 1 vCJD-positive sheep. Although methodologies to minimize the risk of blood transfusion are improving, there still is concern about whether an epidemic could occur. The good news is that the number of people affected with vCJD seems to be less than was predicted. The good news also is that many lessons are being learned about transmissible diseases, which is important for future public health practices.

Judith G. Hall, OC, MD

## Effect of Growth Hormone Treatment on the Adult Height of Children With Chronic Renal Failure

Previous studies have demonstrated that GH therapy increases the growth rate and improves standardized height in prepubertal children with chronic renal failure. What has not been known, however, is whether such therapy actually improves final height. It has been speculated that GH therapy could accelerate the onset or progression of puberty and negate any effect of early prepubertal treatment. Haffner et al report for the German Study Group for Growth Hormone Treatment in Chronic Renal Failure their analyses of 38 initially prepubertal children with chronic renal failure who were treated with GH for 5.3 years until they reached their adult height. Their growth was compared with 50 matched children with chronic renal failure who were not treated with GH. Of note, the 50 children who did not receive GH had growth retardation that was less marked than that of the treated children.

All subjects in the study had chronic renal failure with a height SD of  $-2$  or below and a height velocity below the 25th percentile during the year prior to the onset of treatment. The 38 children (32 boys and 6 girls) who were treated with GH were  $10.4 \pm 2.2$  years at the initiation of GH and their bone age was  $7.1 \pm 2.3$  years with an SD score of  $-3.1 \pm 1.2$ . During the study, 11 of the children were started on dialysis and 9 subsequently received a renal transplant. GH was administered in a total weekly dose of  $0.33$  mg/kg body weight. Fifty children (31 boys) in the control group were matched with respect to age at first observation, underlying renal disease, treatment, residual renal function, and cumulative dose of glucocorticoids. They were not treated with GH because they had relatively little or no growth retardation at baseline. Standard anthropometric measurements were obtained at 3- to 6-month intervals during the study and bone age was determined by the Tanner-Whitehouse II (TW2) method approximately every 12 months. The genetic target was calculated as a midparental height  $+10$  cm for boys and  $-2.6$  cm for girls.

During the prepubertal observation period, height velocity in the GH-treated children increased over baseline and exceeded

values in both the controls and in normal children. After the prepubertal peak, the height velocity decreased until the start of the pubertal growth spurt. The total height gained during the prepubertal observation period was twice as much as that

Table  
Predictors of Growth During the Observation  
Period in the Growth Hormone-Treated  
and Control Children Combined

| Period and Predictor  | Effect   | Partial R <sup>2</sup> | Cumulative R <sup>2</sup> | P Value |
|---|----------|------------------------|---------------------------|---------|
| Prepubertal period (change in cm of height)                   |          |                        |                           |         |
| Increased duration of prepubertal period                      | Positive | 0.67                   | 0.87                      | <0.001  |
| Increased duration of growth hormone therapy                  | Positive | 0.13                   |                           | <0.001  |
| Greater initial target-height deficit                         | Positive | 0.04                   |                           | <0.001  |
| Greater % of time spent on dialysis                           | Negative | 0.03                   |                           | 0.006   |
| Pubertal growth period (change in cm of height)               |          |                        |                           |         |
| Increased duration of pubertal period                         | Positive | 0.45                   | 0.61                      | <0.001  |
| Increased duration of growth hormone therapy                  | Positive | 0.11                   |                           | <0.001  |
| Male sex  | Positive | 0.05                   |                           | 0.005   |
| Total observation period (change in cm of height)             |          |                        |                           |         |
| Greater initial target-height deficit                         | Positive | 0.68                   | 0.78                      | <0.001  |
| Increased duration of growth hormone therapy                  | Positive | 0.06                   |                           | 0.002   |
| Greater % of time spent on dialysis                           | Negative | 0.04                   |                           | 0.004   |
| Total observation period (change in standard deviation score) |          |                        |                           |         |
| Increased duration of growth hormone therapy                  | Positive | 0.58                   | 0.64                      | <0.001  |
| Greater initial target-height deficit                         | Positive | 0.06                   |                           | 0.008   |

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in the control children. During puberty, peak height velocity was not significantly higher in the GH-treated children than in the controls. The onset of the pubertal growth spurt was delayed in these children by approximately 2½ years (compared with normal children) and the duration of the growth spurt was 1.6 years shorter compared with that of normal children.

The total pubertal height gain was similar in the GH-treated and the control children, but was 65% of that in normal children because the pubertal growth spurt was shorter.

Catch-up growth was sustained in the GH-treated children whereas the control children had progressive growth failure. The standardized height increased from the baseline mean of -1.4 SD. The mean final height was 1.6 SD below normal in the treated group, whereas in the control children the standardized height decreased by a mean of 0.6 SD to a final mean adult height of 2.1 SD below normal. Sixty-five percent of the GH-treated children reached an adult height within the normal range, but the mean final adult height was approximately 10 cm below the genetic target height for boys and 12 cm below the genetic target height for girls. The final height in the control children was 15.8 cm lower than the genetic target in boys and 16.1 cm lower than the genetic target in girls. Although the bone age increased faster during the prepubertal period in the GH-treated children than in the controls, it did not reduce overall height gain. Multiple regression analysis revealed that the absolute as well as the standardized height gain during the observation period was significantly associat-

ed with the longer duration of the prepubertal and pubertal observation periods, a longer duration of GH therapy, a greater initial target height deficit, a lower percentage of time spent on dialysis, and male sex. These factors explain 61% to 87% of the variability in the outcome data.

The authors point out that this study provides evidence that GH treatment can sustain catch-up growth in the majority of children with growth failure due to chronic renal failure.

Haffner D, et al. *N Engl J Med* 2000;343(13):923-929.

**Editor's comment:** This is a particularly important study because it is the first to look at final height achieved in this population. Clearly, GH therapy is of significant benefit to final height in children with chronic renal failure. The particular strengths of this study are the variety of causes of chronic renal failure in these children and the careful matching of the etiologies between the treated and control groups. An unanswered question is the effect of GH therapy on adult height in children who begin such treatment during their pubertal years. The data in the current study cannot be used to answer this question. The children in this study had glomerular filtration rates of <60 mL/min/m<sup>2</sup>. It also will be important to evaluate the effect of GH therapy on children with lesser degrees of renal insufficiency but similar degrees of growth retardation.

William L. Clarke, MD

## The Impact of Recombinant Human Growth Hormone Treatment During Chronic Renal Insufficiency on Renal Transplant Recipients

Fine et al described the posttransplant outcome for renal transplant patients who were treated with GH therapy during the course of their chronic renal insufficiency. Subjects were identified from 2 control studies (n=194) and matched with patients in the North American Pediatric Renal Transplant Cooperative Study (NAPRTS) database; 95 "likely" matches and 7 "possible" matches were made. These 102 patients formed the GH-treated cohort group and were compared with a control group of 4913 transplant recipients in the database who did not receive GH therapy during their chronic renal insufficiency. Interestingly, the treated cohort tended to have more males, a larger percentage of subjects between 6 and 12 years of age, and more (67% vs 45%) living parent donors.

Two deaths occurred in the cohort, after 78 days and 5 years. The survival rate for the cohort at 3 years was 98.9%, while that for the control group was 95.1%. In the cohort group, 11.8% of grafts failed; 21% of the grafts failed in the control group. There is no statistically significant difference between graft survival rates for either donor source. The percentage of failed grafts with chronic rejection as the cause was marginally significantly higher than in the control group ( $P=0.05$ ). However, the percentage of all grafts that failed as a result of chronic rejection was similar for the 2 groups (6.9 for the GH-treated cohort and 6.5 for the control).

The mean height Z score at 60 months was slightly improved in the treated group compared with a slight worsening in the control group. In both groups, the delta Z score was positive, indicating continued improvement from baseline. Adverse events in the treated cohort included 2 posttransplant lymphoproliferative disorders and 38 other events, including appendicitis, gastroenteritis, pneumonia, other infections, and hypertensive crisis.

There was no core of adverse events but a broad spectrum of unrelated events. The authors' data did not support the assertion that recombinant human growth hormone (rhGH) treatment during the course of chronic renal insufficiency predisposed to the development of malignancy after transplant.

The authors conclude that GH therapy was not associated with an increase in adverse effects on graft function, nor were there more malignancies posttransplantation. There were concerns that "catch-down" growth would occur after renal transplantation in individuals who received GH during renal insufficiency, which might nullify gains in height. These data do not substantiate these concerns.

Fine R, et al. *J Pediatr* 2000;136(3):376-382.

**Editor's comment:** The results are reassuring to physicians treating short children with chronic renal insufficiency with rhGH. Data from this study do not suggest a negative effect of such pretransplant therapy. Mean height scores in the treated group at baseline and 60 months posttransplant were  $-1.92$  and  $-1.90$ , as compared with the control group at  $-1.88$  and  $-2.10$ . Thus, gains made in height were not lost. This article and an accompanying article by Haffner et al (N Engl J Med 2000;343[13]:923-930) provide

significantly helpful and reassuring information regarding the safety and effectiveness of treating children with rhGH. The reader also is referred to a lead article in GGH (Vol. 12, No. 4, p 49) titled, "Recombinant Human Growth Hormone Therapy for Children With Chronic Renal Insufficiency: An Update 1996," which addressed the subject of rhGH treatment in chronic renal insufficiency.

William L. Clarke, MD

## Treatment of Acromegaly With Pegvisomant, a Genetically Engineered Human Growth-Hormone Receptor (hGHR) Antagonist

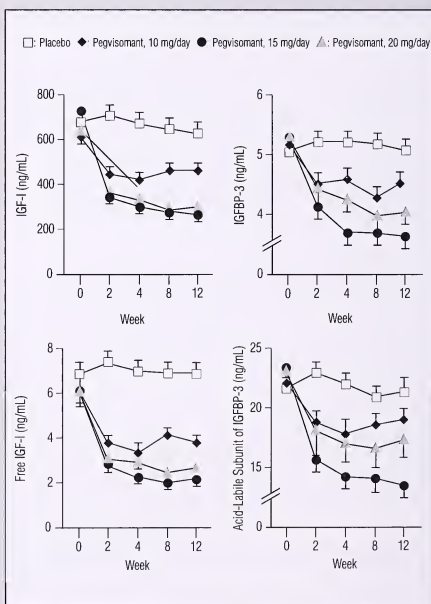
The present investigators report the beneficial effects of a GH receptor (GHR) antagonist in adults with acromegaly. Genetic engineering has permitted development of a mutated GH molecule with replacement of 9 amino acids that increases its affinity for one of the binding sites on the receptor and abolishes binding to the second site, thereby preventing functionally correct dimerization of the receptor. Polyethylene glycol polymers, which are covalently bound to a protein, are stated to be pegylated, thus, the name pegvisomant. Since the GHR is unable to dimerize, signal transduction is inhibited, leading to decreased IGF-I production.

In short-term (12-week) studies, 112 acromegalic adult subjects who had failed previous treatment (surgery and/or radiation and/or dopaminergic agonists, but not long-acting analogues of somatostatin) were divided into 4 groups, including a control group and 3 groups receiving different doses of pegvisomant. IGF-I concentrations (Figure) fell in a dose-dependent manner. Symptoms of GH excess ameliorated as there were significant decreases in soft tissue swelling, diaphoresis, and fatigue. The score for total symptoms and signs of acromegaly decreased significantly in all groups receiving the drug. As expected, serum concentrations of GH increased substantially during treatment in the patients who received 15 or 20 mg of pegvisomant. Anti-GH antibodies were noted in 5 patients but were without physiologic consequence. No patient had a significant change in tumor volume during the study. One patient had alterations in liver function while receiving this agent. No serious adverse effects were otherwise noted. The long-term consequences of the elevated GH concentrations remain to be determined.

Trainer PJ, et al. N Engl J Med 2000;342:1171-1177.

**Editor's comment:** Neurosurgical removal of GH-secreting pituitary adenomas has been and remains the primary mode of therapy for acromegaly. Medical treatment of hypersomatotropism has been reserved as secondary management; estrogens, dopaminergic agonists (bromocriptine, cabergoline), and short- and long-acting somatostatin analogues (depot preparations of octreotide and lanreotide) that impair GHRH release and inhibit its function at the somatotroph membrane have been employed to lower GH production and decrease IGF-I generation. The introduction of a GHR antagonist has expanded the therapeutic boundaries for this disease, which is so difficult to treat. In another study,

Figure  
Serum Concentrations of Insulin-Like Growth Factor I (IGF-I), Free IGF-I, IGF-Binding Protein 3 (IGFBP-3), and the Acid-Labile Subunit of IGFBP-3 in Patients With Acromegaly.



For all 4 measures, the values at all visits after baseline (week 0) were significantly lower ( $P \leq 0.05$ ) in the 3 pegvisomant groups than in the placebo group. T bars indicate means  $\pm$  SE.

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*Pegvisomant also lowered IGF-I concentrations and ameliorated symptoms in acromegalic subjects resistant to treatment with octreotide. Whether this GHR antagonist or later generations of GH antagonists will be useful in children is a matter for study. One hopes that such agents will not be employed to alter the growth of normally tall children, but its*

*use in other overgrowth syndromes will be of interest to explore in controlled settings.*

Allen W. Root, MD

Herman-Bonert VS, et al. Growth hormone receptor antagonist therapy in acromegalic patients resistant to somatostatin analogs. *J Clin Endocrinol Metab* 2000;85:2958-2961.

## Normal Growth Velocity Before Diagnosis of Celiac Disease

Celiac disease has been shown to result in nutritional growth retardation even in asymptomatic patients. However, there are instances in which this disease does not alter normal physical growth.

To evaluate height velocity of patients with confirmed celiac disease before and after diagnosis, anthropometric measurements were taken in 23 patients aged 0.1 to 10.66 years of age. All patients studied during the first 6 months of life showed normal growth velocity, and 6 of 10 patients showed normal growth velocity during the second 6 months of life. Ten of 12 patients between 1 and 2 years of age showed normal growth velocity and 7 of 9 patients aged 2 to 10 years also showed normal height velocity. The authors concluded that celiac disease could be present in children who are growing at a normal rate and that appropriate height and growth should not be factors that exclude the possibility of celiac disease.

*ence of short stature and delayed growth as 2 of the most important clinical manifestations of celiac disease, it is important to be aware of the existence of untreated patients who grow at normal rates. This paper clearly documents that this indeed occurs but is contrary to the usual clinical presentation. Normal growth found in patients with celiac disease requires an explanation. The length of the lesion in the small bowel could be a factor leading to normal or abnormal growth. In countries where the prevalence of celiac disease is high, clinicians should be alerted to the possibility of this disease in a normal, asymptomatic, short-statured child with a previous history of diarrhea or iron deficiency anemia.*

Fima Lifshitz, MD

**2nd Editor's comment:** Unfortunately, the authors made only a minimal statement regarding the weight-to-height relationship. Twelve of the 23 patients had normal height and height velocity at diagnosis. Of all the children, 6 also showed normal weight increments before diagnosis. We can only assume that the phenomenon described occurs in children of normal weight for height and in children of low weight for height.

Robert M. Blizzard, MD

Lejarraga H, et al. *J Pediatr Gastroenterol Nutr* 2000;30:552-556.

**Editor's comment:** This paper is interesting as patients with confirmed celiac disease were followed longitudinally with reliable anthropometric data. While most of us have stressed the pres-

## Nutritional Rickets in African-American Breast-Fed Infants

Kreiter and associates report the characteristics of infants and children diagnosed with nutritional rickets at 2 medical centers in North Carolina in the 1990s. Records of 30 children were reviewed; 57% of these presented in 1998 and 1999. All were black and all were breast-fed (average duration of breast-feeding, 12.5 months). Breast-feeding has increased significantly since 1988 (Figure) in North Carolina in both black and white women. Children older than 1 year had a history of poor intake of fortified cow's milk or other dairy products. The age of diagnosis ranged from 5 to 25 months, but one third presented at 12 months of age or younger. Sixty-three percent were diagnosed between April and October, some of the warmer spring/summer months in this southern area. As expected, presenting signs included skeletal abnormalities (n=16) such as bowing of the legs, flaring of the wrist, costochondral beading, fractures, failure to thrive (n=13), hypocalcemic tetany/seizures (n=2), and developmental delay (n=1). Length was <5th percentile in 17 of 26 of the infants (65%), and only 2 patients had a length >50th percentile. With the exception of 1 patient who had

recently begun vitamin D treatment, all patients had hypophosphatemia. Sixty percent had hypocalcemia, and 100% had elevations in alkaline phosphatase.

All of the children with rickets were breast-fed without vitamin D supplementation. A survey of 400 pediatricians in North Carolina revealed that 42% prescribed vitamin supplements for all breast-feeding infants, whereas 42% prescribed supplemental vitamins only for selected breast-feeding infants (ie, those with dark skin who are being exclusively breast-fed for more than 4 to 6 months or who are premature). The authors also note that the 1997 American Academy of Pediatric Policy Statement indicates that "vitamin D and iron need to be given before 6 months of age in selected groups of infants (vitamin D for infants whose mothers are vitamin D deficient or those infants not exposed to adequate sunlight)" but that no guidance is given as to how to test mothers for vitamin D deficiency.

Kreiter S, et al. *J Pediatr* 2000;137(2):153-157.

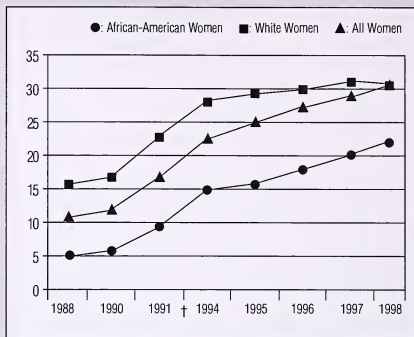


**Editor's comment:** Although this is primarily a descriptive report, the information provided is of significant importance not just to pediatric endocrinologists but to all physicians. In this editor's personal experience, I have seen 2 such children in the past 6 months (1 who was 13 months of age and 1 who was 4 years old). Of interest, the 4 year old was referred for evaluation of short stature and failure to thrive. His lower limb bowing and metaphyseal flaring were obvious at cursory inspection.

With the significant increase in breast-feeding, accompanied by a significant increase in public health warnings regarding the effects of excessive sunlight and the subsequent use of sunscreen on many infants, it is important that all physicians be aware of the possibilities of vitamin D-deficient rickets and that children be supplemented appropriately. In addition, it is important that the community and physicians be reminded of the signs and symptoms of this easily treatable cause of short stature. A study of subclinical rickets in both white and black infants who are breast-feeding would very possibly determine that the incidence of clinical or subclinical rickets is very significant in the latter group.

William L. Clarke, MD

Figure



Incidence of breast-feeding in African-American women in North Carolina, 1988 to 1998. Information for women seen for the maternal postpartum WIC visit. †Data not available for years 1992 to 1993.

Reprinted with permission from Kreiter S, et al. *J Pediatr* 2000;137(2):153-157.

## The Central Melanocortin System Affects the Hypothalamo-Pituitary Thyroid Axis and May Mediate the Effects of Leptin

In the fasted rodent, in the genetically leptin-deficient mouse (*ob/ob*), and in the genetically leptin-resistant mouse (*db/db*), there is secondary hypothyroidism. Kim and collaborators hypothesize that leptin may act through the melanocortin system (MS) upon pituitary thyroid-stimulating hormone (TSH) secretion in adult rats. The basis for the hypothesis is that the MS is known to mediate the inhibitory actions of leptin on feeding.  $\alpha$ MSH was administered by cannulae into the third intracerebroventricular (ICV) or into the intraparenchymal nucleus (IPVN), which regulate the secretion of pituitary TSH. Also, similarly injected was the *agouti*-related peptide (Agrp), which is an endogenous antagonist of melanocortin 3 and 4 receptors (MCR-3, MCR-4). When activated by  $\alpha$ MSH, these receptors inhibit feeding.

In vitro, Agrp significantly decreases plasma TSH concentrations in the *fed* animal when injected into the ICVN or IPVN. In contrast,  $\alpha$ MSH increased TSH levels in fasted rats. In vitro,  $\alpha$ MSH increased the release of thyrotropin-releasing hormone (TRH) from hypothalamic slices (Figure), an effect blocked by Agrp. In this in vitro system, leptin increased and Agrp blocked the release of  $\alpha$ MSH and TRH.

Therefore, the investigators concluded that leptin stimulates thyroid function by enhancing the production of  $\alpha$ MSH from pro-opiomelanocortin and possibly by blocking the synthesis of Agrp.  $\alpha$ MSH stimulates release of TRH, which increases TSH secretion. Consequently, the regulatory pathways for the

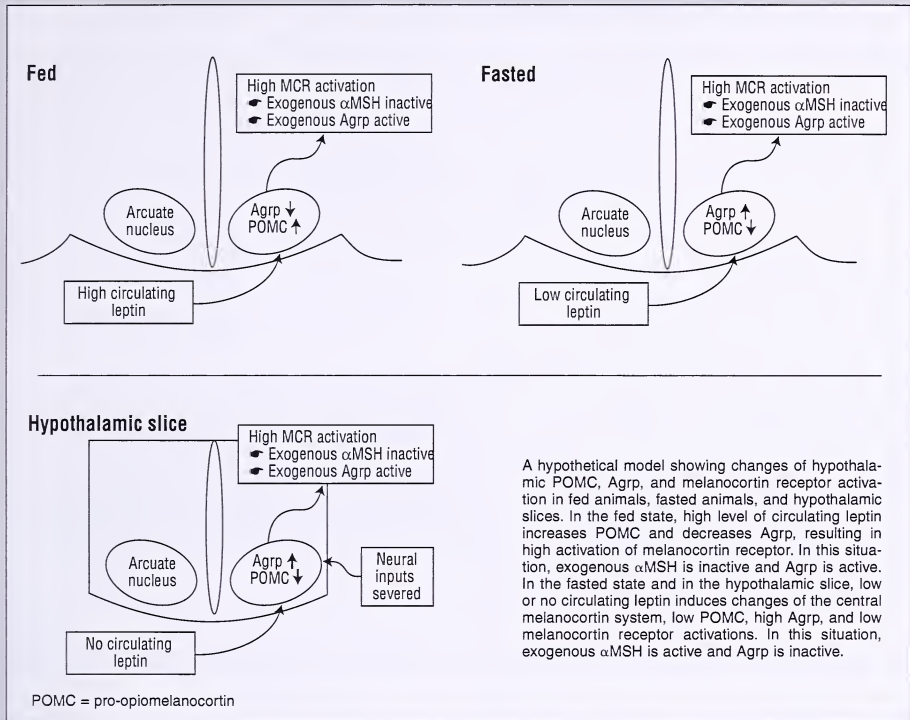
control of energy balance via food intake and food metabolism are linked.

Kim MS, et al. *J Clin Invest* 2000;105:1005-1011.

**Editor's comment:** In the starved state, the expression of TRH in the paraventricular nucleus is dramatically decreased, a response that can be reversed by the administration of leptin. In addition to the pathway through pro-opiomelanocortin and  $\alpha$ MSH synthesized in the arcuate nucleus, leptin likely acts directly on transcription of the gene encoding TRH (Figure).<sup>1</sup> Thus, changes in leptin secretion mediate the metabolic responses characteristic of the fed or starved states. Interestingly, MCR-3 and MCR-4 mediate different aspects of leptin- $\alpha$ MSH actions: MCR-3 affects feed efficiency—that is, the quantity of weight gained per calorie ingested—while MCR-4 influences the quantity of food ingested (or appetite) and energy utilization.<sup>2</sup> Not only does leptin mediate feeding behavior and energy expenditure by its central action, this fat-derived protein also influences bone mass in this manner. ICV administration of leptin inhibits bone formation in *ob/ob* mice by unknown mechanisms, while patients with a loss-of-function mutation in MCR-4 are obese and have a high bone mass.<sup>3</sup>

Besides its effects on melanin synthesis and dispersal by keratinocytes and on feeding mediated primarily by MCR-4,  $\alpha$ MSH, acting through 1 of 5 MCRs, reduces a number of

Figure



A hypothetical model showing changes of hypothalamic POMC, AgRP, and melanocortin receptor activation in fed animals, fasted animals, and hypothalamic slices. In the fed state, high level of circulating leptin increases POMC and decreases AgRP, resulting in high activation of melanocortin receptor. In this situation, exogenous  $\alpha$ MSH is inactive and AgRP is active. In the fasted state and in the hypothalamic slice, low or no circulating leptin induces changes of the central melanocortin system, low POMC, high AgRP, and low melanocortin receptor activations. In this situation, exogenous  $\alpha$ MSH is active and AgRP is inactive.

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inflammatory processes by lowering the production of several proinflammatory cytokines (including interleukin-1 $\beta$  and -6, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$ ) and the nuclear transcription factor NF- $\kappa$ B.<sup>4</sup> Serum concentrations of  $\alpha$ MSH are elevated in a number of inflammatory illnesses, including HIV infection, suggesting that  $\alpha$ MSH may be an important component of our innate host defense mechanisms. Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide that in fish causes the aggregation of melanin within melanophores and thus lightens the color of the fish scale, an effect opposite to that of and opposed by  $\alpha$ MSH.<sup>5</sup> Acting through G-protein coupled receptors, MCH increases food intake in rodents, an effect opposite to that of leptin and  $\alpha$ MSH. MCH also acts within the pituitary, where it stimulates corticotropin secretion. Thus, the regulation

of feeding and energy metabolism is becoming ever more complex.

William L. Clarke, MD

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**GROWTH, Genetics, & Hormones Volume 17, Number 1**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of this issue. Please follow the instructions listed there to receive CME Category 1 credit. Please note that a question may have more than one correct answer.

1. The adult with TS who has a ring X chromosome is more likely to have more congenital anomalies than one with a 45,X karyotype.
- True
  - False

2. (A) The surviving fraction of TS patients alive at 60 years of age in the study conducted by Price et al was \_\_\_\_\_.
- 52%
  - 68%
  - 88%

(B) The surviving fraction of the general UK population at age 60 years was \_\_\_\_\_.

- 52%
- 68%
- 88%

3. The areal BMD, which is the usual BMD measured, has been reported to be low in TS patients. However, the volumetric BMD, which is a calculated method, was not reported to be low.
- True
  - False

4. The short stature in TS patients is believed to be related to which one of these?
- Chondrodystrophy
  - GH deficiency
  - Absence of a *SHOX* gene that is located on the sex chromosome
  - Absence of the *SRY* gene
  - Autoimmune disease

5. The following diseases are frequently found in TS patients. Which is/are believed to be of autoimmune origin in these patients?
- Insulin-dependent diabetes mellitus
  - Non-insulin-dependent diabetes mellitus
  - Hypothyroidism

6. (A) In the literature in 1990 a total of \_\_\_\_\_ pregnancies were reported.
- <100
  - 100 to 150
  - >150

- (B) Of the pregnancies reported, approximately \_\_\_\_\_ produced a live neonate.
- 40%
  - 60%
  - 80%

7. Which of the following statements is/are correct?
- Aortic aneurysms occur with and without aortic coarctation.
  - The increased risk of heart disease and atherosclerosis in TS is consistent with the recent finding from death certificates that approximately 50% of all deaths in TS patients were caused by cardiovascular disease.
  - TS women should be considered at high risk for coronary thrombosis.
  - Between 30% to 60% of TS patients have renal anomalies; 6% to 10% of adult TS women may have silent hydronephrosis.

Answer Key: 1.b 2Ab 2Bc 3a  
4c 5c 6Ac 6Bb 7a,b,c,d

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Ross, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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## Androgens in Puberty: Roles in Metabolism and Growth

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### INTRODUCTION

Testosterone is the predominant hormone of male puberty, and it greatly impacts the transformation of a boy to an individual with full adult body composition and reproductive maturity. Most of its production comes from the testes in males, but some also comes from the adrenal glands. Androgens also play a role in female reproduction, and excessive production is a common reason for endocrine referral of adolescent girls. Many of the metabolic and growth effects of androgens in normal individuals and in those with altered physiology during this critical period of childhood are reviewed here. For brevity, most of the data reviewed apply to males unless otherwise stated.

### PHYSIOLOGY OF ANDROGEN PRODUCTION IN MALE PUBERTY

The prepubertal gonad is relatively quiescent prior to the onset of puberty in terms of sex steroid output. Testosterone concentrations are typically undetectable from about 3 months of age until puberty; however, it is clear that some testosterone must be produced to suppress gonadotropin output. This is evidenced by the marked increase in gonadotropins in patients with anorchism studied in early childhood, suggesting that the prepubertal gonad is active, albeit minimally, substantially before the onset of puberty.

At a mean of 11.2 years, the gonadotropin hormone-releasing hormone (GnRH) pulse generator (gonadostat) increases the amplitude of its pulses, generating increased production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), *particularly at night*. This generates

a marked increase in gonadal steroid production, with daily testosterone production rates in mature males of ~5 to 6 mg/d. Testosterone concentrations change rapidly from levels <10 ng/dL in most conventional assays in prepubertal males to concentrations of 350 to 970 ng/dL when the boy reaches Tanner stage V of sexual development. The pattern of testosterone production has both ultradian and circadian rhythms. Minute-to-minute changes in testosterone concentrations can be detected by using frequent sampling methods. Much greater concentrations are found in the early morning, as compared with the afternoon, in normal youngsters studied with blood withdrawn every 20 minutes for 24 hours.<sup>1</sup> These changes parallel the minute-to-minute changes observed in LH and FSH concentrations, which illustrates the functional coupling of these neuronal and hormonal events (Figure 1, page 22). We have observed levels of testosterone in the mid 600 ng/dL range in the early morning in normal boys in late puberty which drops to 50 to 60 ng/dL in the afternoon of the same day.<sup>2</sup> This underscores the need for early morning sampling of random testosterone concentrations when studying the pubertal progress of an adolescent male. What effect, if any, these marked hormonal changes may have on the metabolic effects of testosterone on, or on the typical mood swings of, the teenage boy awaits further study. These changes in testosterone concentrations within the day are not observed in the adult male, who characteristically has relative stability of testosterone concentrations throughout the day.

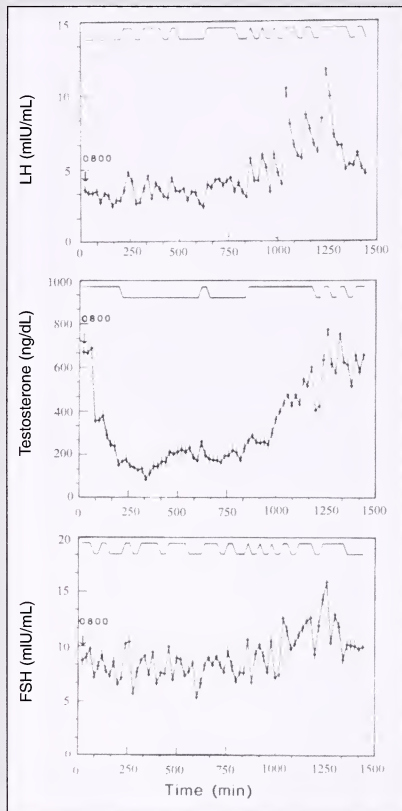
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Figure 1

**Luteinizing Hormone (LH), Testosterone, and Follicle-Stimulating Hormone (FSH) Concentration Profiles Measured Every 20 Minutes for 24 hours in 1 Pubertal 14-Year-Old Boy**



Note the striking decline in serum testosterone concentrations in the afternoon. Note the deflections at the top of each panel. These represent pulses detected by cluster methods.

Reprinted with permission from Mauras, et al.<sup>1</sup>

Testosterone concentrations markedly impact growth hormone (GH) release in puberty. Comparison of the pulsatile profile of GH concentrations between prepubertal and fully mature (Tanner stages IV and V) boys of similar age reveals that during puberty there is a substantial augmentation (more than doubling) of GH production

rates, mostly as an amplitude-modulated phenomenon, that is relatively independent of changes in GH pulse frequency.<sup>2</sup> This rise in GH production is replicated when testosterone is administered to hypogonadal boys<sup>2</sup> but not when nonaromatizable androgens like oxandrolone or dihydrotestosterone (DHT) are administered to similar subjects.<sup>3,4</sup> The impact of testosterone on GH release in puberty is abolished when pubertal boys are given an estrogen receptor blocker (tamoxifen)<sup>5</sup> and is increased when a nonsteroidal androgen receptor blocker (flutamide) is administered, the latter increasing testosterone and hence peripheral aromatization of androgens to estrogen.<sup>6</sup> Taken in aggregate, these data strongly support the concept that the impact of testosterone on GH release is mediated via aromatization to estrogens during puberty.<sup>7</sup>

## IN VIVO EFFECTS

Testosterone and its 5 $\alpha$  reduced metabolite, DHT, share a common androgen receptor (AR) transcribed from a single-copy gene in the X chromosome. The AR is a member of a superfamily of intranuclear receptors, including receptors for estrogen, progesterone, vitamin D, glucocorticoids, thyroid hormone, and retinoic acid. Testosterone binds to the AR and then activates a number of specific DNA sequences called androgen response elements. These initiate a complex signaling transduction cascade of events that result in the modulation of gene transcription and protein synthesis.

## GROWTH

Androgens are critical for a normal and timely pubertal growth spurt in males. This effect is largely mediated by the impact of gonadal steroids on GH production, as discussed above. However, there is evidence for a local effect of androgens on epiphyseal cartilage. Experimental data in human fetal epiphyseal chondrocytes reflect that both testosterone and DHT promote DNA synthesis and that DHT rather than testosterone appears to be the active androgen.<sup>8</sup> Also, using specific monoclonal antibodies against the human AR, experiments demonstrate that there are ARs in human bone in situ, providing evidence for a direct action of androgens on bone and cartilage cells.<sup>9</sup>

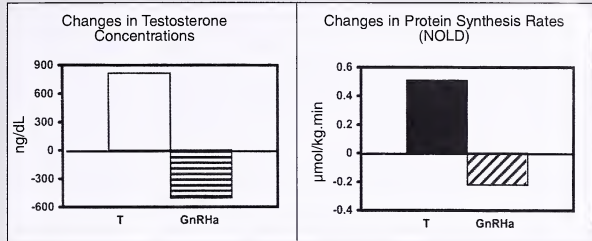
Both GH and testosterone appear to be indispensable for normal pubertal growth. This is evidenced by the lack of pubertal growth spurt in children with isolated GH deficiency<sup>10</sup> and by the much slower than normal pubertal growth pace of GH- and testosterone-deficient children who are treated with testosterone but not GH.<sup>11</sup> Even though these observations may be explained by the lack of increase in GH production typically observed in normal puberty, sex steroids probably directly influence the pubertal growth spurt even in GH-deficient states.<sup>12</sup> This apparent synergy of effects of GH and androgens on the pubertal growth spurt has prompted the study of the proper dosing of GH-deficient children in puberty. In a recent

Figure 2  
Effects of GnRHa Administration in Young Eugonadal Males

Left panel: Absolute changes in testosterone concentrations after 4 weeks of testosterone treatment in young prepubertal boys, and after induction of hypogonadism with a GnRH analogue in young men studied after 10 weeks.

Right panel: Absolute changes in nonoxidative leucine disposal (NOLD), a measure of whole-body protein synthesis in the same groups of subjects.

Reprinted with permission from Mauras N, et al<sup>15,16</sup>



study, GH-deficient pubertal children treated with high doses of daily GH (0.7 mg/kg/wk) grew taller than those treated with conventional doses (0.3 mg/kg/wk), suggesting that during the narrow window of puberty, higher GH doses may be beneficial, particularly for those suffering the most growth retardation at the start of puberty.<sup>13</sup>

The process of epiphyseal fusion in males has been well characterized. Estrogens are essential in this process and are synthesized by aromatization of androgens, primarily testosterone.<sup>14</sup> Whether timed aromatase blockade in pubertal males will safely increase the height potential of growth-retarded boys in this period awaits further study.

### PROTEIN METABOLISM AND SKELETAL MUSCLE

Androgens have potent effects, increasing lean body mass, muscle bulk, and skeletal muscle strength in humans. Using stable isotopes of leucine and glutamine, we have previously shown that testosterone administration to prepubertal males markedly increased whole-body protein turnover and decreased protein oxidation, resulting in a net increase in rates of whole-body protein synthesis.<sup>15</sup> The administration of a GnRH analogue (GnRHa) to eugonadal young men resulted in opposite results, ie, decreased whole-body protein turnover and protein synthesis rates with a marked increase in protein oxidation and decreased lean body mass (Figure 2).<sup>16</sup> The latter was observed despite invariant GH and insulin-like growth factor 1 (IGF-1) concentrations. Testosterone treatment of elderly men, however, is associated with increased mRNA expression of IGF-1 in skeletal muscle,<sup>17</sup> effects opposite of those observed in GnRHa-treated healthy young males, who had decreased mRNA gene expression for IGF-1 after induction of testosterone deficiency.<sup>16</sup> Taken collectively, these data suggest that testosterone *per se* can affect protein metabolism and body composition, independent of changes in GH production at the systemic level. However, it appears that testosterone is necessary for the normal function of the intramuscular IGF-1 system.

These effects of testosterone are likely direct and not secondary to aromatization, as evidenced by the documented increase in skeletal muscle protein synthesis that occurs after the short-term administration of a nonaromatizable androgen, oxandrolone, to healthy young men.<sup>18</sup> In addition, estrogen therapy does not seem to affect large protein pools at the whole-body level. This deduction is supported by the lack of effect of estrogen administration to hypogonadal girls treated for 4 weeks with oral estrogen<sup>19</sup> and the lack of change in protein synthesis of young males treated with an aromatase inhibitor.<sup>20</sup>

The administration of physiologic or supraphysiologic doses of testosterone has been shown to increase skeletal muscle strength in both elderly and young men,<sup>17,21</sup> and the induction of a hypogonadal state with GnRHa results in a quantifiable loss of muscle strength as measured by isokinetic dynamometry.<sup>16</sup> This effect of testosterone is principally responsible for the marked increase in strength in male puberty. Despite these physiologic effects, the administration of testosterone as an ergogenic agent to young boys is not warranted because of the potentially negative impact of accelerating epiphyseal fusion.

Recently, we compared in prepubertal GH-deficient boys the effects of testosterone administered alone and testosterone and GH administered in combination for 4 weeks, each in random order. We observed a marked increase of the effects of these hormones when given together on IGF-1 production, protein synthesis rates, and body composition, supporting further the concept that these 2 hormones are synergistic in their metabolic effects during puberty.<sup>22</sup>

### LIPID AND CARBOHYDRATE METABOLISM

Lipolysis has been shown to occur with androgen stimulation in a variety of experimental situations in both animals and humans. Treatment of rat adipocyte precursor cells with testosterone results in an increase in the number and externalization of  $\beta$ -adrenergic receptors and increases

forskolin-induced (cyclic adenosine monophosphate [cAMP]-mediated) lipolysis.<sup>23,24</sup> Testosterone also increases triacylglycerol lipase activity.<sup>25</sup> When testosterone is administered to hypophysectomized rats, it does not affect lipolysis. However, when given in conjunction with GH, it normalizes rates of lipolysis *in vitro* more than GH alone.<sup>26</sup> When young men were rendered hypogonadal by the administration of a GnRHa, we observed marked changes in body composition, with decreased lean body mass and increased adiposity.<sup>16</sup> This was associated with decreased lipid oxidation rates, suggestive of decreased free fatty acid mobilization and substrate availability for oxidation in the absence of testosterone.<sup>16</sup> These and other data support the concept that testosterone and GH have additive effects on lipolysis and help explain the large changes in body composition, increased lean body mass, and decreased adiposity characteristic of male puberty.

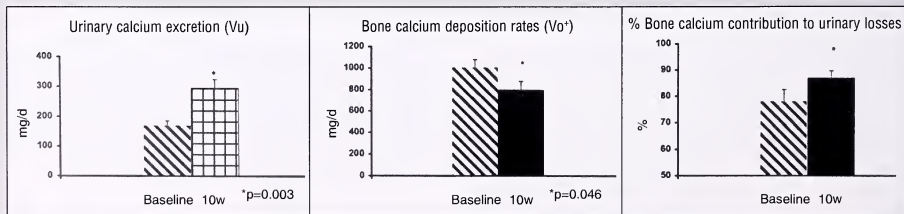
During human puberty there is a decrease in insulin sensitivity, as measured by hyperinsulinemic clamp techniques. Puberty is a state of relative insulin resistance.<sup>27,28</sup> Whether these changes are secondary to the increase in GH and/or gonadal steroids is not entirely clear, however. The effects of androgens on glucose metabolism and insulin action have yielded conflicting results. Whereas testosterone treatment of oophorectomized rats resulted in decreased insulin sensitivity,<sup>29</sup> studies in women with polycystic ovary syndrome have shown either an improvement in insulin sensitivity with antiandrogens<sup>30</sup> or no change.<sup>31</sup> We studied adolescent girls with ovarian hyperandrogenism using isotopic infusions of glucose and indirect calorimetry and observed no changes in measures of glucose production or in glucose oxidation rates after normalization of the testosterone levels with oral estrogen/progesterone treatment.<sup>32</sup> These and other data suggest that androgens may not have a critical effect on carbohydrate metabolism. Whether there is a less than critical effect remains a possibility.

## BONE METABOLISM

Androgens are important anabolic agents in bone and are important in bone remodeling. Hypogonadal men have relative osteopenia, and testosterone replacement has marked beneficial effects on bone in males with delayed puberty or with pathologic hypogonadism.<sup>33-35</sup> Androgen deficiency in males induces an initial, rapid increase in bone loss and increased remodeling, followed by a diminished rate of bone formation.<sup>36</sup> We studied bone turnover using stable tracers of calcium in young boys treated with testosterone for 4 weeks and observed significant increases in intestinal calcium absorption and kinetic measures of bone calcium accretion.<sup>15</sup> These changes were opposite to those observed in young males treated with a GnRHa, who experienced marked urinary calcium losses after only 10 weeks of sustained hypogonadism (Figure 3).<sup>37</sup> These effects of testosterone are only partly due to aromatization to estrogen, as supported by several lines of evidence. First, the osteopenia observed after orchiectomy is prevented by the administration of nonaromatizable androgens;<sup>38</sup> second, there is bone loss in female animals given an antiandrogen (flutamide) despite estrogen replacement.<sup>39</sup> In addition, we observed a preservation of bone calcium turnover rates by using an aromatase blocker in young men,<sup>20</sup> contrary to the marked bone calcium loss that was observed after gonadal axis suppression with a GnRHa.

Males continue to actively accrue bone mass even after the completion of linear growth. Peak bone mass in males is not achieved until they are in their mid-20s.<sup>40</sup> Hence, any delay in the timing of sexual maturation can have negative consequences for bone health and potentially increase the risk for osteoporosis.<sup>41,42</sup>

Figure 3  
Changes Before and After 10 Weeks of Sustained Hypogonadism In Young Men



Urinary calcium excretion (Vu) (left panel), bone calcium deposition rates (Vo+) (middle panel), and the contribution of bone calcium to the urinary losses (right panel) (n=7).

Reprinted with permission from Maura N et al.<sup>37</sup>



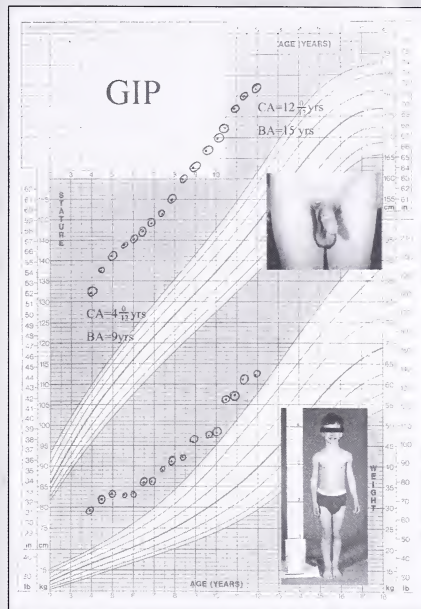
## DISORDERS OF ANDROGEN DEFICIENCY AND EXCESS

The treatment of disorders of androgen production in children requires precise knowledge of the metabolic actions of these steroids and of the dynamic interactions between androgens and GH. Boys with delayed puberty present with sexual infantilism during the teenage years, at the time of the anticipated development of puberty. Low doses of long-acting preparations of testosterone (50 to 100 mg of testosterone enanthate or cypionate, depending on weight) for 6 to 12 months are frequently useful in beginning the process of virilization without negatively impacting skeletal maturation and ultimate height.<sup>43</sup> This also may be achieved with low doses of a nonaromatizable androgen like oxandrolone.<sup>44</sup> Neither testosterone patches nor gels are warranted in this patient group until such a time when the concentrations available for use are low enough in these preparations to prevent premature fusion of the growth plates.

For permanently hypogonadal youngsters who do not have short statures, low doses of testosterone should be used until near adult height is achieved, at which time much higher and fully virilizing doses of androgens should be used, either by injection (depot testosterone, 200 mg every 2 weeks) or via testosterone patches (5 mg/d). The use of testosterone gels in this age group has not been adequately studied to date. Careful assessment of bone mineralization using dual-energy X-ray absorptiometry (DEXA) scanning also can be performed to possible advantage.

Precocious puberty or pseudopuberty in the male, in contrast to girls, is usually secondary to identifiable organic pathology. The differential diagnosis includes adrenogenital syndrome, central precocious puberty due to brain tumor, infiltrative diseases of the brain, cranial irradiation, and CNS trauma. Extracranial germ cell and adrenal tumors also can present with sexual precocity. This is considered when signs of puberty are present prior to age 9 years in boys. The treatment of each of these conditions is disorder-specific; therefore, cortisol supplementation in adrenal hyperplasia, surgical removal or irradiation of brain tumors, and/or excision of extracranial tumors should be considered. When there is premature activation of the hypothalamic-pituitary-gonadal axis, either as a primary cause of the sexual precocity or secondary to the impact of chronic androgens on the hypothalamic gonadostat as in untreated adrenal hyperplasia, treatment with a GnRHa is indicated. In cases of familial male precocious puberty (testotoxicosis), the gonadal activation is independent of gonadotropins and secondary to a constitutive activation of the LH receptor due to a mutation in the LH receptor gene. Attempts to suppress androgen production and androgen effects have proven difficult in this condition. At times striking virilization and temporary tall stature occurs in

Figure 4  
Growth Curve



Growth curve for a boy with gonadotropin-independent precocity (GIP), also known as familial male precocious puberty (testotoxicosis). Pictures are at presentation at the age of 4 years. CA, chronologic age; BA, bone age.

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young boys, and very pronounced bone age advancement occurs (Figure 4), which produces ultimate adult short stature. Treatment with earlier generations of weak androgen receptor blockers (eg, spironolactone) leads to excessive aromatization of circulating androgens, necessitating aromatase blockade (testolactone), thus making treatment cumbersome.<sup>45</sup> Alternative approaches with newer, more potent aromatase inhibitors (eg, Arimidex®) as well as nonsteroidal androgen receptor blockers (eg, flutamide) are being studied. Alternatively, the use of the antifungal itraconazole, which blocks the cytochrome P450 enzyme involved in androgen synthesis, and the testicular desmolase has been proven useful.<sup>17,20,46</sup> The latter, however, requires careful monitoring of liver function and cortisol production.

Lastly, the treatment of girls with virilization disorders not due to adrenal hyperplasia syndromes can be challenging



Many times these girls present with premature pubarche, excess body hair, severe acne, and irregular periods. The most common disorder is ovarian hyperandrogenism, in which the ovary is the predominant source of androgens. This is typically, although not always, associated with obesity and hyperinsulinemia. The role of insulin in the pathogenesis of this disease has only begun to be unraveled.<sup>47</sup> Suppression of excess androgens by putting the ovary "at rest" with the use of birth control pills is effective in controlling excess androgens, and is commonly combined with an antiandrogen, most commonly spironolactone or more recently flutamide. Newer strategies such as suppression of the cytochrome P450 enzyme with metformin<sup>48</sup> or insulin-sensitizing agents like rosiglitazone, typically used to treat type 2 diabetes mellitus, are offering additional choices for the treatment of these virilized girls.<sup>49</sup>

## SUMMARY

This brief review of a variety of in vitro and in vivo studies permits us to conclude that androgens are critical for the normal development of puberty in males. Androgens

potentiate linear growth, mostly indirectly via enhancement of GH's production by way of aromatization to estrogens and, to a lesser extent, directly via the effects on the growth plate. Testosterone potentially stimulates whole-body and muscle protein synthesis, improves skeletal muscle strength, and facilitates lipolysis. Yet it has less important effects on carbohydrate metabolism. Androgens appear to be critical for bone health in males. Treatment of children with androgens in deficiency states and the use of GnRH analogues and antiandrogens in males, and suppression of androgen production in females, requires careful assessment of the endocrine mechanisms operative in the given disease state and a thorough understanding of the metabolic actions of these potent anabolic hormones in childhood.

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## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

## Height Outcome in Congenital Adrenal Hyperplasia Caused by 21-OH Deficiency: A Meta-Analysis

There are reports in the literature of significant short final height associated with virilizing congenital adrenal hyperplasia (CAH). These final heights, which average  $-2$  standard deviations (SD) or lower, are not related to the dose of glucocorticoid, degree of hormonal control achieved, or age at initiation of therapy. Eugster and colleagues review their experience regarding adult height in 65 of their CAH patients over a 20-year period. To be included in their study, children had to be 5 years of age or older. Age at diagnosis, target height, and adult final height were examined. Early diagnosis was defined as a diagnosis made at less than 1 year of age. Actual and predicted height values were expressed as SD scores, and compliance was assessed by querying physicians. For patients who had not yet reached adult height, predictions of adult height were derived using the child's most recent bone age. Of the 65 patients whose charts were examined, 23 had completed their linear growth, and compliance was judged to be good in 28. The overall mean final height SD score minus the target height SD score was  $-1.03$  ( $-4.21$  to  $-2.32$ ). There was no difference seen between males and females. However, a trend (not statistically significant) for better height outcome was seen in patients with good compliance. Those patients identified as having been diagnosed early tended to have a better final height minus target height SD score than those identified later (again, not statistically significant).

In addition, a Medline database search was conducted of all publications reporting height outcome in CAH patients. The meta-analysis identified 16 studies with data that could be used to provide similar outcome information. The current study was added to those data (see Figure). The mean weighted final height SD score for all studies was  $-1.7$ ; in the subset of studies in which target height could be determined, the final height minus target height SD score was  $-1.21$ . In this larger group, a statistically significant difference was seen between patients who were diagnosed early versus late.

The authors state that their data, as well as that from the meta-analysis, demonstrate that adult stature within 1 SD of genetic target may be achieved by many CAH patients with the use of traditional therapy. Also, those subjects diagnosed at an early age have a significantly better outcome than those identified later, and compliance appears to confer some advantage in final height. They correctly point out the flaws in their analyses, including the retrospective design, the prediction of final height in two thirds of the subjects as opposed to actual measurements, and the subjective evaluation of compliance. They stress, however, that rather than pursuing alternative therapies for CAH, efforts should be focused on early detection and improved compliance.

Eugster EA, et al. *J Pediatrics* 2001;138:26-32.

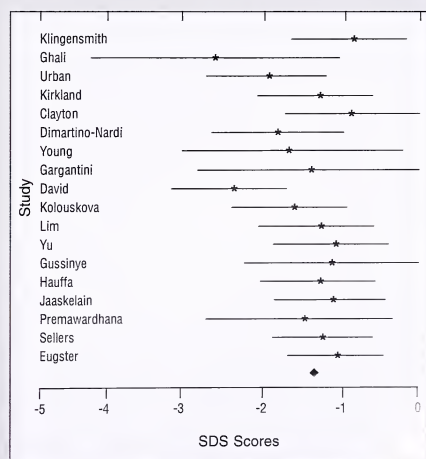
**Editor's comment:** The information provided by this study should be of considerable interest to all pediatric endocrinologists. The flaws in the data, as emphasized by the authors, are significant, even though the results of their particular study are similar to those in the literature. Presenting information regarding final height when that height is a predicted value for more than two thirds of the subjects makes the results highly speculative.

*Indeed, those subjects who are still growing—6 of whom were teenagers—may have had changes in their compliance that later could have affected their adult height. Closing the door on alternative therapy suggests that some physicians may be better at securing compliance in their patients. A previous article in GGH (2001;15(3):33-41) reviews the adult consequences of CAH. This review, which included 5 of the studies listed in the current article, showed that nearly all subjects were shorter than expected, with little influence of age at diagnosis on outcome. Although these data should not be construed as refuting the conclusions provided by Eugster et al, nonetheless there is considerable variability in outcome in different clinics and, presumably, among different patient groups. Indeed, decreased adult height has been correlated with increased body weight and body mass index (BMI) during childhood, suggesting that those children who may be overtreated in attempts to suppress androgen production may have a significant risk of reduction in final height. Clearly, those patients could have benefited from alternative treatments.*

William L. Clarke, MD

**2nd Editor's comment:** Achievement of optimal growth in children with CAH is a well-recognized challenge in the treatment of this disorder. Often there is an inability to adequately suppress

Figure  
Overall Mean SD Score of Final Height for Each Study in Meta-Analysis With 95% CI



Solid diamond indicates weighted mean SD score for all studies. Also demonstrated is lack of correlation between year of publication and outcome.

Reprinted with permission from Eugster EA, et al. *J Pediatrics* 2001; 138:26-32.

corticotropin stimulation without simultaneously incurring the deleterious effect on growth of glucocorticoid overtreatment. This study clearly points out that adult stature in most children with CAH is within 1 SD of the genetic target, with at least one third of the patients achieving their target height. This study reassures pediatric endocrinologists that adequate treatment of patients diagnosed early might lead to achievement of an adult height appropriate for the family. However, there might be opportunities for advances in clinical management combined with diagnostic precision by the molecular genetic characterization of these patients, ie, the CYP21 gene. The heterogeneity of the disease and/or the concept that all patients with CAH need treatment with mineralocorticoid replacement, regardless of their salt-wasting status, needs to be considered to improve the outcome. However, the most practical item is for us to devise ways to

improve compliance with the treatment over prolonged periods. For example, it was recently shown that treatment with dexamethasone in a convenient once-a-day dosage may be easier for the patients and yet allow them to achieve a normal growth (see the next abstract for details).

Fima Lifshitz, MD

**3rd Editor's comment:** The reader's attention is redirected to Dr. Clarke's comments above pertaining to BMI, increased body weight, and adult height. After rereading, proceed to an abstract in this issue entitled, "Body Mass Index in Childhood and Its Association With Height Gain, Timing of Puberty, and Final Height."

Robert M. Blizzard, MD

### Dexamethasone Treatment of Virilizing Congenital Adrenal Hyperplasia (VCAH): The Ability to Achieve Normal Growth

The authors summarize their 2 decades of experience with the long-term, routine use of dexamethasone (DEX) in the treatment of children with 21-hydroxylase- and 11-hydroxylase-deficient CAH (N=26 [23 with salt loss] and 5, respectively). Administration of DEX began as early as birth and continued for an average of 7 to 8 years and for as long as 20 years. DEX elixir was administered once daily (0.1 mg/mL) at a mean dose of 0.27 mg/m<sup>2</sup>/d (range, 0.24 to 0.33 mg/m<sup>2</sup>/d). Fludrocortisone was given as needed. The hypothalamic-pituitary-adrenal axis was effectively suppressed with this regimen.

In the 19 subjects whose bone age was within 2 years of chronologic age at the initiation of DEX, there were comparable increases in chronologic, height, and bone ages in both males and females. Achieved or predicted adult heights were similar to estimated target heights (see Figure).

In 7 children in whom bone ages were more than 2 years in advance of chronologic age when treatment with DEX was begun, linear growth relative to advancement in bone age improved but did not achieve unity. The authors conclude that DEX is an effective and safe glucocorticoid for the management of CAH in childhood.

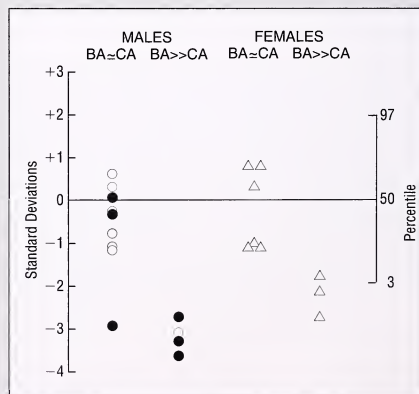
As the authors point out, a comparison of the effects of DEX to those of another group of children with CAH treated more conventionally would have been useful. It would seem reasonable to undertake such a comparative long-term trial, if possible. Assuming these data are confirmed, DEX would seem preferable to the use of androgen receptor blockers and aromatase inhibitors in the management of children with CAH in order to keep treatment as uncomplicated as possible.

Allen W. Root, MD

Rivkees SA, Crawford JD. *Pediatrics* 2000;106:767-773.

**Editor's comment:** Management of infants and children with CAH remains a challenging task primarily because of the need for rigid adherence to the usual therapeutic program—particularly the administration of cortisol at close to 8-hour intervals. While achievable in infancy and early childhood, strict compliance becomes more difficult as the patients' schooling and other activities increase. Thus, the report by Drs. Rivkees and Crawford is welcome and useful. Many of us have been reluctant to utilize DEX in infants and children with CAH, although it is effective in older adolescents and young adults, because of its evident biopotency. Based on their experience, the authors calculated that 1 mg of DEX is 70-fold more effective than 1 mg of cortisol in suppressing adrenal function, rather than the 30-fold potency stated by the manufacturers.

Figure  
Mature and Predicted Adult Heights After  
Treatment With Dexamethasone



Mature heights (solid symbols) or predicted adult heights (open symbols) after treatment with dexamethasone. Heights are given as SDS or percentile.  $\Delta$ , females;  $\circ$ , males; BA, bone age; CA, chronologic age.

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## ***GROWTH, Genetics, & Hormones***

Volume 17, Number 2

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| 1. Educational value of newsletter                         | 5 | 4 | 3 | 2 | 1 |
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## Celiac Disease in Children and Adolescents With Type 1 Diabetes: Importance of Hypoglycemia

This article explores the association of celiac disease and type 1 diabetes mellitus in a retrospective case-controlled study. Patients with type 1 diabetes mellitus were screened for celiac disease by measurements of both serum immunoglobulin (Ig)A antiendomysial (EMA) and anti gliadin (AGA) antibody levels. The diagnosis of celiac disease was confirmed by small-bowel biopsy when testing for EMA and/or AGA antibodies was positive. Patients were matched for age, sex, and duration of disease for the 18 months before and after the diagnosis of celiac disease. Metabolic control was assessed by hemoglobin A<sub>1c</sub>, frequency of hypoglycemia, and total insulin requirements for the 18 months before and after the diagnosis of celiac disease.

There were 20 patients of 434 with type 1 diabetes who had celiac disease. None of them had symptoms or signs typical of this disease. However, during the 6 months before and after diagnosis of celiac disease, these patients had more hypoglycemic episodes than the controls: 4.5 vs 2 severe episodes with a progressive reduction in insulin requirement of 0.6 vs 0.9  $\mu$ g/kg/d. The introduction of a gluten-free diet led to normalization of the intestinal mucosa and reduced the frequency of hypoglycemia in the celiac disease patients. The prevalence of celiac disease in this population of type 1 diabetes mellitus was 4.6%. All 414 control patients had negative tests for EMA and AGA antibodies. The authors concluded that underlying celiac disease should be suspected in patients with diabetes mellitus presenting with symptomatic hypoglycemia.

**Editor's comment:** The association between celiac disease and type 1 diabetes has long been known. The coexistence of these 2 entities appears to be due to a common genetic predisposition attributed to the presence of the locus human leukocyte antigen (HLA) DR3. This report, as well as other studies using serologic data, describe a celiac disease prevalence of 5% to 7% in patients with type 1 diabetes mellitus. Often these patients do not present with any symptoms of overt malabsorption. However, as the authors point out, the occurrence of hypoglycemia in a child with diabetes mellitus should lead to screening for celiac disease. Measurements of EMA or AGA antibodies should be obtained, and, if positive, a confirmatory small-bowel biopsy should be performed even in patients who appear to be asymptomatic. These patients may have malabsorption of a sufficient degree to interfere with carbohydrate absorption with a resultant increased risk for hypoglycemia. It should be kept in mind that the prevalence of celiac disease in normal children might be about 1% (Pediatrics 2001;107:42-45), whereas in type 1 diabetes patients the prevalence is at least 4 times higher. Thus, we should proactively consider routine screening for this disease in type 1 diabetes patients, just as we screen for other diseases (eg, hypothyroidism).

Fima Lifshitz, MD

Mohn A, et al. *J Pediatr Gastroenterol Nutr* 2001;32:37-40.

## Obesity, Increased Linear Growth, and Risk of Type 1 Diabetes in Children

Hyponen and associates report for the Childhood Diabetes Study Group in Finland on their evaluation of the effect of obesity and linear growth on the risk of developing type 1 diabetes during childhood. All children under the age of 15 years who had type 1 diabetes diagnosed between September 1986 and September 1989 were invited to participate in the study. All the study participants were tested for antibodies associated with diabetes. Ninety-eight percent were found to be positive for at least 1 type of antibody, confirming that they had autoimmune type 1 diabetes. Age- and sex-matched nondiabetic control children were randomly selected from the Finnish National Population Registry. Neonatal data and sociodemographic data were collected using structured questionnaires. An equal proportion of the diabetic and control children lived in rural areas. Information regarding height and weight was obtained from well baby clinics and school healthcare units for the 586 children with diabetes and for the 571 controls. Heights were available for both parents for the majority of study subjects. Relative weight calculated as "weight in relation to mean weight for height" and relative height as "a deviation of height in SD scores" were computed using the Finnish growth standards. Statistical analysis was based on relative weight and relative height in relation to age. Three age groups were studied: 2 weeks to 1.9 years, 2 to 9.9 years, and 10 years and older.

Neither the mean relative weight nor the relative height at birth differed between the diabetic and control subjects. But both boys

and girls who developed type 1 diabetes weighed more than the control children from infancy onward. There was a significant difference between the diabetic and control boys with regard to relative height from early infancy on. Among the girls, this significant difference was present until 10 years of age. Unfortunately, there were only limited data available for girls after the age of 10 years. Adjustments for neonatal and sociodemographic characteristics, or target heights, did not affect the results of this study. Both *higher relative weight* and *greater relative height* were associated with an increased risk of developing type 1 diabetes, and the magnitude of the effect was somewhat greater with respect to relative weight in infancy and early childhood. The effect of relative height remained constant throughout all ages.

The authors remind us that obesity is a well-known risk factor for type 2 diabetes, and that obesity is an increasing problem in many countries. In Finland, the annual incidence of type 1 diabetes has increased more than 4 times between 1953 and 1998. The role of obesity in this increase is unclear. Unequivocally, the increase in risk of type 1 diabetes for 1 SDS increment in relative height was 20% to 30%. Obesity or relative weight >120% after 3 years of age was associated with a more than 2-fold risk of developing type 1 diabetes. It is known that there is an association between obesity, accelerated height gain, insulin resistance, or enhanced insulin secretion, and significant subsequent enhanced insulin secretion. Hyperinsulinemia is obviously associated with



active beta cells, and active beta cells have been shown to be more susceptible to cytokine-induced damage than resting cells in vitro.

Hyponen E, et al. *Diabetes Care* 2000;23:1755-1760.

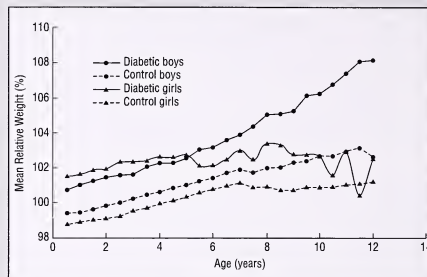
**Editor's comment:** This is a very interesting and important article. The incidence of type 1 diabetes in Finland is exceedingly high, much higher than that in the United States. The association of early childhood obesity and increases in relative height with an increased incidence of type 1 diabetes is significant information and a warning to the pediatric community. Recent reports have documented a significant increase in the incidence of type 2 diabetes among children and adolescents, paralleling the increase in obesity in this group. Hyponen et al's paper is the first to show that an increase in weight also is associated with an increase in type 1 diabetes. The information regarding tall stature is not new, but is consistent with other reports from Europe and the United States.

The Childhood Diabetes Study Group in Finland has presented information that needs to be transmitted to all physicians caring for children. The prevention of childhood obesity may be one of the most important therapeutic activities of pediatricians.

William L. Clarke, MD

Figure

**Cross-Sectioned Mean Relative Weights for Diabetic and Control Groups, Calculated from the Interpolated Values**



Reprinted with permission from Hyponen E, et al. *Diabetes Care* 2000; 23:1755-1760.

## Neonatal Outcome After Preimplantation Genetic Diagnosis by Analysis of the Polar Bodies

New reproductive technologies have increased the options available to couples. Preimplantation genetic diagnosis (PGD) was developed for couples at high genetic risk to avoid establishing pregnancies with genetic diseases. PGD is performed by blastomere biopsy or polar body removal (PBR) for mendelian or chromosomal disorders. Mothers who are heterozygotes for a mutation are good candidates for this procedure. Primordial germ cells will contain 1 chromosome carrying the affected allele and another carrying a normal allele. During meiosis, the oocyte will double its genetic material, yielding 2 chromosomes with normal alleles and 2 that contain the mutant allele. At the conclusion of meiosis I, the oocyte extrudes half of its chromosomes in the form of the first polar body. When the first polar body is removed before fertilization, it can be analyzed for the presence of the normal or mutant allele. Subsequently, fertilization occurs, the oocyte completes a second meiotic division, and then the second polar body is extruded containing 1 set of chromosomes. The second polar body also can be analyzed, and it will usually be identical to the 1 that remains in the egg. If a crossover occurs during meiosis, the first polar body may contain both mutated and normal alleles, in which case it will be necessary to analyze the second polar body to see which allele will be left in the fertilized egg. It is therefore possible to identify embryos developing from oocytes that contain a normal allele and then to transfer the fertilized oocyte back to the mother and establish a pregnancy.

somal disorders, and 18 infants were born where analysis had been done for mendelian disorders (including cystic fibrosis, sickle cell disease, long-chain acyl-CoA dehydrogenase deficiency, and thalassemia). All case analyses also were done postnatally to confirm the prenatal diagnosis. Birth data are available for 98% of the cohort, and developmental assessments are available for 44 children older than 6 months of age (see Table, page 31).

There were 80 singleton pregnancies, 9 twins, and 7 triplets, of which 3 were reduced to twins. One gestation with 5 fetuses

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miscarried in the first trimester. There was an increased occurrence of prematurity, and 1 neonate died as a result of placental abruption. The mean singleton birth weight was at the 47th percentile, and the mean singleton birth length was at the 57th percentile. Forty percent of births were by cesarean section, which is comparable to other in vitro fertilization (IVF) studies. There were 6 infants with birth defects: 1 with a unilateral transverse limb reduction (amniotic band syndrome); 1 with neonatal seizures who had 3 cerebral infarcts on imaging; 1 with a minor hemangioma; 1 with minor strawberry hemangiomas on both arms; 1 with thickening of the tricuspid valve that did not require surgery; and 1 with bilateral webbed toes. Only 1 child of the 44 who had been followed up to 6 months of age was reported to have developmental delay. This was 1 of twins who had had no perinatal complications. This child had speech delay and was receiving speech therapy. This frequency of birth defects is certainly not out of line of what would be expected.

The financial cost of PGD by PBR is reported to be \$8500 for 1 typical cycle. Diagnostic testing for mendelian traits may involve as much as another \$1000 in laboratory costs.

The authors point out that in addition to the financial costs, there are some intrinsic risks of multiple gestations and the complications associated with them. Nevertheless, polar body prenatal diagnosis does provide families with another option in terms of prenatal diagnosis.

Strom CM, et al. *Pediatrics* 2000;106:650-653.

Table  
Summary of Preimplantation Genetics  
Pregnancies

| Number of Fetuses | Number of Pregnancies | Number of Spontaneous Abortions | Number of Live Births |
|-------------------|-----------------------|---------------------------------|-----------------------|
| 1                 | 80                    | 5                               | 75                    |
| 2                 | 9                     | 1                               | 16                    |
| 3                 | 7                     | 0                               | 18*                   |
| 5                 | 1                     | 1                               | 0                     |
| Total             | 97                    | 7                               | 109                   |

\*Three couples had reduction to twins; 4 couples delivered triplets.

Reprinted with permission from Strom CM, et al. *Pediatrics* 2000;106:650-653

**Editor's comment:** The data from this large center are reassuring. The reliability of testing for mendelian disorders needs further study since there are really only 18 cases. The procedure certainly allows individuals to obtain a diagnosis before implantation, if that fits with their particular ethical stance. Clearly, the cost is much higher than that associated for prenatal diagnosis which is performed later in pregnancy. However, it does not involve termination of pregnancy, and only those embryos which do not have a detectable abnormal test would be used for implantation. The reader may wish to extend his/her knowledge of this alternative diagnostic technique as it undoubtedly will become a common tool of IVF.

Judith G. Hall, OC, MD

## Spectrum of the Tricho-Rhino-Phalangeal Syndromes

Three types of tricho-rhino-phalangeal syndrome (TRPS) have been clinically defined. The features characterizing these syndromes, but described initially in TRPS I, include sparse, slowly growing scalp hair; sparse eyebrows laterally; bulbous tip of the nose; protruding ears; brachydactyly and mild to moderate short stature; and the presence of cone-shaped epiphyses of the middle phalanges on X-ray films. TRPS II is distinguished from TRPS I by the occurrence of exostoses; mental retardation often is present. TRPS III is distinguished by the greater severity of the characteristics of TRPS I.

Mutations of a gene designated *TRPS1*, which encodes a zinc finger transcription factor, were recently identified in patients with TRPS I. Microdeletions of chromosome 8q24.1 that include both *TRPS1* and *EXT1*, the gene mutated in hereditary multiple exostoses type I, are responsible for TRPS II. The current study by Lüdecke et al was done to determine if TRPS III is due to *TRPS1* mutations, representing the severe end of a clinical spectrum of TRPS I, or, alternatively, results from mutations of another gene. The results confirmed the former possibility and demonstrated a correlation between the type of mutation and the severity of clinical phenotype.

*TRPS1* was screened by direct sequencing of the coding and flanking intron sequences for mutations in 79 patients with TRPS, including 57 unrelated individuals with either TRPS I or

TRPS III. Thirty-five different mutations were found in 44 of 51 unrelated patients. The majority were deletions or disruptions, nonsense and splicing mutations. These would be expected to truncate the transcription factor protein, leading to loss of function, since the resulting proteins would lack a nuclear localization signal needed for nuclear entry and the C-terminal zinc finger domain required for dimerization. These mutations would, therefore, act through haploinsufficiency. Missense mutations were identified in 8 cases. They all mapped to exon 6, which encodes the GATA zinc finger domain necessary for DNA binding. The resulting proteins would be expected to enter the nucleus and form complexes with other transcription factors that would function poorly because of defective DNA binding. They are predicted to exert a dominant negative effect, which as a disease-causing mechanism generally has a greater impact than haploinsufficiency.

The patients also were evaluated clinically, mainly in terms of height and severity of brachydactyly as judged from hand X-rays films. The results showed a continuous spectrum of severity. They further revealed that nonsense and disruption mutations, which would be predicted to cause haploinsufficiency of *TRPS1*, were associated with the range of severity typical of TRPS I. In contrast, the missense mutations predicted to act in a dominant negative fashion correlated with the severe end of the spectrum characteristic of TRPS III.

Thus, *TRPS1* mutations account for TRPS. Loss of function of 1 *TRPS1* allele gives rise to mild to moderate manifestations associated with the diagnosis of TRPS I. Missense mutations that act in a dominant negative manner account for the severe features observed in TRPS III. Chromosomal deletions that cause haploinsufficiency of *TRPS1*, *EXT1*, and potentially other neighboring genes are responsible for TRPS II.

Lüdecke H-J, et al. *Am J Hum Genet* 2001;68:81-91.

**Editor's comment:** This study nicely demonstrates how different types of mutations of the same gene can produce clinical phenotypes that appear to be different. The authors acknowledge that no mutations were detected in a few patients, making it possible that 1 or more other genes could harbor mutations that lead to a TRPS clinical phenotype. However, their conclusion that TRPS1 is the major, if not only, gene locus responsible for this constellation of features cannot be disputed. It will be interesting to learn the function of TRPS1 in skeletal growth and maturation.

William A. Horton, MD

## BMI in Childhood and Its Association With Height Gain, Timing of Puberty, and Final Height

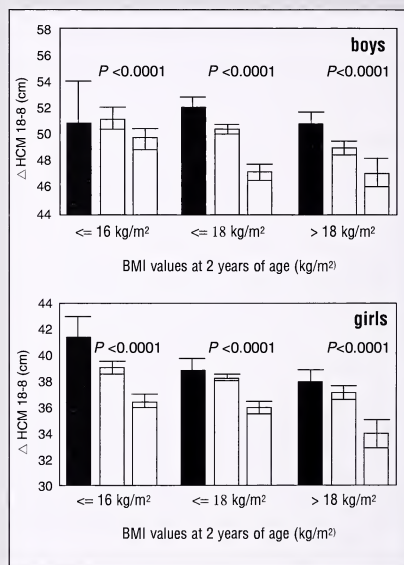
This study was undertaken to ascertain the effects of overnutrition in childhood on height, final height, and timing of puberty. This study was performed in 5111 grade-school children born in the early 1970s in Goteborg, Sweden. The final analysis was made in 3650 full-term healthy children whose growth information was accurate from birth to 18 years of age. The others were eliminated from the final analysis due to a variety of factors and/or illnesses. A computer-generated growth chart was produced for each child, and their nutritional status was assessed by body mass index (BMI) changes between 2 and 8 years of age. Mean parental heights were adjusted to assess genetic influences of the linear growth. Childhood BMI gain was related to an increased height gain during the same period (ie, an increase of 1 BMI unit was associated with an excess increase in height gain of 0.23 cm in boys and 0.29 cm in girls). The BMI also was linked to an earlier onset of puberty; the impact on the timing of puberty was 0.6 years in boys and 0.7 years in girls. Each increased unit of BMI gain in childhood also reduced the height gain in adolescence by 0.88 cm for boys and 0.51 cm for girls. However, no direct effect was found between childhood BMI gain and final adult height. The authors conclude that overnutrition between 2 and 8 years of age may lead to earlier onset of puberty and earlier achievement of adult height, but not greater height.

He Q, Karlberg J. *Pediatr Res* 2001;49:244-251.

**Editor's comment:** Overnutrition and/or obesity in childhood is a worldwide health concern because it may produce several adverse physical and psychosocial developmental consequences. Moreover, the obese child is at a higher risk of remaining obese throughout adulthood. Several studies have shown that overnutrition accelerates linear growth. This large population study certainly adds support to this concept. However, postnatal linear growth is complex, resulting from genetic, nutritional, and endocrine system influences. The BMI does not necessarily represent the only variable affecting growth, nor does it represent the true nutritional status of an individual. The effect of dietary attempts to lose weight was not investigated in this study. Usually, children who are obese tend to be on and off diets. This may lead to poor nutrition and potential growth deceleration. However, it is reassuring to know that this large population of obese children did not experience a reduction in final adult height.

Fima Lifshitz, MD

Figure  
The Mean and Its 95% Confidence Interval  
of  $\Delta$ HCM 18-8 for Boys and Girls in 3 Different  
Groups of BMI Values at 2 Years of Age



The cut off points, 16 and 18 kg/m<sup>2</sup>, represent the 25th and 75th centile values at 2 years of age. Within each BMI group at 2 years, the values of height gain between 8 and 18 years are also shown separately in 3 childhood BMI change groups. The *P* values refer to the ANOVA to compare the differences in central tendency of height gain among the 3 BMI change groups.

Reprinted with permission from He Q, Karlberg J. *Pediatr Res* 2001;49:244-251.



## Genetic Ablation of Parathyroid Glands Reveals Another Source of Parathyroid Hormone

Glial cells missing-2 (*Gcm2*) is 1 of 2 mouse homologues of *Drosophila Gcm*, a gene encoding a transcription factor that is involved in the differentiation of neural cells. In *Drosophila*, loss of *Gcm* leads to decreased numbers of glial cells and their presumptive conversion into neurons, while its overexpression increases the number of glial cells. In the mouse, *Gcm2* is expressed only in the parathyroid gland. From embryonic stem cells, Gunther et al knocked out *Gcm2* and created mice heterozygous for loss of this gene by injecting the altered DNA of these stem cells into blastocysts; the trait could then be transmitted as a germline mutation. The heterozygous mice (*Gcm2*<sup>-/-</sup>) were phenotypically normal.

Mice homozygous for loss of *Gcm2* (*Gcm2*<sup>-/-</sup>) were produced by mating of heterozygous animals. Because of marked hypocalcemia (3.0 mg/dL), 30% of the homozygous animals died within 8 hours after birth; however, 70% survived with subnormal serum calcium levels of 6 to 7 mg/dL. The surviving *Gcm2*<sup>-/-</sup> animals were viable and fertile despite hypocalcemia. They had bone abnormalities consistent with an increase in bone volume such as with hypoparathyroidism. Absent parathyroid glands were determined by histologic examination, and lack of parathyroid hormone (PTH) expressing cells in the thyroid and surrounding tissue. These hypocalcemic rodents had serum levels of immunoreactive PTH, which in an assay that did not cross-react with PTH-rP, were comparable to those in wild-type mice with serum calcium values of 10 mg/dL. However, these PTH levels were too low to restore eucalcemia. No other structural abnormalities were present in the *Gcm2*<sup>-/-</sup> mice.

Search of multiple tissues revealed expression of *PTH* in the hypothalamus (confirming a 1990 report) and in a small group of cells in the subcapsular region of the thymus. This small group of cells colocalized with expression of *Gcm1*, the second mouse homologue. DNA sequencing confirmed that thymic *PTH* was identical to that in the parathyroid glands. It could be downregulated by administration of calcitriol but could not be upregulated by further lowering of serum calcium concentrations (by infusion of phosphate). The investigators suggested that thymic PTH secretion was maximal following ablation of the parathyroid glands.

Gunther T, et al. *Nature* 2000;406:199-203.

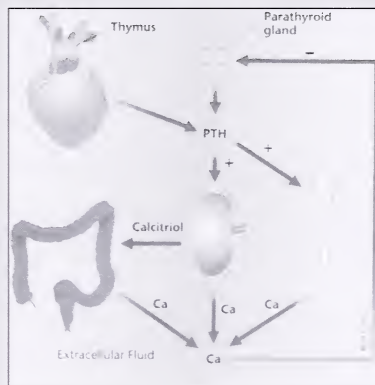
**Editor's comment:** The identification of *Gcm2* as an essential factor for differentiation of the parathyroid gland in mice suggests that its human homologue (*GCM2*,

chromosome 6p24.2, OMIM 603716) may have the same function. The identification of a patient or of a family with this genetic mutation may be anticipated. The affected members of the family would join the gain-of-function mutation in *CASR* (encoding the membrane calcium sensing receptor) and the loss-of-function mutations in *PTH* and *UFDIL* (which is the mutation in the DiGeorge syndrome) as documented familial forms of hypoparathyroidism. The relationship between neural cell differentiation in insects and parathyroid gland development in mammals is intriguing and unexplained at present. That the thymus and the immune system are secondary sources of PTH synthesis and secretion is consistent with their production of other peptide hormones (corticotropin, GH, etc).

Allen W. Root, MD

Balling R, Erben RG. *Nature Med* 2000;6:860-861.

Figure  
Endocrine Control of Calcium Homeostasis



Parathyroid hormone (PTH) is secreted from the parathyroid glands (four circles). A new auxiliary source of PTH has been located in the thymus. PTH increases mobilization of calcium (Ca) from bone by enhancing bone turnover. In the kidney, PTH stimulates tubular reabsorption of Ca and favors the synthesis of the steroid vitamin D hormone, calcitriol. The main physiologic function of calcitriol is to increase intestinal Ca absorption. Therefore, all effects of PTH act to directly or indirectly increase the calcium concentration in the extracellular fluids. An increase in the concentration of ionized Ca in the extracellular fluids is the major feedback mechanism that inhibits PTH secretion from the parathyroid glands and possibly also from the thymus by a Ca-sensing receptor expressed in the membrane of PTH-secreting cells. In the absence of parathyroid glands, thymic PTH secretion seems to be a backup mechanism for emergency regulation of Ca metabolism.

Reprinted with permission from Balling R, Erben RG. *Nature Med* 2000;6:861.

Please Send Correspondence to:

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## Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism

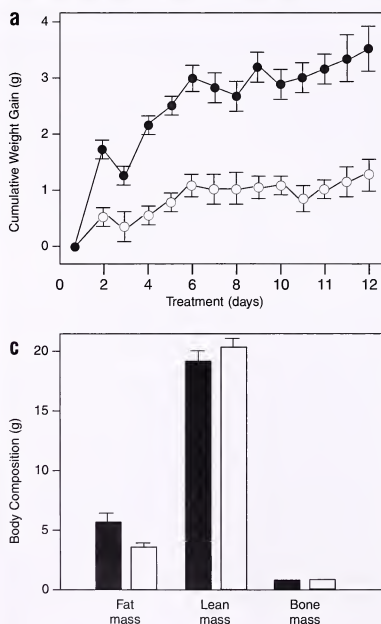
Ghrelin, a 28 amino acid peptide synthesized by the gastrointestinal tracts and hypothalamic arcuate nuclei of rodents and humans, is the natural ligand for the GH secretagogue receptor (GHS-R). Since synthetic GH secretagogues increase weight in experimental animals, both groups of investigators studied the effect of ghrelin on feeding and weight gain in intact adult male rats.

Tschöp et al demonstrate that once-daily *subcutaneous* administration of synthetic rat ghrelin (2.4  $\mu\text{mol/kg/d}$ ; MW 3313.85) for 14 days doubled the rate of weight gain without inducing hyperphagia and increasing food intake; the increase in weight was due to accumulation of fat without alteration in lean body mass or bone density (see Figure). The isolated increase in fat was related to an increase in respiratory quotient (RQ) of ghrelin-treated rats, indicating that this peptide stimu-

lated carbohydrate utilization while decreasing the rate of fat utilization. Ghrelin did not increase the rate of energy expenditure or the motor activity of recipients. The mechanism by which ghrelin enhanced fat accumulation was not due to its GH-releasing effects as GH was lipolytic in control animals and was similarly effective in GH-deficient dwarf rats and wild-type animals. Its effect was not mediated by the orexigenic neuropeptide Y (NPY), as ghrelin stimulated fat accumulation in *NPY*<sup>-/-</sup> animals. *Continuous intracerebroventricular (ICV) infusion* of ghrelin for 7 days enhanced weight gain in wild-type rats and increased their RQ and food intake. Tschöp et al also observed that fasting increased and feeding decreased serum concentrations of ghrelin in these animals.

Wren et al report that *intraperitoneal administration* of ghrelin (3, 10, and 30 nmol) *acutely* increased food intake only in the

Figure  
Ghrelin-Stimulated Adiposity in Mice



**a.** Ghrelin induces body weight gain in male wild-type mice ( $n=10$  per group,  $P=0.0001$ ). Mice treated once daily for 2 weeks with ghrelin (2.4  $\mu\text{mol kg}^{-1}$ , subcutaneously) gained 13.9% of their initial body weight ( $24.4 \pm 1.0$  g), while vehicle-injected control animals gained 5.6% of their initial body weight ( $25.1 \pm 1.0$  g). **b.** Ghrelin treatment did not change food intake rate in wild-type mice. **c.** Body composition of wild-type mice was measured by DXA after 2 weeks of treatment with ghrelin (2.4  $\mu\text{mol kg}^{-1}$ , daily subcutaneously) or vehicle ( $n=10$  per group). Mice treated with ghrelin had a greater fat mass ( $6.34 \pm 0.50$  g) than vehicle-injected control animals ( $3.72 \pm 0.29$  g,  $P=0.002$ ). Symbols or bars represent the mean  $\pm$  the standard error of the mean (s.e.m.). Filled symbols or bars, ghrelin treated; empty symbols or bars, controls.

Reprinted with permission from Tschöp M, et al. *Nature* 2000;407:908-913.

first hour after injection; its *ICV injection* (0.3, 1.0, and 3.0 nmol) acutely increased food intake, with maximum intake in the first hour after injection but with a duration of effect of 24 hours. *ICV ghrelin* increased serum concentrations of GH and corticotropin and decreased those of thyrotropin.

Tschöp M, et al. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908-913.

Wren AM, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000;141:4325-4328.

**Editor's comment:** Ghrelin is now added to the complex of neuroendocrine and transcription factors that affect feeding behavior and energy metabolism, including peroxisome-proliferator-activated receptor- $\gamma$ 2, leptin, NPY, melanin-concentrating hormone, pro-opiomelanocortin and melanocortin, the agouti protein, and so forth, and provides another site at which weight-control pharmacologic therapeutics may be targeted. The mechanism by which ghrelin selectively spares fat metabolism, thus increasing its accumulation, is unknown at present. Increased RQ without an increase in energy intake might be due to decreased activity of the sympathetic nervous system or to hypothalamic stimulation. The bioeffects of ghrelin are uniquely suited to enhance the anabolic effects of GH, which is maximally effective in the well-nourished recipient.

Date et al have identified the rat and human gastrointestinal X/A-like cell of the oxyntic gland as the site of synthesis of ghrelin; these cells are located primarily in the fundus of the stomach. Apparently, more than 18 cell types that synthesize

endocrine-like hormones have been identified to date in the gastrointestinal tract. There are 4 distinct endocrine cells, each synthesizing a specific product in the oxyntic mucosa of the rat: ECL-histamine; D-somatostatin; enterochromaffin-serotonin; and X/A-like-ghrelin.

Allen W. Root, MD

Date Y, et al. Ghrelin, a novel growth hormone-releasing acetylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000;141:4255-4261.

Kojima M, et al. Ghrelin is a novel growth hormone-acetylated peptide from stomach. *Nature* 1999;402:656-660.

**2nd Editor's comment:** A third article, by Nakazato et al in (*Nature* 2001;409:194) supplements the above. Rats were injected with ghrelin in the cerebral ventricles, which produced significantly greater weight gain than was observed in controls infused with saline. The authors demonstrated that the increased eating observed was not related to GH secretion. However, ghrelin stimulates not only food intake but also GH secretion. These mechanisms are not interdependent. However, in the normal creature with the capability to respond in a dual manner to ghrelin, the growth action of GH may be enhanced by the increased food ingestion. These early reports are not necessarily synchronous. Confirming and additional studies are needed and undoubtedly clarification will occur.

Robert M. Blizzard, MD

## Autosomal Dominant Hypophosphataemic Rickets Is Associated With Mutations in *FGF23*

Clinical and biochemical manifestations of autosomal dominant hypophosphatemic rickets (ADHR) are same as to those of X-linked hypophosphatemic rickets (XHR), ie, deformities of the lower extremities, short stature, rickets, hypophosphatemia. XHR has been attributed to loss-of-function mutations in *PHEX*, a gene encoding an endopeptidase that may serve to activate or degrade an as yet uncharacterized protein involved in phosphate transport termed "phosphatonin."

Studies of families with multiple members affected with ADHR by collaborating investigators of the ADHR Consortium linked this disorder to chromosome 12p13.3. Utilizing publicly available genomic sequences from chromosome 12p13, the authors found 37 genes in this region, 13 of which were previously unrecognized. With more discriminating linkage analysis, a segment of chromosome 12p13.3 encoding 11 genes was identified; screening of these genes for mutations revealed 1 with homology to those encoding the fibroblast growth factor (FGF) family that was mutated in patients with ADHR. Previously undescribed, *FGF23* has 3 exons with 1612 bp encoding a peptide with 251 amino acids that has a similar 3-dimensional configuration and 25% to 36% homology with other members of the FGF family; *FGF23* is the largest FGF described to date. In subjects with ADHR, mutations in *FGF23* that segregated with the disease include: NT 527G→A → Arg176Gln; NT 535C→T → Arg179Trp; and NT 536G→A → Arg179Gln.

These changes were not polymorphisms. No mutations of *FGF23* were detected in patients with hypophosphatemic bone disease or in subjects with apparent XHR with normal *PHEX* analyses. In normal human tissues, *FGF23* was expressed predominantly in heart, liver, and thyroid/parathyroid tissue. The physiologic function of *FGF23* was not identified in this report. The investigators speculate that it might be related to or perhaps even be the elusive phosphaturic substance "phosphatonin."

ADHR Consortium. *Nat Genet* 2000;26:345-348.

**Editor's comment:** This work illustrates the treasure trove of genetic data already available from the Human Genome Project waiting to be mined for relevance to human physiology and pathophysiology. *PHEX* is expressed by osteoblasts, and it has been hypothesized that "phosphatonin" also may be synthesized by these cells. In normal mouse embryos, the murine homologue *Fgf23* maps to chromosome 6. The present investigators were unable to demonstrate expression of *Fgf23* in the tibiae of embryonic mice, perhaps suggesting that *FGF23* is not "phosphatonin."

Allen W. Root, MD

Ecarot B, Desbarats M. 1,25-(OH) $_2$ D $_3$  down-regulates expression of *PHEX*, a marker of the mature osteoblast. *Endocrinology* 1999;140:1192-1197.

**GROWTH, Genetics, & Hormones Volume 17, Number 2**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Follow the instructions listed there to receive CME Category 1 credit.

1. Gonadotropins are suppressed by testosterone in childhood.
  - a. True
  - b. False
2. Testosterone and growth hormone each have a direct effect on bone growth in the early pubertal boy.
  - a. True
  - b. False
3. A growth spurt at adolescence does *not* occur in the hypogonadal male.
  - a. True
  - b. False
4. Testosterone and its 5 $\alpha$  reduced metabolite, dihydrotestosterone, share an androgen receptor which is transcribed from a single-copy gene in the Y chromosome.
  - a. True
  - b. False
5. Aromatization of an androgenic steroid means that it is converted, at least in part, to an estrogenic steroid, usually estradiol.
  - a. True
  - b. False

6. Testosterone given to prepubertal males markedly inhibits whole body protein turnover.
  - a. True
  - b. False
7. The marked increase in strength in males at puberty is due to the increased production of:
  - a. Growth hormone
  - b. Testosterone

1, a, 2, a, 3, a, 4, b, 5, a, 6, b, 7, b  
**Answer Key:**

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The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.



The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Mauras, Lifshitz, Clark, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Inc.'s National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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## Endocrine Complications of the Successful Treatment of Neoplastic Diseases in Childhood

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### INTRODUCTION

Cancers are relatively rare in children and adolescents, with approximately 12,500 individuals younger than 20 years of age diagnosed with a new malignancy yearly.<sup>1</sup> The most prevalent cancers observed before 20 years of age include leukemias (25%), of which most are acute lymphoblastic leukemia (ALL); tumors of the central nervous system (CNS; 17%), lymphomas, including Hodgkin's disease (15%); and tumors of bone and soft tissue (13%). The remaining 30% are of other origin.

Mortality rates in the past 25 years have decreased dramatically in contrast to incident rates, which have increased slightly or remained steady. The current 5-year overall survival rate for childhood cancers exceeds 70%, and survival rates currently are 80% for children with ALL and greater than 90% for children and adolescents diagnosed with Hodgkin's disease.<sup>1</sup>

The remarkable improvements in survival result from advances in supportive care such as prevention and treatment of infections; the improved utilization of blood and blood products; and, most importantly, changes in therapy occurring over the past 30 years. Included are the use of combined modality therapies such as the use of surgery with chemotherapy and radiation therapy and the use of aggressive multiagent chemotherapeutic regimens. From our experience at Memorial Sloan-Kettering Cancer Center, as well as the experience of others, approximately two thirds of pediatric cancer survivors will develop medical complication or disabilities attributed to their previous cancer treatment.<sup>2</sup> Endocrine disturbances have been documented in 20% to 50% of

survivors and frequently occur as late effects of cancer therapy. An overview of the endocrine complications that develop following successful treatment of childhood cancer will be presented. Emphasis is placed on the observations at the Memorial Sloan-Kettering Cancer Center and from those reported in the literature during the past several years.

### GROWTH FAILURE

Impaired linear growth with resultant adult short stature occurs frequently in survivors of childhood cancer, particularly in individuals treated at a young age. The incidence is greater in females than in males. A variety of factors, including high-dose radiation therapy, particularly to the brain and the spinal cord, early pubertal development, hypothyroidism, and growth hormone deficiency (GHD), contribute to the short stature in adult survivors. As is apparent, both nonendocrine and endocrine factors can contribute to growth retardation.

The *nonendocrine factors* affecting growth are primarily intensive oral or parenteral chemotherapy and irradiation of skeletal structures. The administration of chemotherapy is often associated with mild to moderate reduced growth, which in many instances is only temporary. However, the adverse effects on growth can persist more long term.<sup>3</sup> The deleterious effects on growth are dependent on the number and dosage of the drugs and

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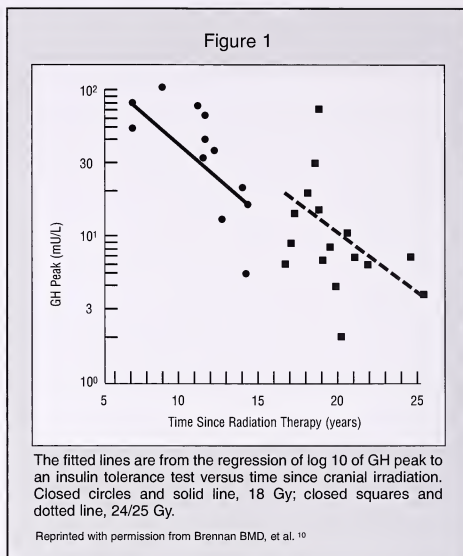
the duration of treatment, all of which reflect the intensity of the regimen. Glucocorticoids, mercaptopurine, and methotrexate are specific drugs implicated in the inhibition of normal growth. While the mechanism(s) of chemotherapy-induced growth failure remain uncertain, the data suggest that chemotherapy may act both directly on bone growth by suppressing osteoblast and osteoclast activity and through alterations of the growth hormone–insulin-like growth factor 1 (GH–IGF-1) system.<sup>4,5</sup>

Direct external beam radiation to the spine and, to a lesser degree, to the long bones can produce profound losses in growth potential in children. The ultimate impact on final height depends on the dose of radiation therapy, the volume irradiated, and the age of the subject at the time of treatment. The height reduction that occurs following contemporary radiation regimens for the treatment of diseases such as Hodgkin's disease<sup>6</sup> and Wilms' tumor,<sup>7</sup> where direct external beam radiation to the spine and bones is not intense, is generally quite modest and usually not clinically important.

The *endocrine factors* that disrupt the normal pattern of growth in survivors include GHD and premature sexual development (PSD). Both of these neuroendocrine disturbances usually are the consequence of hypothalamic-pituitary irradiation. Primary hypothyroidism also may contribute to poor linear growth in these children and will be discussed in the section on thyroid abnormalities.

Tumors such as germinomas and optic nerve gliomas, which arise in or near the region of the hypothalamus and pituitary, produce GHD as a direct result of the tumor or as a consequence of the surgery required to remove the tumor. More frequently, however, GHD is diagnosed after exposure of the hypothalamus or, less commonly, after exposure of the pituitary to high-dose, external beam radiation therapy. GHD is most often seen following whole brain irradiation for acute leukemia or for a variety of CNS tumors and after localized radiation therapy for sarcomas and carcinomas of the orbit, face, and nasopharynx. Additionally, GHD does occur following total body irradiation, which is used as preparative therapy for bone marrow/stem cell transplantation.<sup>3,8,9</sup>

Radiation therapy is followed by GHD in both a dose- and time-dependent relationship. External beam radiation doses >30 Gy typically produce GHD within 5 years of treatment; after lower doses, such as 18 to 24 Gy, GHD may not become evident for 10 or more years (Figure 1).<sup>10</sup> Once established, however, radiation-induced GHD is usually permanent. Establishing a diagnosis of GHD can be problematic in this population. First, neither plasma concentrations of IGF-1 or IGF-binding protein-3 (IGFBP-3) appear to be reliable indicators of the GH status following cranial irradiation and, thus, cannot be recommended as screening tests for the presence of GHD in irradiated subjects.<sup>11</sup> Second, the standard provocative tests to release GH can produce false-negative results (ie, normal GH levels despite low spontaneous secretion of GH). Differentiation from normal requires the use of 12- to 24-hour frequent sampling



studies, particularly in subjects treated with doses <30 Gy. False-negative results, however, appear to be less common if one utilizes the insulin tolerance test.<sup>12</sup>

Final height is most affected in individuals who are diagnosed with cancer at a young age and who are treated with high doses (>30 Gy) or who receive radiation to the whole brain and/or spine. Additionally, there is some evidence that suggests that females do worse than males, presumably because girls are more likely than boys to enter puberty at an early age following hypothalamic irradiation. Over the past several years, it has become evident that cranial irradiation at both lower and higher doses (35 to 50 Gy) is associated with the development of precocious puberty.<sup>13,14</sup> Age of onset of puberty is directly correlated with age at treatment but indirectly correlated with body mass index. While earlier studies suggested that the tempo of puberty also is accelerated in these patients, recent data have been unable to confirm this. The vast majority of patients with early onset of puberty also will suffer from GHD. Clinical signs of GHD may be obscured by the seemingly normal rate of growth these children manifest, owing to the inappropriate production of sex steroids. However, when viewed within the context of their prepubertal status and bone age, these children usually are found to be growing at a suboptimal rate. It is very important that physicians following these children keep in mind the possibility of these phenomena existing and obscuring GHD.

GH improves the growth rate of children who develop GHD following cancer therapy, at least in the short term. Data accumulated several years ago suggested that most patients, however, achieved a final height significantly below their target height. The poor response

to GH therapy has been attributed both to patient factors such as spinal irradiation, early pubertal onset, and variables in treatment such as suboptimal dosing schedules and to the older age of most patients when started on GH. Recent data suggest that improvements in growth and final height can be achieved with contemporary dosing regimens.<sup>15</sup> Moreover, the addition of a gonadotropin-releasing hormone (GnRH) agonist to suppress puberty in individuals who have sexual precocity may augment final height, but this is based on data derived solely from retrospective, uncontrolled studies.<sup>16</sup> GH-releasing hormone (GHRH) therapy also may improve growth in subjects with radiation-induced GHD, but the data are quite limited.

Concerns over the safety of GH therapy relate to the fact that GH is a potent growth-promoting agent with mitogenic and proliferating properties. However, large-scale studies assessing the risk of tumor recurrence in brain tumor survivors treated with GH have now been reported. All have consistently reported no increased risk associated with GH replacement therapy.<sup>17,18</sup> Because of the paucity of data, uncertainty remains about the risk of disease recurrence when GH therapy is administered to survivors of pediatric cancers other than brain tumors. Similarly, little information is available about the effect of GH replacement on the risk of developing secondary neoplasms in pediatric cancer survivors. The risk of developing slipped epiphyses may be increased in cancer survivors, particularly survivors of leukemia, who were treated with GH compared with children treated with GH for idiopathic GHD.

Young adult survivors with either childhood- or adult-onset GHD, such as that following low-dose cranial irradiation at a young age, also may benefit from GH therapy, especially if they manifest any of the metabolic derangements such as increased body fat, raised plasma lipids, and decreased bone density and/or quality-of-life issues that have come to be recognized as the adult GHD syndrome.

To date there are no studies, however, that have addressed the risks and benefits associated with long-term GH therapy in adult survivors of childhood cancer.

## HYPOTHALAMIC-PITUITARY DYSFUNCTION

A variety of neuroendocrine abnormalities result from external radiation to the whole brain, orbit, face, or nasopharynx, with resultant pathophysiology in the hypothalamic-pituitary axis. The larger the dose of radiation and the longer the time interval since completion of therapy, the greater the likelihood of developing any of the given problems. In the majority of instances, the site of damage appears to be at the hypothalamus rather than the pituitary gland. Early puberty and GHD are the most common neuroendocrine disturbances. The threshold dose necessary to induce these problems appears to be about 18 Gy when given in conventional daily fractions.

Clinically evident deficits of luteinizing hormone/follicle-stimulating hormone (LH/FSH), thyrotropin (TSH), and corticotropin occur less often than GHD, and generally only following doses of radiation in the range of 30 to 40 Gy.<sup>19</sup> Deficits of these hormones usually occur several years following irradiation. GHD ordinarily is the first recognizable hormonal deficiency. Interpretation of the available literature about these trophic hormones is complicated by the fact that different investigators employ different hormonal tests and use varying criteria for what constitutes "abnormal." For example, Rose et al<sup>20</sup> report a very high incidence of "hidden" central hypothyroidism secondary to TSH deficiency following cranial irradiation. According to the authors, the establishment of a diagnosis of TSH deficiency often requires performing both a thyrotropin-releasing hormone stimulation test and an assessment of the nocturnal TSH surge. These tests involve obtaining multiple blood samples during the day and night. At present it is unclear whether this subtle form of TSH dysfunction correlates with any clinical findings and, thus, whether

## CME CERTIFICATION

The GGH Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACME Essentials.

**Overview:** This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

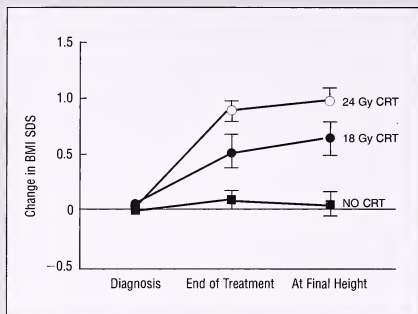
**Target Audience:** This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

**Method of Physician Participation:** Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

**Learning Objectives:** Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Figure 2



Change in body mass index (BMI)-standard deviation score (mean  $\pm$  SEM) in survivors of acute lymphoblastic leukemia according to type of central nervous system prophylaxis. CRT, cranial irradiation.

Reprinted with permission from Sklar C, et al.<sup>23</sup>

one can justify on clinical grounds the time and expense involved in this diagnostic protocol. Hyperprolactinemia also can be observed following high-dose irradiation, particularly when more than 50 Gy are used to the hypothalamus. Associated with hyperprolactinemia, clinical symptoms such as secondary amenorrhea and galactorrhea occasionally occur.

**Obesity** is a well-established sequela of cancer therapy and is often observed in survivors of acute leukemia and various brain tumors. Sklar et al<sup>21</sup> and others suggest that in survivors of ALL a high incidence of obesity is seen but confined to those survivors who received cranial irradiation (Figure 2). Additional risk factors for obesity other than cranial irradiation include female gender and exposure to dexamethasone. The mechanisms underlying these propensities remain unsolved. It is unlikely, however, that the weight gain observed in the majority of individuals is of an endocrine basis. One possible explanation is that radiation damages centers within the brain that normally control eating behaviors and/or regulate body composition. Preliminary data suggest that cranial irradiation may even induce a state of relative leptin resistance.

## PRIMARY DISORDERS OF THE THYROID

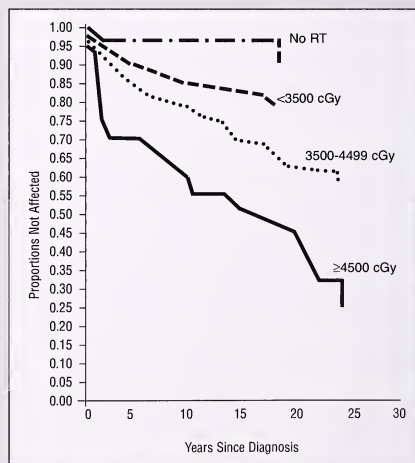
**Primary hypothyroidism** is the most common thyroid disturbance that occurs in patients whose thyroid gland has been irradiated. Primary hypothyroidism generally results from direct damage to the gland following external beam radiation. Thus, it is often detected in survivors who have been treated with neck/mantle irradiation for Hodgkin's disease, cranial-irradiation for brain tumors, or total body irradiation for cytoreduction before bone marrow/stem cell transplantation.<sup>8,22,23</sup> Primary hypothyroidism also has been described in individuals

treated with a radiolabeled monoclonal antibody such as <sup>131</sup>I-MIBG for neuroblastoma.

As in other dysfunctions following chemotherapy or radiation therapy, the dysfunction is determined primarily by the total dose to the thyroid and by the duration of follow-up. In a recent study of 1791 young adult survivors of Hodgkin's disease, a cumulative incidence of hypothyroidism of 28% was observed.<sup>23</sup> Moreover, the actuarial risk of developing an underactive thyroid 20 years after treatment was 50% for survivors who had received thyroid irradiation with doses  $\geq 45$  Gy (Figure 3). Additional risk factors for developing hypothyroidism included female gender and/or being older than 15 years of age at the time of diagnosis. Of great clinical importance, new cases have been observed more than 25 years following diagnosis and treatment of Hodgkin's disease. Consequently all patients undergoing radiation therapy in the thyroid area deserve many years of annual observation.

**Hyperthyroidism**, while far less prevalent than hypothyroidism, does develop at an increased rate in certain subsets of childhood cancer survivors. A common setting is following external beam radiation to the neck region for Hodgkin's disease, where the chances of becoming hyperthyroid are 8 times greater than that observed in the general population.<sup>23</sup> The major risk factor for development of hyperthyroidism is irradiation of

Figure 3



Probability of developing an underactive thyroid after diagnosis of Hodgkin's disease. Patients are grouped according to dose of thyroid irradiation. RT, radiation therapy.

Reprinted with permission from Sklar C, et al.<sup>23</sup>



the thyroid involving doses >35 Gy. A second but less common cause of hyperthyroidism is the appearance of autoimmune thyroid disease following allogeneic bone marrow/stem cell transplant. The published data are most consistent with the hypothesis that the thyroid disorder is due to adoptive transfer of abnormal clones of T or B cells from donor to recipient.<sup>8</sup> Various types of autoimmune disease have been demonstrated to occur at increased frequency following bone marrow/stem cell transplants.

**Thyroid neoplasms**, both benign and malignant, do occur following irradiation of the thyroid gland. Children at greatest risk are those <5 years of age at the time of treatment and those treated with doses of radiation <20 Gy. Nonetheless, the risk of developing a thyroid neoplasm remains elevated following even relatively high-dose radiation therapy. Thyroid nodules are particularly common in females and often occur after a long latency period (>10 years). Recently, Sklar et al<sup>23</sup> reported that the risk of thyroid cancer was increased 18-fold in a large cohort of young adult survivors of Hodgkin's disease. The median dose of radiation to the thyroid was 35 Gy, with a range of 25 to 35 Gy. Fortunately, the vast majority of cancers noted after radiation therapy are well differentiated and have an excellent prognosis.

## PRIMARY GONADAL DYSFUNCTION

**Treatment-induced Leydig cell failure** and/or dysfunction results from damage or loss of the machinery required for testosterone synthesis and release. Leydig cell failure and androgen insufficiency are relatively uncommon compared with damage to germ cells and infertility following cancer therapy. Chemotherapy-induced Leydig cell failure resulting in androgen insufficiency and requiring testosterone replacement therapy is quite rare.<sup>24</sup> As the majority of males undergo a normal puberty and most produce normal adult levels of testosterone, Leydig cell dysfunction is generally subclinical when it occurs. Subtle forms of Leydig cell dysfunction may be observed following chemotherapy protocols utilizing high doses of one of several alkylating agents.

**External irradiation** is more likely than chemotherapy to cause Leydig cell damage. The doses required are much higher than the doses needed to cause germ cell failure. The data obtained from individuals treated with radiation therapy for a variety of malignancies show that the likelihood of sustaining radiation-associated Leydig cell failure is directly related to the dose delivered and inversely related to age at treatment. Normal amounts of testosterone are produced by the majority of males who receive ≤20 Gy fractionated radiation to the testes.<sup>24</sup> Since raised concentrations of LH at baseline and following GnRH stimulation are found in many of these young men, one must assume that subclinical injury to the Leydig cells occurs even at these low levels of radiation exposure. The clinical importance of this phenomenon is unclear, but there are data to suggest that subtle forms of Leydig cell insufficiency may predispose to decreased bone density and changes

Table 1  
**Chemotherapeutic Agents Associated With Germ Cell Damage**

| Alkylating agents | Nitrosoureas      |
|-------------------|-------------------|
| Cyclophosphamide  | BCNU (carmustine) |
| Ifosfamide        | CCNU (lomustine)  |
| Procarbazine      |                   |
| Busulfan          | <b>Cisplatin</b>  |
| Meiphalan         |                   |
| Thiotepa          | <b>Etoposide</b>  |

in body composition over time.<sup>25</sup> A dose of >24 Gy fractionated irradiation as therapy for young males with testicular relapse of ALL is associated with a very high risk for Leydig cell dysfunction. One should anticipate that all boys who are prepubertal at the time that they receive 24 Gy testicular irradiation will also develop frank Leydig cell failure and require androgen replacement. Most but not all boys who are older and/or in early puberty at the time they are treated with 24 Gy will also ultimately need therapy with testosterone.

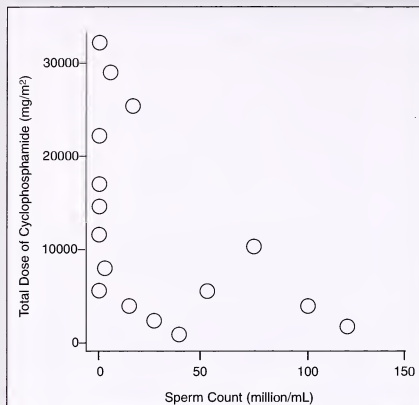
**Treatment-induced germ cell failure** in males occurs frequently, in contrast to what occurs in Leydig cells, which are resistant to damage from most chemotherapeutic agents and lower doses of radiation. The chemotherapeutic agents most commonly associated with impaired male fertility include the alkylating agents listed in Table 1. Importantly, the concept derived from earlier studies suggesting that the germ cells of younger males were less vulnerable to the toxic effects of chemotherapy compared with older boys and young adults has been called into question by recent studies.<sup>26</sup> Impaired fertility occurs in 40% to 60% of young adult male survivors of childhood cancer. A high probability of oligospermia azoospermia and infertility exists in those exposed to >20 g/m<sup>2</sup> of cyclophosphamide. In contrast, many individuals treated with a cumulative dose of 7.5 to 10 g/m<sup>2</sup> or less retain normal sperm production (Figure 4, page 42).<sup>26,27</sup>

Testicular irradiation in doses as low as 0.15 Gy has produced impaired sperm production. If the dose is under 1 to 2 Gy, recovery is generally common. At doses >2 to 3 Gy, recovery of sperm production is rare.<sup>28</sup>

Infertility resulting from radiation therapy or chemotherapy is often associated with reduced testicular volume, increased FSH concentrations, and reduced plasma concentrations of inhibin B. While there are good correlations overall between these markers and sperm counts in large groups of survivors, considerable overlap occurs between normal and abnormal individuals. *Many male survivors with documented azoospermia fail to manifest either a reduced testicular volume or an elevated level of FSH.* Thus, currently there is no substitute for sperm analysis to determine a male's current fertility status.<sup>29</sup>



Figure 4



Relation between total dose of cyclophosphamide ( $\text{mg}/\text{m}^2$ ) and sperm count.

Reprinted with permission from Relander T, et al.<sup>27</sup>

**Ovarian failure** results in disruption of and damage to both ovarian germ cells and the hormone-producing cells. This results from the structural and functional interdependence within the follicle between sex hormone-producing cells and oocytes.<sup>24</sup> This contrasts with testicular pathology, where despite the loss of germ cells following cytotoxic therapy, production of sex hormones is often preserved.

The ovaries of prepubertal females are relatively resistant to chemotherapy-induced damage compared with the ovaries of adults. Nonetheless, alkylating agents (Table 1, page 41) given at high doses can be toxic to the young ovary. Fortunately, the majority of prepubertal girls and adolescent females receiving standard combination chemotherapy will retain or recover ovarian function during the immediate posttreatment period.<sup>24</sup>

In young women treated with alkylating agents for acute leukemia, brain tumors, and Hodgkin's disease, increased plasma concentrations of FSH have been reported. Fortunately, normalization of FSH levels occurs in a majority, and only a minority experience irreversible ovarian failure. Recovery may not occur for many years following completion of therapy.<sup>30</sup> Even with FSH elevated at 5 years following completion of therapy, normalization and subsequent pregnancy have been reported. Some of these women, however, experience premature menopause when they reach their 20s and 30s.<sup>31</sup>

Females who receive high-dose myeloablative therapy such as busulfan, melphalan, and thiotepa with alkylating

agents in the context of bone marrow transplantation are at high risk of developing ovarian failure.

Females receiving abdominal, pelvic, or spinal irradiation are at increased risk of ovarian failure, especially if both ovaries were within the treatment field. As is true for chemotherapy, damage from radiation therapy seems to be less severe in younger individuals than in older (adult) individuals. Thus, while radiation doses of 6 Gy may be sufficient to produce irreversible ovarian damage in women >40 years of age, doses in the range of 10 to 20 Gy are needed to induce permanent ovarian failure in the majority of females treated during childhood.<sup>24,32</sup>

## SUMMARY

Our understanding of the endocrine consequences of cancer therapy has increased substantially over the past few years. Radiation therapy and chemotherapy are capable of causing damage that is often subtle; endocrine abnormalities may remain subclinical for many years. Physicians who follow children or adolescents who have been treated with chemotherapy and/or radiation therapy for cancer must encourage the patients to continue lifelong surveillance for potential endocrine disease.

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## Cranial Irradiation and Central Hypothyroidism

Survivors of therapy for tumor infiltration of various sorts in the hypothalamic-pituitary area frequently have tropic hormone deficiencies. These deficiencies are known as central hormone deficiencies and produce "secondary" hormone deficiencies of the peripheral endocrine glands (thyroid, adrenal, gonads). Destruction of these peripheral glands directly results in primary thyroid, adrenal, and/or gonadal deficiency. Patients receiving irradiation of the head often end up with either primary or secondary thyroid deficiency—or both—because the thyroid and pituitary glands both receive irradiation as a result of the proximity of the thyroid gland to the hypothalamic-pituitary area. Rose reviews in this article the effects of cranial irradiation on regulatory cells of the pituitary gland. She then summarizes the characteristics of mild hypothyroidism (primary, central, and mixed) resulting from radiation therapy, discusses diagnostic methods, and recommends guidelines for the treatment of central hypothyroidism.

Rose's review is too extensive to abstract for *GROWTH, Genetics & Hormones*, but its thoroughness and importance must be brought to the attention of any physician working with children or adults who have received intracranial irradiation. Early in the article the effect of different types and doses of radiation upon various physiologic parameters is covered. The following section pertains to regulation of the thyroid axis and emphasizes the circadian pattern of thyrotropin secretion and how measurement of the normalcy or abnormalcy of this parameter is helpful in differentiating central, primary, and mixed hypothyroidism. The third section discusses primary hypothyroidism resulting from mantle irradiation for Hodgkin's disease,

cranial irradiation for medulloblastoma, and total body irradiation in preparation for bone marrow transplant. Dr. Rose emphasizes that primary hypothyroidism is very frequently associated with secondary hypothyroidism in these instances. Central or secondary hypothyroidism then is considered; emphasis is placed on the frequent occurrence of free thyroxine ( $T_4$ ) levels in the low normal range and on the absence of elevated thyrotropin levels. A blunted or absent nocturnal thyrotropin surge is a characteristic of central hypothyroidism, suggesting loss of the normal circadian variation in thyrotropin-releasing hormone (TRH) release. Mild hypothyroidism, both central and primary as well as mixed, is considered. Dr. Rose urges treatment for all patients with mild hypothyroidism, whether primary or secondary. "Even mild TSH (thyrotropin) rises might be a sign of possible thyroid dysfunction and should not be ignored. The opportunity to improve growth rate will be missed."

A subsequent section considers *mixed hypothyroidism*, which is a newly named syndrome consisting of central hypothyroidism associated with elevated thyrotropin. Secretory dynamics are abnormal.

A subsequent section deals with treatment of central hypothyroidism. One recommendation is that  $T_4$  therapy in patients with central hypothyroidism should be adjusted to keep the free  $T_4$  values at 1.4 to 1.6 ng/dL.

Dr. Rose observes that the cause of poor growth in childhood cancer survivors cannot always be identified. Although often caused by toxic effects of chemotherapy, radiation effects on bone growth centers, or GH deficiency, poor growth also can in many cases be caused by undiagnosed central hypothyroidism. Central hypothyroidism is much more common after radiation therapy for childhood cancer than has generally been recognized. Early identification and treatment of hypothyroidism can improve the quality of life and optimize the final adult height of these patients.

Rose SR. *Trends in Endocrinol & Metab* 2001;12(3):97-104.

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**Editor's comment:** The use of free  $T_4$  screening and of confirmatory testing that combines the thyrotropin surge test with the TRH test should improve the sensitivity with which central hypothyroidism is diagnosed. The thyrotropin surge and TRH tests should be used to assess thyroid status in cancer survivors whose free  $T_4$  value is in the lowest third of the normal range, whose basal thyrotropin concentration is normal, and whose growth rate is slowed. Other hypothalamic-pituitary axes should be evaluated concurrently as clinically indicated. Much improvement in diagnosing and treating primary, secondary, and tertiary hypothyroidism has occurred in the last 10 years. Dr. Rose's article is an excellent summary of these advances and how to apply them.

Robert M. Blizzard, MD

## Final Height of Short Subjects of Low Birth Weight With and Without Growth Hormone Treatment

Zucchini et al report on their analyses of final heights in 2 groups of short children who were below the 10th percentile for weight. The 49 subjects presented at approximately 10 to 11 years of age. Thirty-five were below the 3rd percentile for height and 15 were between the 3rd and 10th percentiles for height. The latter were growing <3 cm/y. All had predicted heights lower than target heights, which were defined as sex-corrected midparental height (father's + mother's height)  $\div 2 + 6.5$  cm for males and  $-6.5$  cm for females, expressed in SDS units. Each subject underwent 2 tests for GH release, arginine and levodopa stimulation. Those (29) with a peak of  $<8$   $\mu\text{g/L}$  were classified as GH deficient (GHD) and treated with

above their target height. In the untreated group, the height for CA SDS at diagnosis was the largest contributor to the variance in final height, followed by CA at diagnosis. In the treated group, height for BA SDS was followed by height for CA SDS and then CA at diagnosis, in respect to contributing variance. The Figure below graphically displays the lack of effect on the statistics of the 2 groups.

The authors state that their study confirms a negative prognosis for adult height when postnatal short stature persists, and that short subjects with low birth size will not reach their target height regardless of treatment with GH. They compare their data to that of Coutant and colleagues (*J Clin Endocrinol Metab* 1998;83:1070), who used lower doses of GH (0.4 U or 0.13 mg/kg/wk/  $\text{m}^2$  for a child) in 70 intrauterine growth retarded (IUGR) children with alleged GHD (not supported with retesting as adults) and compared the resultant data with an untreated comparable group. Final heights were comparable in both groups. Treatment was associated with a suggestive height gain of about 3.4 cm. The authors concluded that GH at this dosage level in IUGR GHD-classified patients had a limited effect on the final height of short children born with IUGR. Only those children starting treatment from a greater height for CA, and BA, and those with shorter parents had a chance of becoming taller than their parents in this study.

Zucchini S, et al. *Arch Dis Child* 2001;84:340-343.

**Editor's comment:** This interesting study confirms the clinical observations of many pediatric endocrinologists, ie, most

Table  
Results in the 2 Groups of  
Subjects Studied

|                           | Final Height | Target Height-<br>Final Height | Cases With<br>Final Height><br>Target Height |
|---------------------------|--------------|--------------------------------|--|
| Untreated group<br>(n=20) | -1.87 (0.21) | 0.65 (0.20)                    | 6/20 (30%)                                   |
| Males (n=9)               | -1.81 (0.31) | 0.56 (0.30)                    | 3/9 (33%)                                    |
| Females (n=11)            | -1.92 (0.30) | 0.75* (-0.33 + 1.35)           | 3/11 (27%)                                   |
| Treated group (n=9)       | -1.78 (0.18) | 0.61 (0.18)                    | 7/29 (24%)                                   |
| Males (n=16)              | -1.77 (0.25) | 0.63 (0.27)                    | 4/16 (25%)                                   |
| Females (n=13)            | -1.80 (0.25) | 0.83* (0.07 + 1.20)            | 3/13 (23%)                                   |

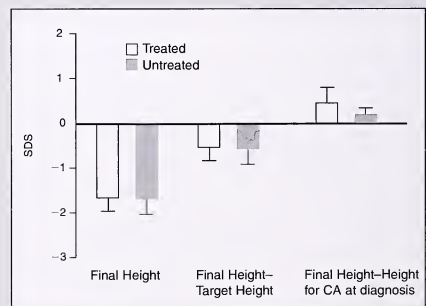
In the first 2 columns data are expressed in SDS as mean (SEM) or median\* (interquartile range).

Reprinted with permission from Zucchini S, et al. *Arch Dis Child* 2001;84:340-343.

GH 20 U ( $\sim 7$  mg/ $\text{m}^2$ /wk), which at the average weight per m<sup>2</sup> equals 28k. On the average, such a child has a height age of 8 years. Therefore, for this size child, administration of 0.25 mg/kg/wk of GH is slightly less than the usual dose of 0.3 mg/kg/wk given in the United States to GHD patients. Treatment ranged from 36 to 84 months, with a median of 55.7 months. Final height was determined when growth was less than 0.5 cm in the last 6 months of GH treatment or at a chronologic age (CA) greater than 16 years (females) or 18 years (males). All subjects went through puberty spontaneously and had completed pubertal development by the end of the study.

Both groups were similar at the initiation of the study with regard to birth weight, CA, height for CA SDS, height for bone age (BA) SDS, predicted height SDS, and target height SDS. Unfortunately, there was no statistical difference between the 2 groups when final height was measured (Table). Final height was significantly lower than the target height in both groups, and fewer than one third of the subjects reached a final height

Figure  
Lack of Effect in the  
2 Groups of Subjects Studied



Final height, final height-target height, and final height-height for CA at diagnosis in untreated and treated subjects.

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children with IUGR do not grow well even when given GH. Although the current study was in part retrospective, in that the physicians did not examine the children at the time of birth, they were able to determine that none of the children had any syndromes associated with short stature. The strength of the study is that it is one of the first to examine final height in these children. However, before placing undue credence on the findings, it should be noted that the children in both groups were relatively old (~10.8 years) when they presented for evaluation, and, therefore, there was little time for GH treatment prior to the onset of puberty. Despite these drawbacks, this relatively large study with final heights provides important information for physicians trying to determine whether to treat similar children with GH.

William L. Clarke, MD

**Second editor's comment:** Although substantial data indicate that administration of rhGH increases growth rate and height in short children and adolescents with IUGR selected on the basis of low birth weight or short birth length,<sup>1</sup> there are few data concerning the adult height of such subjects.<sup>2</sup> (The term "near adult" height rather than "final" height is preferred by this commentator as the latter conjures up a vision of the ultimate "finality.") Sas et al<sup>3</sup> note that administration of rhGH over 6 years to children with IUGR (birth length <3rd percentile) has no apparent deleterious effect upon glucose disposal, although fasting insulin and glucose concentrations, the insulin:glucose

ratio, and the insulin secretory response to oral glucose increased. Given the increasing evidence that impaired insulin sensitivity in subjects with IUGR untreated with rhGH may have possible long-term adverse consequences (hypertension, hypertriglyceridemia, ischemic heart disease, impaired glucose tolerance<sup>4</sup>), augmenting this potential problem with rhGH is an area of concern. Lastly, present data demonstrate once more (as if further evidence is necessary) the fallibility of provocative tests and the arbitrariness of GH concentrations in the assessment of GH secretory status in the absence of known anatomic, infectious, radiation, or neoplastic insults to the hypothalamic-pituitary axis.

Allan W. Root, MD

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## Short Stature in Noonan Syndrome: Response to Growth Hormone Therapy

Noonan syndrome is a common syndrome occurring in both males and females; prevalence is approximately 1:1000. The gene for Noonan syndrome is found on chromosome 12p. Eighty-three percent of affected children in one series had short stature. Birth weight is usually normal but growth falls off before puberty, which is delayed. Final height is often compromised; mean adult height for males is 162.5 cm and for females 152.7 cm. There is no evidence of GH deficiency. Cardiac anomalies are frequent.

Kirk et al report on change in height SDS of 66 patients (54 males, 12 females) with Noonan syndrome, of whom 10 were treated with GH for up to 6 years. Seventy-eight percent of the subjects had a cardiac malformation, and 67% of the males suffered from cryptorchidism. The assessment of anterior pituitary function in 55 patients demonstrated normal GH secretion in all. Children with Noonan syndrome in one series had a height SDS of -2.9 compared with the normal population. The mean age at initiation of treatment was 10.2 years ( $\pm 3.3$ ). Seven of the 66 were experiencing pubertal development. The mean dose of GH was 0.79 U/kg/wk. Therapy with GH induced a significant increase in linear growth the first year, with subsequent falloff by the 4th year so that there was pretreatment growth velocity from year 4 on. The height SDS increased from -2.9 at the start of therapy to -2.3 after 6 years. The final height data were available only for 10 patients who were treated to near final height. The mean

final height was 147.2 cm in girls and 159.9 cm in boys. These results are not greater than the average height of girls and boys with Noonan syndrome who are not treated with GH.

The authors note that information on long-term therapy is often limited to small numbers of patients in other studies. The National Cooperative Growth Hormone Study in the United States has registered 150 patients treated with GH. The data in that study were similar to those in the study reported here. In the Kabi International Growth Study (KIGS) from Europe, there were 143 patients in the registry treated with GH with an increase in height SDS of 0.5 for boys and 1.1 for girls after 3 years of therapy.

The authors conclude that GH therapy for up to 6 years in a group of short patients with Noonan syndrome has been shown to increase height velocity and height SDS compared with both normal and Noonan children, although there is a waning of effect after 3 years. Only a minority of patients improved their height prediction by more than 5 cm even though treated for longer than 3 years. This is similar to the response to GH seen in patients with Turner syndrome. Further prospective studies are required to see whether GH has a long-term benefit in Noonan patients.

Kirk J, et al of the UK KIGS Executive Group on Behalf of the Participating Centers. *Arch Dis Child* 2001;84:440-443.



**Editor's comments:** This study is important for the data it presents on long-term GH treatment of Noonan syndrome. A recent article by MacFarlane et al (*J Clin Endocrinol Metab* 2001;86:1953) noted a waning of growth effect after 3 years of GH treatment. It is possible that the optimal dose of GH for Noonan syndrome has not yet been determined and that, as in

the treatment of Turner syndrome, it is a greater dose (based on kilogram of body weight) than usually prescribed for children with idiopathic GH deficiency. Unfortunately, the studies to date do not show an extremely positive response for patients with Noonan syndrome.

William L. Clarke, MD

## A Comparison of hGH and IGF-I as Growth-Promoting Agents in Children

Messina et al report the near adult stature of 2 children with isolated GH deficiency type 1A due to partial or complete deletion of the gene complex encoding the human *GH* gene cluster on chromosome 17q22-q24. In the first patient, only the gene encoding *CS-B* was retained; she was treated with rhGH for 12 years and achieved a near adult stature greater than her target height (153 cm vs 149 cm). This patient developed only a low titer of rhGH antibodies with low binding capacity. In the second subject, only the *GH-N* gene was deleted; the patient responded well to the administration of rhGH for 4 years (0.6 to 4.6 years) without development of antibodies to rhGH (height SDS increased from -5.0 to -1.4), but then abruptly developed a high titer of rhGH antibodies with high binding capacity that severely restricted the linear growth response to further rhGH administration (7.3 cm between 4.6 to 8.6 years). This child then received recombinant human insulin-like growth factor 1 (rhIGF-1) (8.6 to 13.9 years; 40 to 120  $\mu\text{g/kg}$  SC twice daily); height increased only 21.2 cm during rhIGF-1 administration and the achieved near/adult height was far less than target height (128.6 cm vs 153.6 cm).

Backeljauw et al describe the linear growth response to rhIGF-1 (80 to 120  $\mu\text{g/kg}$  SC twice daily) in 5 children with loss-of-function mutations in the GH receptor (Laron syndrome) and 3 with deletion of the *GH* gene and acquired GH insensitivity due to development of high titers of antibodies to rhGH during treatment with this agent. The response to rhIGF-1 was similar in the 2 groups. Overall, the mean pretreatment height SDS was -5.6, (range, -3.4 to -7.0); after 6.5 to 7.4 years of rhIGF-1 administration, mean height SDS was -4.2 (range, -1.5 to -6.6), and only 1 child had achieved a height SDS greater than -2.0. The mean pretreatment growth rate was 4.0 cm/y and increased to 9.3 and 6.2 cm/y during the first 2 years of rhIGF-1 administration but slowed thereafter. Head circumference, weight and fat mass, spleen and kidney size, nasopharyngeal lymphoid tissue, facial soft tissues, and bone mineral density increased during treatment with rhIGF-1.

The authors of both articles concluded that the linear growth response to rhIGF-1 of GH-insensitive subjects is far less than that of GH-deficient patients to rhGH. They attribute the variation in response, in part, to the different effects of GH and IGF-1 on early chondrocyte differentiation and later clonal proliferation, respectively.

Messina MF, et al. Final height in isolated GH deficiency type 1A: effects of 5-year treatment with IGF-I. *Eur J Endocrinol* 2001;144:379-383.

Backeljauw PF, Underwood LE, and the GHIS Collaborative Group. Therapy for 6.5-7.5 years with recombinant insulin-like growth factor I in children with growth hormone insensitivity syndrome: a clinical research center study. *J Clin Endocrinol Metab* 2001;86:1504-1510.

**Editor's comment:** It was disappointing to learn that administration of rhIGF-1 did not restore normal linear growth in children with GH insensitivity. It is now apparent that the circulating concentration of IGF-1 is not as important a determinant of linear growth as is its tissue level. In mice without hepatic IGF-1 production, serum IGF-1 concentrations are low but linear growth is normal, suggesting that it is the local synthesis of IGF-1 that is critical for cartilage proliferation and bone growth. Since serum concentrations of "free" IGF-1 are normal in the animals without hepatic IGF-1 production, they might have accounted for the normal growth of these animals. However, the present studies in humans, in whom it is likely that during treatment "free" IGF-1 values were normal if not high (as IGF-binding protein-3 levels are low in these patients), suggest that it is not circulating but tissue IGF-1 values that are of greater importance for cartilage proliferation and linear growth. It will be of interest to examine the phenotype and response to therapy of the experimental mouse with dual knock-out of the genes encoding the GH receptor and hepatic IGF-1 synthesis.

Allen W. Root, MD

Butler AA, LeRoith D. Minireview: tissue-specific versus generalized gene targeting of the *igf1* and *igf1r* genes and their roles in insulin-like growth factor physiology. *Endocrinology* 2001;142:1685-1688.

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## FGF23, PEX and Hypophosphatemic Rickets

Hypophosphatemia occurs in a number of clinical settings, perhaps most apparent to pediatric endocrinologists and medical geneticists in X-linked and in the less common autosomal dominant forms of hypophosphatemic rickets, XLHR and ADHR, respectively. Both conditions are characterized by short stature, bow legs, hypophosphatemia, and radiographic changes of rickets and osteomalacia. A picture that explains the pathogenesis of these 2 inherited disorders and relates them to tumor-induced osteomalacia is beginning to emerge, and it involves an unlikely candidate, a relatively new member of the fibroblast growth factor (FGF) family, FGF23.

The recent story begins in 1995 with the identification by the HYP Consortium of mutations in a gene that maps to chromosome Xp22.1 in patients with XLHR.<sup>1</sup> The gene, which encodes a protein whose amino acid sequence suggests it is a neutral endopeptidase, was called *PEX* for "phosphate regulating gene with homologies to endopeptidases on the X chromosome." However, the substrates for PEX were not known. A number of mutations have subsequently been found that predict loss of function for the putative enzyme.<sup>2</sup>

The next chapter occurred in late 2000 with the positional cloning of FGF23 as the gene that harbors mutations responsible for ADHR.<sup>3</sup> Of note was that the mutations in 4 families studied mapped to 1 of 2 closely spaced arginine residues at positions 176 or 179 of FGF23.

Most recently, Shimada et al have shown that FGF23 is produced abundantly in tumor-induced osteomalacia.<sup>4</sup> They first cloned a highly expressed cDNA from an osteomalacia-inducing tumor, showing that it encoded FGF23. Next, they demonstrated that injection of FGF23 into mice reduced serum phosphate levels within 12 hours. They then showed that trans-

plantation of CHO cells expressing and secreting FGF23 into nude mice led to hypophosphatemia; increased phosphate renal clearance; high alkaline phosphatase and inappropriately low 1,25-dihydroxy-vitamin D levels in association with bone deformities; osteomalacia; and widening of the growth plate typical of rickets. Shimada et al were unable to demonstrate direct effects of FGF23 on phosphate transport in renal epithelial cells (OK cells) in culture, raising the possibility that FGF23 acts indirectly on renal phosphate transport. However, in an independent study, Bowe et al documented that FGF23 does block phosphate resorption in this cell culture model of renal proximal tubule epithelia.<sup>5</sup>

As this story evolved, the idea emerged that FGF23 is a substrate for PEX and that loss of PEX function in XLHR leads to an accumulation of FGF23 in serum and in kidney tissues, where it blocks renal phosphate resorption. Supporting this possibility is that the arginine residues (Arg176 and Arg179) that are mutated in all 4 families with ADHR are part of a consensus recognition sequence for endopeptidases, such as PEX. Indeed, Bowe et al have now confirmed that FGF23 is a substrate for PEX cleavage and that FGF23 harboring the Arg179Gln missense ADHR mutation is not cleaved in an *in vitro* assay.<sup>5</sup>

1. HYP Consortium. *Nat Genet* 1995;11:130-136.

2. White KE, et al. Autosomal dominant hypophosphatemic rickets is associated with mutations in FGF23. *Nat Genet* 2000;26:345-348.

3. Sabbagh Y, Jones AO, Tenenhouse HS. *Hum Mutat* 2000;16:1-6.

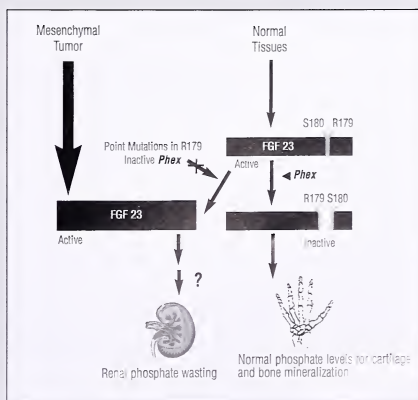
4. Shimada T, et al. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci USA* 2001;98:6500-6505.

5. Bowe AE, et al. FGF-23 inhibits renal tubular phosphate transport and is a PEX substrate. *Biochem Biophys Res Commun* 2001;284:977-981.

Figure  
Proposed Pathogenesis of Renal  
Phosphate Wasting

Mesenchymal tumors produce renal phosphate wasting by overproduction of FGF23 levels can also be increased by mutations in *Phex*, a protease that cleaves and inactivates the molecule, or by mutations at key arginine residues that render FGF23 resistant to cleavage by *Phex*. FGF23 excess causes phosphate wasting either directly or by inducing another phosphaturic factor.

Reprinted with permission from Strevler GJ. *Proc Natl Acad Sci USA* 2001;98:5945-5946.



**Editor's comment:** These articles document that FGF23 is an important modulator of phosphate homeostasis and that this process is regulated at least in part by PEX through degradation of the growth factor. They further demonstrate that FGF23 levels and resulting phosphate homeostasis can be altered through several mechanisms, including excess production by tumors and by slowed degradation either because the enzyme that normally cleaves FGF23 is ineffective due to mutation or because the growth factor itself is mutated so that it is resistant to degradation. This concept is discussed in depth by Strewler and depicted in the Figure on page 47.

William A. Horton, MD

Strewler GJ. FGF23, hypophosphatemia, and rickets: has phosphatonin been found? *Proc Natl Acad Sci USA* 2001;98:5945-5946.

**Second editor's comment:** This work illustrates the treasure trove of genetic data already available from the Human Genome Project waiting to be mined for relevance to human physiology and pathophysiology. PEX is expressed by osteoblasts, and it has been hypothesized that "phosphatonin" also may be

synthesized by these cells.<sup>1</sup> In normal mouse embryos, the murine homologue *Fgf23* maps to chromosome 6. The present investigators were unable to demonstrate expression of *Fgf23* in the tibiae of embryonic mice, perhaps suggesting that FGF23 is not "phosphatonin." Tumors that secrete a phosphate-wasting product leading to rickets or osteomalacia have been demonstrated by the same group to express FGF23 mRNA and to synthesize FGF23 protein.<sup>2</sup> However, it has not as yet been shown that FGF23 has phosphaturic activity or acts upon yet another molecule, the still elusive "phosphatonin."<sup>3</sup>

Allen W. Root, MD

1. Ecarot B, Desbarats M. 1,25-(OH)<sub>2</sub>D<sub>3</sub> down-regulates expression of PHEX, a marker of the mature osteoblast. *Endocrinology* 1999;140:1192-1199.

2. White KE, et al. The autosomal dominant hypophosphataemic rickets (ADHR) gene is a secreted polypeptide overexpressed by tumors that cause phosphate wasting. *J Clin Endocrinol Metab* 2001;86:497-500.

3. Quarles LD, Drezner MK. Pathophysiology of X-linked hypophosphatemia, tumor-induced osteomalacia, and autosomal dominant hypophosphatemia: a perPHEXing problem. *J Clin Endocrinol Metab* 2001;86:494-496. Editorial.

## Ethical Issues With Genetic Testing in Pediatrics

Advances in genetic research and emerging genetic technology are enabling testing and screening to be implemented before a full understanding of the ramifications has been developed. Clearly, new developments in genetics should be made available if they promote the best interest of the patient, in this case the child. The Committee on Bioethics of the American Academy of Pediatrics (AAP) reviewed the issues involved in genetic testing and put forward principles that should be considered before genetic testing is provided to an infant, child, or adolescent. Their report cites the Institute of Medicine's report of 1994 assessing genetic risks, implications for health, and social policy in which 3 principles were described for the introduction of new genetic tests: (1) Identification of the genetic condition must provide a clear benefit to the child; (2) a system must be in place to confirm the diagnosis; and (3) treatment and follow-up must be available for the affected individuals.

Although genetic research offers great promise for the improvement of health, the use of genetic testing must be considered carefully and only introduced with full and appropriate informed consent for the parents who provide consent for the child to have testing. There are several critical reasons for this. Genetic testing is different than other types of laboratory testing since the information obtained is familial and thus has implications for other family members. The risks of genetic testing may not be obvious but include psychosocial risks such as guilt, anxiety, and impaired self-esteem, social risks such as stigma, and financial risks involving insurance and employment. Genetic information may have limited predictive power since diseases are very complex and there are multiple environmental and genetic variables. Genetic conditions may be difficult to treat or prevent without additional research. The positive aspects of making a diagnosis should be demonstrated before screening tests are implemented.

The AAP committee report points out that there are insufficient numbers of genetic professionals (genetic counselors and

clinical geneticists) to have primary responsibility for managing the use of genetic testing, and, thus, primary care physicians must become knowledgeable about both the limitations and the positive aspects of genetic screening in children. It is particularly important to provide or refer children for counseling and testing only when it is in the best interest of the child and when testing and counseling can be provided without anticipated harm to the child.

The committee report is broken down into newborn screening, carrier screening, and predictive testing for late-onset disorders. Under newborn screening, it is reiterated that the purpose of newborn screening for genetic disorders is to limit the morbidity and mortality attributable to these inherited diseases. The report indicates that mandatory and voluntary screening should be distinguished. It strongly suggests that informed consent and voluntary screening occur rather than mandatory screening. The informed consent improves the efficiency of response to positive results and incorporates outcomes research if parents are already involved in making the decision to screen. Newborn screening protocols for phenylketonuria and hypothyroidism have been the model for early diagnosis, leading to improved treatable outcomes; however, the evaluation of the consequence of informed refusal is not yet available.

Screening programs to detect carriers are associated with significant concerns about the possibility for communities to misunderstand the carrier state, leading to stigma and discrimination against the identified carrier, as well as the possibility of adverse psychological reactions. Nevertheless, carrier testing for pregnant adolescents or adolescents who plan pregnancies may well be appropriate.

Predictive testing for late-onset disorders is as yet poorly understood and in general should be delayed until an autonomous decision by the individual to have this type of



predictive testing can be made. Reduction in morbidity or mortality as a result of genetic testing for late-onset disorders has not yet been demonstrated, and the risk of adverse psychological response and discrimination by insurers and employers appear to be real concerns. Further, the complexities of genetic testing for complex disorders have not been worked out.

In summary, the AAP Committee on Bioethics points out that pediatricians must be well informed about these issues and understand that there are both positive and negative aspects of genetic screening that are part of proper informed consent. Furthermore, potential harm does exist in screening programs, and testing

should be deferred until adulthood unless there would be significant benefit to the child to undergo genetic testing.

Committee on Bioethics. *Pediatrics* 2001;107:1451-1455.

**Editor's comment:** The AAP report on genetic testing should be required reading for pediatricians since there are pitfalls to all genetic testing. These must be understood by both the pediatrician and the person giving permission for testing of a child before testing is undertaken. Therefore, search out the complete article.

Judith G. Hall, OC, MD

## Development of Renal Cell Carcinoma in Living Donor Kidney Grafts (in Association With hGH Administration)

Tyden et al report 2 cases of young boys (~4 years of age) who received kidney transplants from their fathers. De novo development of carcinoma was diagnosed by biopsy 9 and 11 years after transplant. One patient received a new transplant and the other received dialysis therapy. Progressive cyst formation was observed in each kidney for many years before carcinoma was diagnosed. The kidneys remaining in the 2 fathers did not develop cyst formation. The boys received human growth hormone (hGH) for a total of 7 years and approximately 5 years. For the latter, administration was intermittent.

The authors state that although renal cell carcinomas have developed previously in kidney allografts (cadaver source), it is not known whether in those reported cases the carcinomas were de novo or whether they had been present at transplantation. The authors, however, state that these are the first de novo cases reported in living donor transplants. The authors conclude that it is possible that hGH stimulates the growth of renal cell carcinoma, or perhaps induces the development of such carcinoma more quickly, in acquired disease of the kidney transplant. They also state that the findings emphasize the importance of annual ultrasonographic surveillance of renal grafts, especially in the pediatric population.

Tyden G, et al. *Transplantation* 2000;11:1650-1656.

**Editor's comment:** Regardless of whether coincident with, or attributable to, hGH administration, the fact that renal cell carcinoma occurred in these 2 kidney recipients who were receiving hGH deserves significant attention. All transplanted patients should be followed closely for the possible development of renal carcinoma. Development of cysts should prompt suspicion that carcinoma might develop. The development of solid tumors superimposed on the cystic kidney should be reason for immediate surgery. The development of cysts in patients receiving hGH, in my opinion, should prompt prompt discontinuation of hGH. Fortunately, the time intervals appear to be lengthy before renal cell carcinoma develops after transplant. The possibility that hGH might be an inductive agent for renal carcinoma, again in my opinion, should be discussed with the parents, and with the child if he/she is the age of consent, before hGH is administered. hGH should be given under the auspices of a research protocol.

Robert M. Blizzard, MD

## Growth Hormone Deficiency (GHD) Caused by Pituitary Stalk Interruption in Fanconi's Anemia

Fanconi's anemia can be associated with growth retardation. The authors describe the presence of isolated growth hormone deficiency (GHD) or GHD associated with thyrotropin deficiency in the pituitary stalk interruption syndrome, which was demonstrated by magnetic resonance imaging (MRI) in 5 patients with Fanconi's anemia. GH treatment produced catch-up growth in all cases. The authors concluded that the combination of these findings suggests a common genetic origin.

Dupuis-Girod S, et al. *J Pediatr* 2001;138:129-133.

**Editor's comment:** Fanconi's anemia is a rare autosomal recessive disease of variable penetrance that arises from an abnormal processing of DNA. The first of the genes responsible for this syndrome was identified in this decade (Nature

1992;358(6385):434). Fanconi's anemia patients may present with multiple congenital abnormalities, including bone marrow failure, and increased susceptibility to cancer. They have a 15,000 times greater risk of developing acute myelogenous leukemia (*Blood* 1994;84:1650-1655).

It has long been recognized that growth retardation with normal or decreased GH response to pharmacologic stimuli may be present in this disease. The International Fanconi's Anemia registry reported that short stature is a common finding in these patients (mean, 22.37 SDS) with an 81% prevalence of endocrinopathy. Forty-four percent of the tabulated patients had a subnormal response to GH stimulants; 100% had an abnormal response to GH profile (*Pediatrics* 2001;107:744-754). Dupuis-Girod et al in the present paper



demonstrated that Fanconi's anemia is frequently associated with GHD and pituitary stalk interruption syndrome. The demonstration by MRI of the latter abnormality is a new finding, which had not been documented in the past in such patients. The pathogenesis of pituitary stalk interruption syndrome is unknown. It could be related to injury at birth or perhaps to the same deletions in the genes that lead to Fanconi's anemia. It is interesting to note that patients with Fanconi syndrome might not always have the severe type of GHD. Pituitary stalk interruption probably needs to be considered only in patients with Fanconi's anemia who are severely growth retarded and in whom treatment with GH will induce catch-up growth. However, it should be kept in mind that patients with

chromosomal abnormalities, including patients with Fanconi's anemia, in particular are at a higher risk for malignancies when treated with GH. Therefore, the question has been raised about the dilemma of initiating a treatment that may improve growth but also might increase the risk for cancer. Although the incidence of leukemia in GH-treated patients without predisposing risk factors is believed not to be different from that of the general population (J Clin Endocrinol Metab 1996;81[693]:1692-1696 and 1704-1710), in patients with Fanconi's anemia this complication might ensue (Lancet 1994;343:1576).

Fima Lifshitz, MD

## Neonatal Diabetes Mellitus Due to Complete Glucokinase Deficiency

Diabetes mellitus is a heterogeneous disorder. Neonatal diabetes, defined as insulin-requiring hyperglycemia occurring within the first month of life, is a rare form of diabetes but also is heterogeneous. Transient or permanent neonatal diabetes can occur. Recently, it has been recognized that transient neonatal diabetes is often associated with abnormalities of chromosome 6, including imprinting abnormalities. Mutations of insulin promoter factor 1, resulting in pancreatic agenesis, are seen in permanent neonatal diabetes.

This report describes 2 patients with permanent neonatal diabetes due to complete glucokinase deficiency, the result of identified mutations in the glucokinase gene. The affected individuals had poor fetal growth and intrauterine growth retardation, and required insulin in the first days of life. Interestingly, diabetes of many forms was seen within the family among the carriers (heterozygotes) of the gene defects. Among the carriers (heterozygote), maturity-onset diabetes, diabetes of the young, type 1 diabetes, and type 2 diabetes were all observed. One affected infant also had total situs inversus, which was not seen in any other family members.

Glucokinase mutations are relatively common in diabetes, and the homozygous state may actually be a common cause for neonatal diabetes. Glucokinase plays a key role in the regulation of insulin secretion in humans. Thus, the authors tested for mutations in other genes along the pathway, including hepatocyte nuclear factors 1 and 4, insulin promoter factor 1, NK-2 homeobox homologue 2, neurogenic differentiating factor 1-beta-cell, and E box transactivator 2. They found no abnormalities in any of those genes.

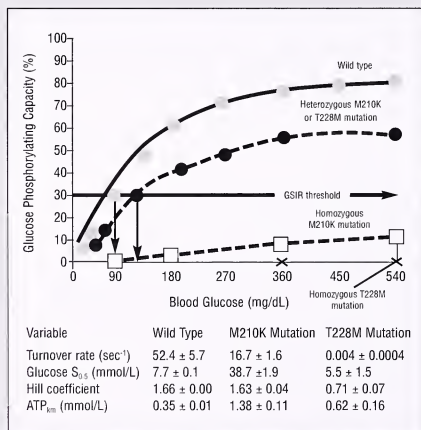
Interestingly, mouse models of glucokinase deficiency also have growth retardation and hypoglycemia at birth, but they also have hypertriglyceridemia, hepatic steatosis, and reduced stores of glycogen, which apparently are not seen with the human mutations.

Njolstad PR, et al. *N Engl J Med* 2001;344:1588-1592.

**Editor's comment:** The variabilities seen in the families of these infants with neonatal prone diabetes are quite remarkable, suggesting that heterozygotes have problems of many varieties. The authors worked out the kinetics of complex control of glucose metabolism and showed very nicely that the homozygous state simply does not produce enough enzyme to have a normal role, whereas the heterozygous state has variable levels and thus must interact with other factors to produce the various types of diabetes seen (Figure).

Judith G. Hall, OC, MD

Figure



Comparison of the modeled functional properties of wild-type glucokinase, glucokinase with the M210K mutation, and glucokinase with the T228M mutation in the homozygous and heterozygous state. GSIR, glucose-stimulated insulin release.

Reprinted with permission from Njolstad PR, et al. *N Engl J Med* 2001; 344:1588-1592.

## Stem Cells to Pancreatic Islet, Insulin Secreting Cells

Stem cells are receiving considerable attention because of the ethical issues they raise and the potential they offer for treatment of many human diseases. The recent report that stem cells can be coaxed to produce insulin may raise the debate to a new level.

The endocrine pancreas (islets of Langerhans) contains 4 cell types that secrete peptide hormones: insulin ( $\beta$  cells), glucagon ( $\alpha$  cells), somatostatin ( $\delta$  cells), and pancreatic polypeptide (PP cells). Because of the close association of these cells with neural cells in the pancreas and their similar embryonic origins, the National Institutes of Health group headed by McKay postulated that experimental strategies that induce embryonic stem cells (ES cells) to become neural cells could be used to induce ES cells to become pancreatic endocrine cells.

Using techniques previously developed, they first induced ES cells to differentiate as neural precursor cells. These cells expressed a marker gene, termed nestin. Their protocol then progressed through a series of steps that sequentially expanded and selected pancreatic endocrine progenitor cells, using various markers to identify these cells and their precursors. By the end of the protocol, which took approximately 3 weeks, they generated relatively large numbers of insulin-positive cells, which resided in clusters in close association with neurons. Confocal microscopy revealed that the insulin-positive cells were located in the centers of the clusters surrounded by neurons. Immunostaining revealed that glucagon, somatostatin, and pancreatic polypeptide also were produced by cells in the clusters that tended to surround the insulin-positive cells. Pancreatic exocrine markers were not detected. Thus, the ES cells generated multicellular structures that resembled pancreatic islets *in vivo*.

The investigators next performed clonal analysis to determine if the islet-like cells and the neurons developed from independent progenitor cells or from a common progenitor cell. The results suggested they arose from a common progenitor cell pool.

Experiments were next carried out to show that the islet-like cells release insulin in response to glucose in a dose-dependent manner with kinetics similar to those of pancreatic islet cells in culture. Quantitation revealed that the ES cell-derived cells contained about 1/50th the amount of insulin that normal islet cells contain. The researchers then examined the effect of several known agonists and antagonists of insulin secretion on insulin release. All of the agents tested produced appropriate responses, indicating that the machinery used to regulate insulin secretion by islet cells is present in the islet-like cells.

Finally, the authors tested the ability of the ES cell-derived clusters to survive and function *in vivo* by grafting the cell clusters subcutaneously into the shoulders of streptozocin-diabetic mice. When harvested later, the clusters were shown to vascularize and to remain insulin-reactive. Although grafted mice were able to maintain body weight and survive longer than sham-grafted controls, they were not able to sustain normal blood glucose levels, which the authors attributed to

the relatively low levels of insulin per cell of the ES cell-derived islet-like cells.

The researchers concluded that engineering of ES cells to produce an abundant source of immunocompatible tissue for transplantation holds considerable promise as a future strategy for treating diabetes. In an accompanying commentary, Vogel points out that although others have reported promising results in transplanting pancreatic cells from cadavers into diabetic patients, the demand for cells is far greater than the current supply.

Lumelsky N, et al. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001;292:1389-1394.

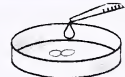
Vogel G. Stem cells are coaxed to produce insulin. *Science* 2001;292:615-617.

**Editor's comment:** As the authors acknowledge, these very promising results are still only preliminary. Nevertheless, they have caused excitement in the scientific community. It will be interesting to see if human ES cells behave like the mouse ES cells. If so, it will add considerable fuel to the debate over the use of ES cells to treat human disease.

William A. Horton, MD

### Figure Turning Mouse Embryonic Stem (ES) Cells Into Insulin-Secreting "Islet Clusters"

Stage 1 (2-3 days):  
Expand ES cells in the presence  
of leukemia inhibitory factor (LIF):



Stage 2 (4 days):  
Removing LIF prompts disorganized  
clumps of differentiating cells  
(called embryoid bodies) to form.



Stage 3 (6-7 days):  
Growing embryoid bodies in  
serum-free medium kills many cells;  
nestin-positive cells remain.



Stage 4 (6 days):  
Nestin-positive cells exposed to basic  
fibroblast growth factor (bFGF)  
and several other proteins become  
pancreatic precursor cells.



Stage 5 (6 days):  
Removing bFGF causes some cells  
to differentiate into insulin-secreting  
clusters of cells resembling  
pancreatic islets.



Reprinted with permission from Vogel G. *Science* 2001; 292:615-617

**GROWTH, Genetics, & Hormones Volume 17, Number 3**  
**Post-Program Self-Assessment/CME Verification**

**Instructions:** The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of this issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Chemotherapy, as suggested by the data, suppresses growth
  - a. through alterations of the GH-IGF-1 system
  - b. by suppressing osteoblast and osteoclast activity
  - c. neither
  - d. both
2. The ultimate impact on final height of direct external beam radiation depends on which of the following:
  - a. the dose of radiation therapy
  - b. the volume irradiated
  - c. the race of the subject
  - d. the gender of the subject
  - e. the age of the subject
3. Endocrine complications as a result of irradiation usually occur in the first 5 years following treatment.
  - a. True
  - b. False
4. External irradiation of the testis is more likely than chemotherapy to cause Leydig cell damage.
  - a. True
  - b. False

5. A normal testosterone level following external irradiation is indicative that Leydig cells are not compromised.
  - a. True
  - b. False
6. Reduced testicular volume and elevated levels of follicle-stimulating hormone (FSH) following irradiation are reliable indicators that azoospermia is present. Azoospermia also occurs frequently in males receiving external beam irradiation whose testicular size and levels of FSH are normal.
  - a. True
  - b. False

1.d, 2.abce, 3.b, 4.a, 5.b, 6.a  
**Answer Key**

**Disclosure:** As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Dr. Sklar reports grant support with Eli Lilly & Company. Drs. Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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# GROWTH

## Genetics & Hormones

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### Circadian Rhythms - Genetic Regulation and Clinical Disorders

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#### INTRODUCTION

Circadian rhythms are endogenously generated rhythms with a period length of about 24-hours. A biological clock in the hypothalamic suprachiasmatic nuclei is responsible for the generation of circadian rhythms. Notable examples of the circadian rhythms include the sleep-wake cycle and rhythms in hormone production. Abnormalities of the circadian system include biological clock lesions that result in arrhythmic behavior and irregular sleep patterns. Abnormalities of the circadian system also occur when there is desynchronization of environmental clock time with the phase of the "internal milieu" resulting in conditions such as "jet lag". Numerous aspects of human physiology are greatly influenced by the time of day, as is the pathogenesis of illness.

This review summarizes our current knowledge of the organization of the circadian system and the generation and regulation of biological clock function. The role the circadian system plays in human physiology along with the detection and treatment of biological clock disorders is also discussed.

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#### Letter From the Editor - *GGH* is on the Web!

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For The Editorial Board,  
Robert M. Blizzard, MD  
Editor-in-Chief



## CIRCADIAN SYSTEM ORGANIZATION

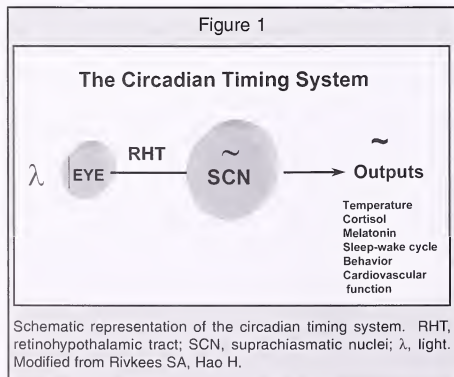
The system responsible for the generation and regulation of circadian rhythms is the circadian timing system. This neural system consists of a biological clock located in the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, of an input pathway from the retina, and output pathways from the SCN (Figure 1).<sup>1</sup>

Because oscillations of the biological clock are not exactly 24-hours, synchronizing (entraining) the circadian pacemaker each day to the 24-hour light-dark cycle is necessary. Otherwise, clock oscillations will drift (free-run) out of phase with that of the environmental cycle. A direct pathway, the retinohypothalamic tract (RHT), from the retina to the SCN mediates photic entrainment of the SCN.<sup>1</sup> Light is the most potent entraining stimulus (Figure 1).

Two types of photic regulation of circadian phase (types 0 and 1) have been described.<sup>2</sup> In humans, strong (type 0) resetting is observed after very bright light exposure (10,000 lux), and modest (type 1) resetting is observed with ordinary indoor lighting (200 lux). Although cutaneous light has been suggested as influencing circadian function in humans, there is little support for the notion that this or other extraretinal photoreception is important in mammals.<sup>3</sup>

## MOLECULAR BASIS OF CIRCADIAN RHYTHMICITY

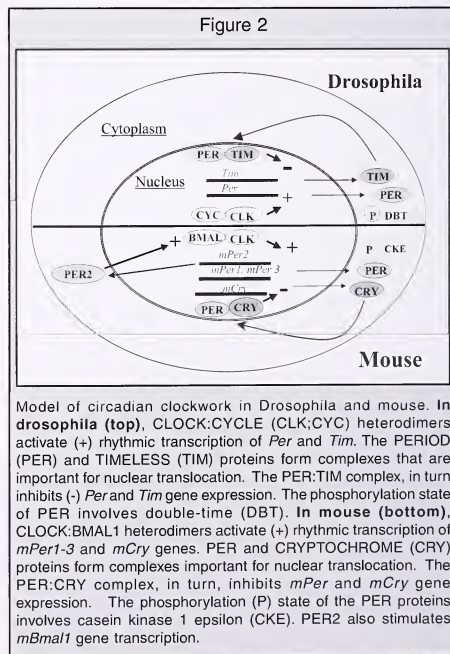
Recent data suggests that the SCN is composed of multiple, single cell circadian oscillators. These oscillate as an ensemble to generate overt rhythms.<sup>4</sup> Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, plays an important role in synchronizing the oscillations of individual clock cells.<sup>4</sup>



Considerable progress has been made over the past several years in defining the molecular mechanisms of clock oscillations.<sup>5</sup> In yeast, drosophila, and in mammals, it now appears that the molecular clockwork involves interlocking feedback loops that stimulate or inhibit clock gene expression.<sup>6</sup>

The molecular mechanisms leading to circadian rhythm generation were first detailed in drosophila (Figure 2). In these flies, the circadian feedback loop is generated by the transcriptional regulatory proteins PERIOD (PER) and TIMELESS (TIM) encoded by the *per* and *tim* genes. These are activated in the morning, and their two protein products accumulate in the cytoplasm during the day. In the evening, dimerization of PER and TIM occurs and the complex enters the nucleus. After entering the nuclei, the PER-TIM complex inhibits *per* and *tim* gene expression. In addition to feedback inhibition, the proteins CYCLE (CYC) and CLOCK (CLK) dimerize to stimulate *per* and *tim* gene expression in a rhythmic manner. These processes result in a 24-hour cycle of clock protein oscillations.

In the mammalian clock, several clock genes that are homologous to drosophila clock genes have been recently identified and discovered to play similar roles in clock regulation. Homologous mammalian and



Drosophila clock genes are described in Table 1, and their corresponding roles in circadian rhythm generation are illustrated in Figure 2. The rhythmic transcription of *mPer* genes (murine *Pers* 1-3) and *mCry* (Cryptochromes 1 and 2) are driven by the transcriptional activating factors CLOCK and BMAL1, that interact with specific promoter elements. PER and CRY then accumulate in the cytoplasm to form complexes that enter the nucleus. Within the nucleus, CRY will then directly interact with CLOCK and BMAL1 to turn off transcription of the *mPer* and *mCry* genes. As the levels of PER and CRY fall, CLOCK and BMAL1 will dimerize to restart *mPer* and *mCry* transcription restarting the 24-hour cycle.<sup>5</sup>

In addition to PER:CRY feedback inhibition, other processes contribute to the clock mechanisms. For example, PER2 (Figure 2) stimulates BMAL1 expression so that PER and BMAL1 expression are out of phase. Alteration in the phosphorylation status of PER proteins also influences PER stability and cellular localization. In Drosophila, the kinase double-time alters PER phosphorylation.<sup>6</sup> In mammals, casein kinase 1 epsilon<sup>7</sup> influences PER phosphorylation. Mutations in each of these kinases alter normal rhythmicity.

Evidence suggests that PER proteins also play a role in the photic regulation of clock phase. Following either photic or glutamatergic stimulation of the SCN, a cascade of calcium-mediated events is triggered, leading to activation of the transcriptional regulator CREB.<sup>4</sup> In turn, CREB binds to cAMP-response-element (CRE) sites within promoter regions to induce the expression of *mPer1* and *mPer2*. Alterations in PER protein expression then play a role in resetting clock phase.

## EXPRESSED RHYTHMICITY IN HUMANS AND OTHER MAMMALS

The rhythmic expression of intrinsic clock genes also drives the expression of clock-output genes, which communicate circadian phase to the rest of the organism.<sup>4</sup> This occurs as E-box elements, which are a binding site for PER, and which are present in promoter regions of other genes.<sup>4</sup>

Mutations in clock genes have been recognized in rodents with abnormal rhythmicity. Very recently, the first mutation of a human clock gene *hPER2* has been discovered. This mutation results in the advanced-sleep phase syndrome that is characterized by very early morning awakening.<sup>8,9</sup> As other individuals with abnormal rhythmicity are identified, it is anticipated that additional clock gene mutations will be found.

Table 1  
Homologous Genes in Drosophila and Mice that Play a Role in Circadian Clock Regulation

| Drosophila                               | Mouse                                  |
|--|--|
| period ( <i>per</i> )*                   | mPeriod1 *<br>mPeriod2 *<br>mPeriod3 * |
| Timeless ( <i>tim</i> )*                 | None                                   |
| Time-out                                 | mTimeless**                            |
| Cryptochromes ( <i>Cry</i> )*            | mCry1*<br>mCry2*                       |
| clock*                                   | mClock*                                |
| cycle*                                   | mBmal1 (MOP3)<br>mBmal2 (MOP9)         |
| double-time*                             | casein kinases 1 epsilon (TAU)*        |
| *mutation results in arrhythmic behavior |  |
| **mutation results in embryonic lethal   |  |

Adapted from Reppert and Weaver<sup>1</sup>

Outputs of the circadian system have been widely characterized in human clinical studies. Notable examples include the sleep-wake cycle, daily rhythms in body temperature, and day-night rhythms in cortisol production. Day-night differences in gonadotropin, testosterone, growth hormone and thyrotropin secretion are also recognized.<sup>10</sup> Melatonin production by the pineal gland is also regulated by the SCN, with secretion occurring at night in proportion to the duration of darkness. In seasonal breeding species, changes in the duration of nocturnal melatonin production regulates the activity of the reproductive axis.<sup>11</sup> Melatonin does not appear to influence the human reproductive axis.<sup>12</sup> In humans, the duration of melatonin secretion is related to the length of days. The role of endogenous melatonin secretion in regulating SCN function is also unclear, as pinealectomized animals exhibit normal circadian rhythmicity and normal phase-shifting responses to light.<sup>13</sup>

Day-night differences are recognized for many homeostatic mechanisms such as body temperature, which has a nadir in the early morning hours. Cardiovascular function exhibits diurnal rhythmicity, as

does platelet function.<sup>14</sup> Rhythms in cognitive ability are recognized, and the productivity of shift workers and health care providers varies with the time of day.

There is also increasing recognition that the circadian cycle influences the pathogenesis of many illnesses. Myocardial infarctions and cerebrovascular events occur most commonly in the morning.<sup>14</sup> Croup and certain forms of asthma are associated with evening-hour exacerbations.<sup>15</sup> In some individuals, seizures are related to the time of day. Sudden infant death syndrome (SIDS) has a strong time related component, occurring most frequently in early morning hours.<sup>16</sup> However, we do not know if the circadian system plays a role in SIDS pathogenesis.

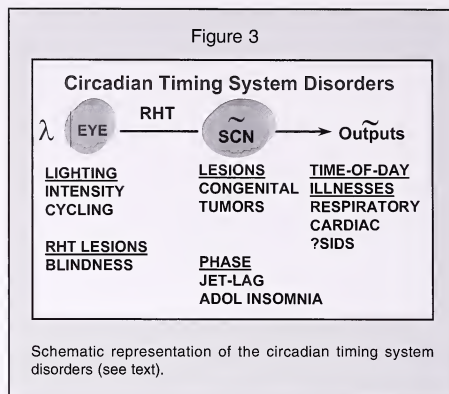
### CIRCADIAN SYSTEM ABNORMALITIES

Since the circadian system exerts potent influences on human behavior and physiology, circadian system disorders will have overt clinical manifestations.<sup>17</sup> Circadian system disorders may be related to abnormal clock function or to abnormal entrainment of the clock (Figure 3).

When more than 90% of the SCN is damaged, arrhythmic behavior may result. Thus, congenital or acquired anterior hypothalamic lesions or tumors may result in the loss of expressed day-night rhythms on sleep-wake disorders.<sup>18</sup> Congenital central system abnormalities may also be associated with clock lesions, as we have discovered arrhythmic activity patterns in a child with septo-optic dysplasia.<sup>19</sup>

Clock disorders include abnormalities in circadian phase, which relate to the timing of expressed rhythmicity (e.g. the onset and offset of sleep-wake cycles) relative to the 24-hour day. Abnormalities of circadian phase occur when the "hands" of the endogenous clock are out of phase with the environmental light-dark cycle. One notable example of this phenomenon is jet lag, which occurs when circadian clock phase does not match that of light-dark cycle after changing time zones.

Another condition in which abnormal phase relationships occur is in delayed-sleep phase insomnia. In this condition that prominently affects adolescents, clock phase is delayed with resultant late sleep-onset and awakening times. Delayed-sleep phase insomnia should be considered when the individual does not fall asleep until after midnight and awakens late in the morning or in the afternoon. This condition becomes exaggerated when the effected individual is allowed to "sleep in" on weekends. Families with abnormally advanced circadian phase have also been described, some with hPER2



mutations, suggesting a strong genetic component for the setting of circadian phase.<sup>8,9</sup>

Entrainment disorders may result from inadequate retinal innervation of the SCN. In blind individuals without intact RHT function, the absence of photic information may result in impaired synchronization of endogenous and environmental phases. The circadian phase of such individuals will free-run, resulting in times when the individuals' sleep-wake cycles do not correspond with the light-dark cycle. Recent evidence shows that timed melatonin administration may help entrain the circadian phase of blind individuals who do not entrain to the 24-hour day. This helps synchronize sleep-wake cycles with the environmental light-dark cycle.<sup>20</sup> Surprisingly there are blind individuals who have intact retinal innervation of the SCN. In these individuals, environmental lighting will entrain the circadian clock so that endogenous rhythmicity is in phase with the light-dark cycle.<sup>21</sup> Unknown non-photic factors may also entrain circadian phase in blind individuals, as we have observed sleep-wake cycles in perfect synchrony with the light-dark cycle in individuals with anophthalmia.

Another cause of entrainment abnormalities is related to problems in environmental lighting conditions. If individuals are exposed to constant indoor lighting or darkness, or to low-intensity cycled lighting that is not potent enough to shift the clock (<200 lux), expressed rhythmicity will free-run. This situation can occur in constantly illuminated intensive care units where the patient's circadian phase will drift from that of care providers. This may result in perceptions of abnormal behavior. The interpretation of time-of-day dependent tests e.g., cortisol levels also will be inaccurate in this setting. Thus, to prevent free-running rhythms, cycled lighting of adequate intensity is needed.



## DETECTING BIOLOGICAL CLOCK DISORDERS

A history of regular sleep and wake times in an individual is reassuring that the biological clock is functioning normally. The lack of regular sleep or awakening time may reflect abnormal clock function. Surprisingly, despite the socially disruptive effects of arrhythmic behavior, clock-related behavioral problems may not be brought to medical attention. Yet upon inquiry, families will give clear histories of abnormal activity patterns.

To assess clock function, diaries of sleep and waking times are useful. If the time the patient awakens and retires to sleep is consistent from day-to-day, this suggests normal clock function. However, if sleep patterns are irregular, or are out of synchrony with those of other family members, clock lesions may be present.

To provide objective assessments of behavior patterns, periods of rest and wakefulness can be assessed using monitors worn on the wrist that collect activity information for extended periods (actigraphy). Analysis of activity patterns collected over 2-3 week periods (actograms) can then be used to determine if there is normal rhythmicity or altered phase-relationships.

## CHRONOTHERAPY

Over the past several years, considerable progress has been made in the treatment of biological rhythm disorders. Light has been recognized to regulate circadian rhythmicity in humans.<sup>2</sup> Exposure to bright light (10,000 lux) during the night is a strong stimulus that produces rapid shifts in circadian phase in humans.<sup>2</sup> Not surprisingly, light therapy is now being considered as a potential therapy for jet lag and other circadian phase disorders.

The concept that bright light resets the circadian clock is also important for night-shift workers. By providing an environment with bright light exposure during work at night and darkness during the daytime when the worker rests, it is possible to shift the endogenous circadian cycle to that of the work schedule.<sup>22</sup> Light therapy is also used in the treatment of certain forms of depression.<sup>23</sup>

Behavioral paradigms can be used to treat circadian-phase disorders. Delayed sleep-phase insomnia can be treated by progressively delaying sleep onset over several days until the patient's sleep-wake cycle is in phase with the desired time of day. Alternatively, imposing regular waking times each morning can help resynchronize circadian phase.

## MELATONIN

Melatonin has received much attention as a "chronotherapeutic". Melatonin is an endogenous indolamine that is produced by the pineal gland at night in proportion to the duration of darkness.<sup>24</sup> In mammals, melatonin exerts its effects through specific high-affinity receptors that include Mel 1a (mel 1) and Mel 1b (mel 2) receptors.<sup>25</sup> These receptors consist of seven transmembrane spanning domains and couple with guanosine nucleotide binding proteins (G proteins).<sup>25</sup> In humans, the melatonin receptors have been identified in the SCN.<sup>26</sup> In non-human primates, melatonin receptors have been identified in the hippocampus, brainstem, thalamus and cerebral cortex.<sup>27</sup>

Melatonin has been touted as a therapy for a variety of conditions ranging from aging to cancer. Yet, as reviewed,<sup>28</sup> most of these claims have little credible scientific support. Melatonin, however, may have legitimate use in treating sleep disorders. Melatonin has well documented hypnotic properties, and is therefore effective in facilitating sleep onset.<sup>29-31</sup> The hypnotic effects of melatonin are most pronounced when melatonin is given in the evening.<sup>32</sup>

It has also been suggested that melatonin can acutely shift circadian phase and may have a role in treating clock disorders such as jet lag.<sup>33</sup> This issue remains controversial. Modest melatonin-induced phase shifts have been detected in some rodent species, but not in others.<sup>34</sup>

In humans, using the onset of melatonin secretion to mark circadian phase, it has been suggested that melatonin induces small shifts in circadian phase.<sup>33,35</sup> However, when primates are studied under rigorous conditions that are very difficult to achieve in humans, no phase shifting effects of melatonin are apparent.<sup>32</sup> These observations suggest that melatonin action in the treatment of jet lag<sup>36,37</sup> may be related to hypnotic effects, rather than phase-shifting properties.

Although melatonin may not acutely shift circadian phase,<sup>32</sup> melatonin administration at the same time each day may entrain free-running circadian phase. In blind individuals, nocturnal melatonin administration has been shown to entrain activity patterns to the 24-hour day.<sup>20,37,38</sup>

## SUMMARY

Increasing evidence show that the circadian system exerts profound effect on human physiology. In parallel with increases in our understanding of the clinical importance of circadian biology, there has been an explosion in our understanding of the genetic



mechanisms that contribute to the workings of the circadian clock. Elucidation of abnormalities of the circadian system has also led to the discovery of new clinical disorders that can now be identified and treated.

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## Letter to the Editor

### Ghrelin-induced obesity

The July issue of *Growth, Genetics & Hormones* (Vol. 17, p 34-35) contains a discussion of the ability of this 28 amino acid peptide to induce body fat accumulation in rodents.

But of great importance to students of human obesity is the observation that the lean weight of these obese animals was probably less, certainly not greater, than that of the controls. This finding puts such ghrelin-treated animals clearly at odds with the human state, for the latter usually have an increase in lean weight, most certainly not a decrement.<sup>1</sup> The only clearly documented exceptions to this rule are patients with the Prader-Willi syndrome<sup>2,3</sup> or Cushing's syndrome. With respect to body composition the human state differs from obesity induced by experimental hypothalamic lesions, from that of the "ob/ob" mouse, and the Zucker rat, all of which are characterized by a subnormal lean weight. Obviously, such animals, and those treated with ghrelin, cannot serve as models for human obesity.

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Gilbert B. Forbes, MD

**Editor's Response:** Dr. Forbes in his talented analytical way has added significantly to the Abstract, Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism which dealt with studies in rats and not humans. With his astute commentary he reminds us that we should not necessarily project data obtained in rodents to humans. Neither of the Editors commenting on this article were so astute as to mention this most poignant point.

Thanks very much, Dr. Forbes. The Editorial Board eagerly invites each reader to write and comment on pertinent points, ask questions or query us concerning what is published in *Growth, Genetics & Hormones*.

Robert M. Blizzard, MD  
Editor-in-Chief

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## Growth Hormone Treatment Enhances Bone Mineralisation in Children with Chronic Renal Failure (CRF)

Van Dyck et al report on bone mineralisation as determined by Dual Energy X-ray Absorptiometry (DEXA), of the whole body and lumbar spine prior, to and one-year after, the initiation of rhGH therapy in 10 pre-pubertal children with stable CRF. Inclusion criteria for the study included: (1) a height SDS of  $< -2$  SD or a height velocity of  $< 25^{\text{th}}$  percentile for age, (2) absence of growth hormone deficiency, (3) normal thyroid function, and (4) normal PTH levels. DEXA was used to measure total body mineral content (TBMC), lumbar spine bone mineral content (LBMC), total body mineral density (TBMD), and lumbar spine bone mineral density (LBMD), in patients and in a control group of 20 healthy children of similar age. DEXA was performed twice in the CRF patients and in the healthy controls. Body height was measured with a stadiometer and bone age was determined by TW2 method at the start and after one-year of treatment. Data were analyzed using Wilcoxon matched pairs.

Growth hormone treatment (1 unit or 0.3 mg/kg/week given in daily divided doses) was associated with an increase in median height velocity from 5.1 cm/year (3.0-8.8 cm/year) to 10.6 cm/year (8.2-12.7 cm/year). Median creatinine clearance remained unchanged as did calcium, phosphorous, and intact PTH levels. There was, however, a marked change in serum alkaline phosphatase. This is a well-known phenomenon in different groups of patients treated with hGH and reflects osteoblastic activity. At the beginning of the study, the median bone age was delayed 1.9 years and increased 0.8 years over the duration of treatment. The patients' TBMC, TBMD, LBMC, and LBMD increased significantly after one-year of rhGH treatment ( $p < 0.05$  for each – see Table). When compared with height/age match controls, these values were not different at the start of treatment, nor at the end of treatment. Yet BMD, TBMD, and LBMD, significantly improved in patients over one year ( $P < 0.05$ ). When compared with age- matched controls, patients had lower TBMC and LBMC at the

start of treatment and experienced a catch-up of LBMC to values similar to controls over the course of the year.

The authors note that there has been discrepancy in results from previous studies of various parameters of BMD in children with CRF treated with rhGH. They speculate that this might be explained by 2 factors - small sample size and selection bias. In the current study, findings demonstrate significantly improved BMD in children with CRF who are growth retarded. All subjects in the current study were on calcium supplements and their bone mineralisation was adequate for their height at baseline. The authors state that homogeneity of their results is most likely due to the homogeneity of the patients studied, that is pre-pubertal with severe renal disease from early years of life without signs of osteodystrophy. They conclude that rhGH treatment has a beneficial effect on BMC and BMD in pre-pubertal children with CRF. This was the finding of Lanes et al (*Horm Res* 1996;46:263-268).

Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

**Editor's Comment:** At first glance, the results of this short paper might not be appreciated as adding significantly to the information with regard to the effects of rhGH on children with renal disease. It is well known that BMC and BMD prior to puberty are important factors of similar measures in adults. Thus, any improvement which might be gained in the pre-pubertal years, could potentially be realized later in adult life. Indeed, the subjects in the Van Dyck study had indices of bone density comparable to those of height matched children at entry into the study and at the one-year follow up. What is significant is the increased BMC and BMD observed. These studies underline the importance of initiating rhGH therapy in children with CRF even when their absolute height deficiency is modest.

William L. Clarke, MD

Table

| Mineralisation parameter  | Baseline            | After 1 year rhGH   | P        |
|---------------------------|---------------------|---------------------|----------|
| TBMC (g)                  | 521 (144-944)       | 589 (225-1139)      | $< 0.01$ |
| TBMD (g/cm <sup>2</sup> ) | 0.750 (0.672-0.888) | 0.775 (0.681-0.995) | $< 0.05$ |
| LBMC (g)                  | 7.5 (3.8-15.7)      | 10.9 (5.9-18.0)     | 0.005    |
| LBMD (g/cm <sup>2</sup> ) | 0.475 (0.281-0.660) | 0.525 (0.333-0.660) | $< 0.01$ |

Adapted from Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

## Adipose Tissue is an Endocrine Gland Secreting Multiple Hormones

*You Are What You Secrete* is a summary and editorial by Saltiel in which he discusses two articles concerning adiponectin.<sup>1</sup> Saltiel emphasizes that our notion of the adipocyte as merely a cargo space for fat has undergone a dramatic change. We now know that adipose tissue is much more complex than previously thought, secreting proteins which include tumor necrosis factor (TNF)- $\alpha$ , leptin, adiponin, resistin and adiponectin known also as Acrp30 or adipoQ. These proteins perform diverse functions but share structural properties of cytokines, and are referred to collectively as "adipokines". Dynamic interactions occur between these proteins and dictate the extent to which insulin is sensed in its target tissues. In an article referred to by Saltiel, Berg et al<sup>2</sup> report that a single injection of adiponectin leads to a 2-3 fold elevation in its circulating levels, which precipitates a transient decrease in basal glucose levels. Similar treatment in ob/ob or streptozotocin - treated mice transiently abolishes hyperglycemia. This relative hypoglycemic effect is not associated with an increase in insulin levels. Moreover, in isolated hepatocytes adiponectin increases the ability of sub-physiological levels of insulin to suppress glucose production. Berg et al propose that adiponectin is a potent insulin enhancer linking adipose tissue and whole body glucose metabolism.

In the article by Yamauchi et al<sup>3</sup> the reversal by adiponectin of insulin resistance associated with both lipotrophy and obesity is described. Yamauchi et al discuss the findings that recent genome-wide scans have mapped a susceptibility locus for type 2 diabetes to chromosome 3q27, where the gene encoding adiponectin is located. This group demonstrated decreased expression of adiponectin and its correlation

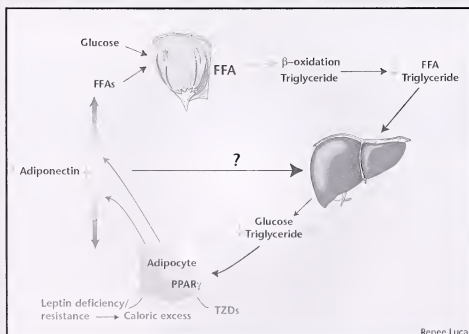
with insulin resistance in mice models of altered insulin sensitivity. Adiponectin decreases insulin resistance in obese mice by decreasing triglyceride content in muscle and liver. Insulin resistance in lipotrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either given alone. Yamauchi et al concluded that decreased adiponectin production is implicated in the development of insulin resistance in mouse models of both obesity and lipotrophy. Their data also indicate that administration of adiponectin might provide a novel treatment modality for insulin resistance in type 2 diabetes.

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2. Berg AH, et al. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Med* 7:947-953,2001.
3. Yamauchi T, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nature Med* 7:941-946,2001.

**Editorial Comment:** Adiponectin is a 247 amino acid protein whose expression in adipose tissue is depressed in obese animals. The plasma concentrations are low in these obese animals and also in obese humans, which is a pattern directly opposite to those of leptin, another adipocyte hormone. As discussed by Yamauchi et al, mice ingesting a high fat diet with increased fat accumulation had low tissue levels of adiponectin mRNA and low serum concentrations. Insulin resistance as reflected by hyperglycemia and hyperinsulinemia occurred.

Figure



From Saltiel AR. You are what you secrete. *Nature Med* 7:887-888,2001.

A hypothetical model for the secretion and action of adiponectin. The synthesis and secretion of adiponectin is increased by activation of the nuclear receptor PPAR- $\gamma$ , and reduced by caloric excess, presumably associated with leptin deficiency or resistance. Once released, adiponectin can directly increase fatty-acid transport, oxidation and dissipation in skeletal muscle, reducing the levels of intramyocellular lipids, thus improving insulin signaling. The protein can also increase the sensitivity of the hepatocyte to insulin, either through a direct action, or indirectly by lowering circulating lipids due to its action on muscle. Thus, administration of adiponectin can result in improved insulin sensitivity and glucose tolerance, and can correct hyperglycemia associated with obesity.



Administration of rosiglitazone, an inhibitor of peroxisome proliferator-activated receptor- $\gamma$  which is an essential element for adipogenesis, increased adiponectin tissue mRNA values and also serum levels. Serum glucose was decreased as were serum levels of insulin.

In other mouse models of obesity (e.g. leptin receptor deficiency), administration of adiponectin lowered blood glucose and insulin values. In another mouse model, a lipodystrophic mouse without fat, serum concentrations of adiponectin were undetectable. Hyperglycemia and hyperinsulinemia were present. Administration of adiponectin lowered serum glucose and insulin levels. Both leptin and adiponectin were required in the lipotrophic mice to restore serum glucose and insulin values to normal.

In the article by Berg *et al*, serum glucose concentrations were decreased with the administration of recombinant adiponectin to wild type, leptin deficient, and insulin deficient mice. Berg *et al* also demonstrated that adiponectin depressed hepatic glucose output *in vitro* which is thus the second physiological effect that might contribute to enhanced insulin sensitivity. In

calorically restricted wild type mice, serum adiponectin concentrations were twice those of freely feeding animals suggesting that this adipokine may be important in prolonging the lives of such animals.

Thus, the data in these manuscripts indicate that adiponectin plays a key role in energy metabolism. It enhances insulin sensitivity by lowering serum and tissue triglyceride values, by uncoupling of oxidative phosphorylation in muscle, and by suppressing hepatic glucose output. In addition to the effects on energy metabolism, adiponectin depresses the inflammatory response that accompanies atherogenesis. Indeed, patients with coronary artery disease have lower plasma adiponectin concentrations than do controls. Adiponectin inhibits inflammation in part by suppressing proliferation of myelomonocytic progenitor cells by accelerating apoptosis. The potential utilization of adiponectin as a therapeutic agent for patients with obesity, diabetes mellitus types 1 and 2, hyperlipidemia, and/or atherogenic disorders is clearly enormous. A lead article regarding Adipose Tissue as an Endocrine Gland will appear soon in GGH.

Allen Root, MD

## Genetic Basis of Stature – Genome-Wide Search for Genes that Influence Normal Adult Height

It is well known that short parents have short children and vice versa, and that variation in normal stature has a strong genetic component. However, despite many decades of interest in the genetics of stature, the relevant genes remain elusive. In fact, the genetics of most common traits and diseases in humans is not well understood. The principal explanation is that the geneticist's primary tool for mapping genes is of only limited power for finding genes that have modest effects, such as those that contribute to common diseases and variable traits such as stature. Recent advances in genomics, however, have made it feasible to apply genome-wide linkage analysis to such entities. Indeed, the group led by Eric Lander has used this approach to identify genetic linkage for adult height.<sup>1</sup>

In total, 2,327 individuals from 483 families were studied. Fifty-eight families resided in the Botnia region of Finland, 183 families were from other areas of Finland, 179 families were from southern Sweden and 63 families were from the Saguenay-Lac-St-Jean region of Quebec. They were originally ascertained to investigate other genetic traits. Males were older than 23.5 years and females older than 21.1 years to exclude individuals still growing. The original genotyping results that were based on average spacing of microsatellite markers from 6.5 cM to 12.5 cM depending on the study population, were reanalyzed using the variance-components method

implemented in the GENE-HUNTER 2 protocol. The method uses nonparametric multipoint approaches to generate LOD scores for chromosomal locations that reflect the likelihood that genotype data being observed is due to linkage relative to the absence of linkage.

Evidence for linkage was detected in four instances. A LOD score of 3.85 was obtained for linkage at chromosome 6q24-25 in Botnia. A score of 3.40 was calculated for a marker located at 7q31.3-36 in Sweden. A LOD score of 3.35 was determined for markers at 12p11.2-q14 in Finland and a score of 3.56 was found in Finland for 13q32-33. The authors note that a companion study also detected linkage at chromosome 7 site.<sup>2</sup>

The authors are optimistic that they have identified chromosomal regions where genes that influence stature reside, especially on chromosome 7. However, they caution that definitive interpretation is difficult in the absence of confirmation of linkage in additional populations. They observe their results were inconsistent across the four study groups, but note that this is typical in linkage studies of common diseases. They discuss possible reasons for the inconsistency including variation in sampling, existence of genetic variation in different populations and statistical fluctuations and false positives due to unknown causes.



**Editor's Comment:** An additional comment is pertinent to this topic. Many genes known to influence stature have been identified by searching for disease genes. Examples include genes that harbor mutations that cause chondrodysplasias and many other syndromes associated with short stature. They range from homeobox-containing genes such as *SHOX* to cartilage matrix protein genes, i.e., *COL2A1* to transcription factor and receptor genes such as *SOX9* and *FGFR3*, respectively. Similarly, mutations of *Fibrillin 1* lead to tall stature in the Marfan syndrome. It seems likely that there are genes that influence stature that are not associated with disease. The approach used here should identify genes in both categories. It will be interesting to see what genes fall into the latter category.

These papers are the first reported genome-wide studies of genetic linkage and stature. They probably represent the tip of the iceberg in terms of what will come as genetic markers become more dense, more

populations are studied and analytical approaches become more sophisticated. As noted by Hirschhorn et al, identifying the genetic basis of variation in height raises important ethical issues as the potential for genetic engineering evolves. However, as they point out, a greater understanding of this subject could be beneficial in the contexts of establishing diagnoses and predicting adult stature of "short" children.

William Horton, MD

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2. Perola M et al. Quantitative-trait-locus analysis of body-mass index and of stature, by combined analysis of genome scans of five Finnish study groups. *Am J Hum Genet* 69:117-123, 2001.

## Short Stature Homeobox-Containing Gene Deletion: Screening by Fluorescence in Situ Hybridisation in Patients with Short Stature

In an attempt to determine when to screen for *SHOX* gene deletion in subjects with short stature, Müsebeck and colleagues determined the frequency of *SHOX* deletions in 50 children with short stature. All children studied had a height < -2 SDS and 3 of the subjects also had the Madelung deformity (shortening and bowing of the radius with dorsal subluxation of the distal ulna and partial foreleg anomalies). Thirty-five of the 50 subjects had idiopathic short stature (ISS) accompanied by the absence of skeletal, endocrine, or organic symptoms and had no family history of short stature. Twelve subjects had upper limb abnormalities such as cubitus valgus. Two subjects had Léri-Weill dyschondrosteosis, and 3 had a congenital heart defect. Blood was analyzed by FISH process (Fluorescence In Situ Hybridization) for the *SHOX* deletion.

Microdeletions of the *SHOX* gene were not detected in any of the 35 patients with ISS. Of the 12 patients with additional upper limb abnormalities 5 (41.7%) displayed *SHOX* signals on only one sex chromosome. Of the 7 with short stature who displayed *SHOX* signals on 2 sex chromosomes, 3 had Madelung deformity and brachymetacarpia was present in the other 4. Point mutations of course are not picked up in the FISH technique. Molecular genetic methods will possibly detect point mutations in patients such as the 7 referred to above. Three patients with congenital heart defects did not carry *SHOX* deletions.

The authors state that their findings provide important guidelines for selecting patients for *SHOX* analysis. They

state that children with ISS are unlikely to carry such a mutation of the *SHOX* gene. Indeed, other studies have shown the *SHOX* mutation in about 1% of all patients with ISS. The combination of short stature and skeletal abnormalities of the forearm, however, makes the *SHOX* mutation much more probable. The authors caution that a father carrying a *SHOX* mutation on the X chromosome could transmit these mutations to his son because of crossing over between the pseudoautosomal regions of the X and Y chromosomes during paternal meiosis.

Müsebeck J, et al. *Eur J Pediatr* 2001;160:561-565.

**Editor's Comment:** *SHOX* gene deletion determinations have become increasingly popular in endocrine/genetic clinics evaluating children with short stature. Although, the number of subjects studied by Müsebeck et al is relatively small (n=50), their data are convincing. Apparently, *SHOX* gene determinations have little place in the evaluation of the child with ISS and should be reserved for those children who have deformities of the upper extremities even when those are very mild. Hopefully, data can be pooled in the future from numerous centers so that definitive guidelines for evaluation of *SHOX* gene determinations are more clearly defined.

William L. Clarke, MD

## Growth Hormone in Short Children: Beyond Medicine?

The increasing use of rhGH in short children with non-GH deficient (GHD) short stature, whether or not data support the efficacy of such treatment, may lead to its use being perceived as a cosmetic "enhancement". Drs. Bolt and Mul discuss the merits of the use of rhGH in such children and whether such treatment is "in the medical realm". Employing a disease-oriented model, rhGH would be administered only to patients with documented GHD or identified abnormal state (e.g., Turner syndrome) to restore health and normal functioning. The authors reject this approach because the differences between normal and abnormal growth and function are often indistinct. On the other hand, they also reject the "client approach" to prescribing of rhGH in which one would administer it "on demand" for any and all types of short stature including familial and idiopathic, because this approach might lead to "medicalization" of many perceived and apparent differences between individuals and make patients of otherwise healthy persons. Bolt and Mul believe the proper goal of medicine is to prevent or relieve suffering, both demonstrable and subjective, and advocate this approach to deciding when the administration of rhGH is or is not warranted. Suffering, while perhaps not always quantifiable, can be perceived by the family and physician. Thus, children with non-GHD short stature may be eligible for treatment with rhGH if s/he demonstrates present suffering or the potential for future suffering. They conclude that because the impact of short stature upon the functional status of normal adults is minor, treatment with rhGH "should take place in a research setting".

Bolt LLE and Mul D. *Acta Paediatr* 90:69-73,2001.

**Editor's Comment:** *The suffering individual is anguished, tortured, bitter and sad. However, it may not always be easy to identify the suffering short child.*

*Firstly, the majority of short, otherwise normal children are brought to the office of the pediatric endocrinologist by their parents who are often more concerned about the height of their child than is the child himself. Thus, it is likely that it is the parent who is "suffering" rather than the child. Drs. Bolt and Mul do not address the issue of whether rhGH should be administered to a short child to alleviate parental suffering. Secondly, suffering related to short stature is seldom due exclusively to height, but reflects a constellation of behavioral, learning and social problems. As Macklin<sup>1</sup> points out in a companion commentary, the discomfort of the short-statured child may pale when compared to the physical suffering imposed by the numerous medical procedures that accompany treatment with, and the administration of rhGH. Although the "goal of medicine" involves all of the interrelated components delineated by the authors - disease-oriented, client-related, relief of suffering - this reviewer adheres to the precept that medicine is primarily a science and that medical decision making should be based upon valid scientific data. To date, there are limited and conflicting data relative to the growth promoting efficacy of rhGH therapy of the non-GHD short child and even fewer data concerning any psychosocial benefits of treatment.<sup>2</sup> Thus, I concur with the recommendation of Drs. Bolt and Mul that such treatment be undertaken in the context of a research environment.*

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2. Guyda HJ. Four decades of growth hormone therapy for short children: what have we achieved? *J Clin Endocrinol Metab* 84:4307-4316,1999.

Allen Root, MD

## Extended Life-Span Conferred by Cotransporter Gene Mutations in *Drosophila*

These investigators demonstrate that in the adult fruit fly, *Drosophila melanogaster*, heterozygous inactivating mutations in a newly identified gene *Indy* (for *I'm not dead yet* from the film "Monty Python and the Holy Grail") double the active, fertile, and fecund life span of this insect. *Indy* encodes a 572 amino acid sodium dicarboxylate cotransporter, a membrane protein that shepherds the uptake and re-uptake of di- and tricarboxylic acid intermediate metabolites (e.g., succinate, citrate) of the Krebs cycle across cell membranes of organs responsible for metabolism and storage of fat, glycogen, and protein (e.g., the liver in

mammals). The investigators suggest that heterozygous loss-of-function mutations in *Indy* decrease the rate of absorption and utilization of metabolites, thus acting functionally to extend life span in a manner similar to that of partial caloric restriction.

Rogina B, et al. *Science* 290:2137-2140, 2000.

**Editor's Comment:** *Energy restriction has been demonstrated to extend life span in worms, mammals, and insects, but the mechanism(s) by which decreased calories does (do) so have not been identified. It may*

be that caloric restriction down regulates the expression of sodium dicarboxylate cotransporter(s) genes thus decreasing the rate of intracellular metabolism and consequently increasing cellular life. These observations suggest that perhaps some obese subjects possibly have gain-of-function mutations in one or another sodium dicarboxylate cotransporter that enhance intracellular intermediary metabolism leading to accumulation of fat, while other individuals (who can

"eat a tone and never gain an ounce") may have a variant that impedes metabolism. The data also suggest that it may be possible to modify the activity of these cotransporter molecules chemically - opening a portal for treatment of a group of obese subjects.

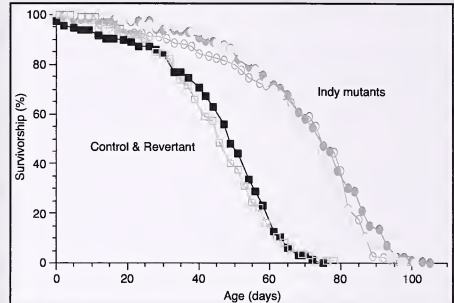
Pennisi E. Old files may hold secrets of aging. *Science* 290:2048, 2000.

Allen Root, MD

Figure

Life-span extension in *Indy* mutants. Survival curves of males heterozygous for three different *Indy* mutations, a precise excision of the P-element from *Indy* 302 (revertant), and an enhancer-trap control are shown. All flies were tested as heterozygotes over a wild-type Canton-S strain. The *Indy* mutants are *Indy*302 (open white circles), *Indy*206 (solid gray circles), and *Indy*159 (striethrough circles). The excision line (striethrough squares) is one of four exact excisions (sequence confirmed) of the P element obtained by mobilizing the P element from either the *Indy*302 or *Indy*206 line, using delta 2-3 transposase. The control (solid black squares) is one of four other enhancertrap control lines from the same mutagenesis that generated *Indy*302 and *Indy*206, tested as a heterozygote over Canton-S.

From Rogina B, et al. *Science* 290:2137-2140, 2000.



## Insulin Resistance and Insulin-Like Growth Factors in Children with Intrauterine Growth Retardation

The authors recently proposed that when tissues in utero are chronically depleted of insulin and IGF1, but subsequently exposed after normalization of nutrient supply in postnatal life to increased levels, insulin resistance often develops. Carrying this thesis forward, they postulate that postnatal "catch-up" growth might, therefore, be associated with a higher risk of developing insulin resistance, especially when other risk factors such as genetic predisposition and/or obesity coexist.

To investigate this possibility, 49 children with IUGR (22 boys) with birth weight <10th percentile for gestational age were studied. Children with malformations and/or genetic disorders were excluded. Stature was corrected for mid-parental height. Children were divided into two groups according to their corrected height; specifically, those with corrected height z-score  $\geq 0$  and those <0. Insulin resistance was evaluated using OGTT, fasting glucose and insulin levels, and a G/I <6 to interpret insulin resistance. Thirty-nine percent (19/49) of the children with IUGR had a corrected stature >0 z-score and 61% had not reached their genetic height, as expressed as MPH z-score. Corrected stature at the age evaluated correlated with birth weight, whereas actual height was related to birth length, MPH

and BMI. Twenty-two percent or 11 of 49 IUGR children had a G/I <6. The endocrine variables in children as divided on the basis of G/I <6 and >6 are provided in Table 1. All the parameters related to insulin resistance correlated with alanine aminotransferase (ALT) and gamma glutamyltransferase ( $\gamma$ -GT) levels. IGF system parameters were in the normal range and correlated neither with growth nor with insulin sensitivity.

The first aim of the study was to assess the prevalence of insulin resistance in children and adolescents with IUGR. The authors considered that insulin resistance was at a high prevalence since 22% of the children were so classified, and these data are consistent with previous studies reporting impairment in insulin sensitivity in children with IUGR. The second objective was to prove the catch up growth hypothesis that catch up growth induces insulin sensitivity. The data in this study suggest that catch up growth is not a risk factor. They further comment that the finding of high prevalence of insulin resistance did not show a significant influence over postnatal growth - is consistent with the intrauterine reprogramming previously postulated by the authors and is consistent with a genetic predispositioning determining both low birth weight and



insulin resistance. The authors also postulate that obesity may be an additional risk factor during childhood. One of the most important findings was the close relationship observed between insulin resistant parameters and liver function tests; this suggests that the liver might be a target organ of the reprogramming process. The authors did not find any indications that the IGF systems (IGF1, IGFBP-3, etc) are related to the insulin sensitivity status, at least during childhood. The latter data are in accord with those of at least two other authors.

Cianfarani S, et al. *Horm Res* 2001;55(suppl 1):7-10.

**Editorial Comment:** The authors have provided excellent data on a large number of small for gestational age infants. I have not used the term *intrauterine growth retarded* children as in the title of the article, as I believe

that term should be reserved for children who are <3rd percentile. I remain skeptical that one out of every 10 children is *intrauterine growth retarded*, which would be the case if one uses the 10th percentile as cutoff. The article as presented does not indicate to me what percentages of the children born <3rd percentile had insulin resistance. The authors and others are invited to comment to the Editor concerning which criteria are appropriate to use for determination of metabolic alterations in IUGR children, as much confusion now exists among data stated to be that of IUGR.

Regardless of what I consider this limitation, the data are worthwhile and provide interpretations to postulated metabolic alterations in children who are small for gestational age.

Robert M. Blizzard, MD

Table

Anthropometric and endocrine variables in children with IUGR glucose/insulin (G/I) ratio <6 (n = 11) or >6 (n = 38)

|                                   | G/I <6 (n = 11) | G/I >6 (n = 38) | p       |   |
|-----------------------------------|-----------------|-----------------|---------|---|
| Age, years                        | 10.3 ± 3.6      | 8.9 ± 3.3       | n.s.    | ALT = alanine aminotransferase  |
| Birth weight, kg                  | 2.16 ± 0.35     | 2.18 ± 0.38     | n.s.    | AST = aspartate aminotransferase  |
| Birth length, cm                  | 45.4 ± 2.8      | 45.8 ± 2.8      | n.s.    | AUC <sub>ins</sub> = area under the curve of insulin during oral glucose tolerance test |
| Ponderal index, g/cm <sup>3</sup> | 0.002 ± 0.002   | 0.022 ± 0.004   | n.s.    | BMI = body mass index   |
| BMI, kg/m <sup>2</sup>            | 18.5 ± 4.0      | 16.2 ± 3.9      | n.s.    | HOMA-β-cell = homeostasis model assessment β-cell function                              |
| BMI, z-score                      | 1.0 ± 2.6       | -0.29 ± 1.8     | n.s.    | HOMA-IR = HOMA for insulin resistance   |
| Height, z-score                   | -1.08 ± 1.29    | -1.23 ± 1.3     | n.s.    | IGF = insulin-like growth factor  |
| MPH, z-score                      | -1.4 ± 0.6      | -0.8 ± 0.9      | <0.05   | IGFBP = IGF binding protein   |
| Corrected stature, z-score        | 0.36 ± 1.1      | -0.36 ± 1.3     | n.s.    | IUGR = intrauterine growth retardation  |
| Fasting insulin, mU/l             | 12.4 ± 9.0      | 8.2 ± 3.3       | n.s.    | MPH = midparental height  |
| HOMA-IR                           | 3.5 ± 1.0       | 1.5 ± 0.8       | <0.0001 | n.s. = not significant.   |
| HOMA-β-CELL                       | 180 ± 139       | 43 ± 90         | <0.01   |   |
| AUC <sub>ins</sub> , mU/l         | 240 ± 113       | 164 ± 115       | n.s.    |   |
| Proinsulin, pM                    | 9.6 ± 11.2      | 5.0 ± 4.4       | n.s.    |   |
| IGFBP-1, µg/l                     | 83 ± 59         | 119 ± 50        | <0.05   |   |
| IGF-I, z-score                    | 0.41 ± 2.8      | 0.47 ± 3.0      | n.s.    |   |
| IGF-II, z-score                   | 0.56 ± 0.7      | 0.62 ± 0.9      | n.s.    |   |
| IGFBP-3, z-score                  | 0.35 ± 0.7      | 0.23 ± 1.3      | n.s.    |   |
| IGF-I/IGFBP3 ratio                | 61.2 ± 23       | 63.5 ± 35       | n.s.    |   |
| AST, U/l                          | 29.1 ± 8.0      | 27.4 ± 6.7      | n.s.    |   |
| ALT, U/l                          | 27.4 ± 17.1     | 16.3 ± 7.2      | n.s.    |   |
| γ-GT, U/l                         | 15.7 ± 6.6      | 11.7 ± 3.8      | n.s.    |   |

Adapted from Cianfarani S, et al. *Horm Res* 2001;55 (suppl 1):7-10.

## The Molecular Basis of X-Linked Spondyloepiphyseal Dysplasia Tarda

The gene for X-linked form of spondyloepiphyseal dysplasia tarda has been identified as SEDT, a protein that apparently plays a role in endoplasmic reticulum-to-Golgi transport and involves subcellular localization of normal sedlin constructs. The protein is relatively small with 140 amino acids. It is located in the non-X-inactivated part of Xp22. This suggests that female

carriers express sufficient normal gene to avoid the disease.

The present study looked at 36 unrelated cases and attempted to make phenotype/genotype correlations. Mutations could be found in 30 individuals. The 6 individuals in which mutations were not found either lacked a strong family history or convincing physical



features, and therefore, may represent other diseases. Twenty-one different gene mutations were observed among the 30 cases, and in those cases with several identical mutations, hupetype analysis suggests that they arose separately and, therefore, do not represent a founder effect.

Intrafamilial variation was certainly observed; however, mutations occurring toward the five' end of the SEDL gene (mutations in Exons 3 and 4) resulted in kyphosis and scoliosis with severe pain early in life and with more debilitating types of complications. This was observed while mutations in Exons 5 and 6 resulted in milder clinical features.

Mutations were spread throughout the gene, including point mutations, splice alterations, insertions, deletions, and complex rearrangements. The most common type of mutation was a deletion. There was a 10 fold greater occurrence of deletions than would be expected. This may represent slippage during homologous recombination between the Y and X chromosome.

The SEDL phenotype may be explained by reduction in endochondral bone formation in the epiphysis, particularly in the vertebral bodies. A timely switch to up regulate the endogenous expression of a pseudo gene on chromosome 19 might provide gene therapy. The authors are undertaking a study of SEDL mutations in premature osteoarthritis.

Gedeon, AK, et al. *Am J Hum Genet.* 2001;68:1386-1397.

**Editor's Comment:** *When genes are identified for the chondrodysplasias, the possibility of making phenotype/genotype correlations and understanding the basic molecular biology are very enticing. This paper is a lovely demonstration of how a great deal can be learned in rare disorders by large international collaborations. This work hopefully will lead both to a better understanding of disease and to potential therapies.*

Judith G. Hall, OC, MD

## Postnatal Malnutrition and Growth Retardation: An Inevitable Consequence of Current Recommendations in Preterm Infants?

Intake of adequate nutrients in preterm infants is difficult at best, and most often does not accomplish meeting the recommended dietary intakes (RDI). A nutrient deficit therefore accrues, leading to postnatal malnutrition and growth retardation. This study assesses the dietary intake in a prospective single observer design in 105 preterm infants with a body weight of < 1750 grams and a gestational age of < 34 weeks who were admitted to the Neonatal Intensive Care Unit over a 6 month period. Actual intake was subtracted from the recommended energy intake (120 kcal/kg/day) and protein (3 g/kg/day), and nutritional deficits were calculated. Infants were weighed on admission and throughout the hospital stay.

Nutrient intakes meeting current RDI's were rarely achieved during early life. By the end of the first week, cumulative energy and protein deficits were 406 +/- 92 and 335 +/- 86 kcal/kg and 14 +/- 3 and 12 +/- 4 g/kg in infants < 30 and those at > 31 weeks, respectively. By the end of the fifth week, cumulative energy and protein deficits were 813 +/- 542 and 382 +/- 263 kcal/kg and 23 +/- 12 and 13 +/- 15 g/kg. The z scores were -1.14 +/- .6 and -.82 +/- .5 for infants at < 30 and > 31 weeks. Stepwise regression analysis indicated that variation in dietary intake accounted for 45% of the variation in changes in z-score. The authors concluded that preterm infants inevitably accumulate a significant nutrient deficit in the first few weeks of life.

**Editor's Comments:** *This study clearly demonstrated that there is an accumulated nutrient deficit in preterm infants in an NICU setup. It also clearly suggests that the nutritional approach to the care of these infants needs to be re-thought, perhaps with a more aggressive approach, i.e. enteral or parenteral feedings. However, even early parenteral or enteral supplementation might be limited as these infants might not be able to tolerate it. A more aggressive enteral feeding is also hard to attain in the first few days of life, and it could lead to necrotizing enterocolitis or other adverse effects. The long-term consequences of this accumulated nutrient deficit may be important. It is generally assumed that poor growth in the preterm low birth weight infants primarily reflects inadequate nutrient intake, and in this study there was a 45% variation in growth related to such. Nonetheless, despite poor growth during the initial stages of life, most premature infants grow well thereafter and attain a normal height, unless there are other complications. Once the infant matures, the nutrient deficits are recouped and there is nutritional recovery with catch-up growth. However it should be kept in mind that nutrient deficits in early infancy might have other devastating consequences. The data from this study suggest that the clinician is in a quandary and that a more realistic picture regarding the quantity and quality of nutritional care in low birth weight infants needs to be re-thought.*

Embleton NE, et al. *Pediatrics* 107:270-272, 2001.

Fima Lifshitz, MD

## The Land Between Mendelian and Multifactorial Inheritance

Burghes et al discuss the concept that genetic disorders can often be thought of as attributable to Mendelian and/or multifactorial triads. However, we now must consider other possibilities in classifying certain genetic syndromes. One such category has been classified as *triallelic inheritance*. The Bardet-Biedl syndrome, as published by Katsanis et al, is tagged as such. This article prompts a perspective commentary on genetics by Burghes et al.<sup>1</sup>

Although there has been spectacular success in identifying genes responsible for Mendelian inherited disorders, finding *susceptibility* genes involved in multifactorial diseases has been a struggle. How multiple genes interact to give the final phenotype of a multifactorial disease and what we might expect, remains an enigma. The land between Mendelian and multifactorial inheritance is inhabited by genes such as *modifier* genes and *redundant* genes that have many effects on the developing phenotype. Understanding the mode of action of these will help in determining how *susceptibility* genes may interact to give rise to a multifactorial phenomena.

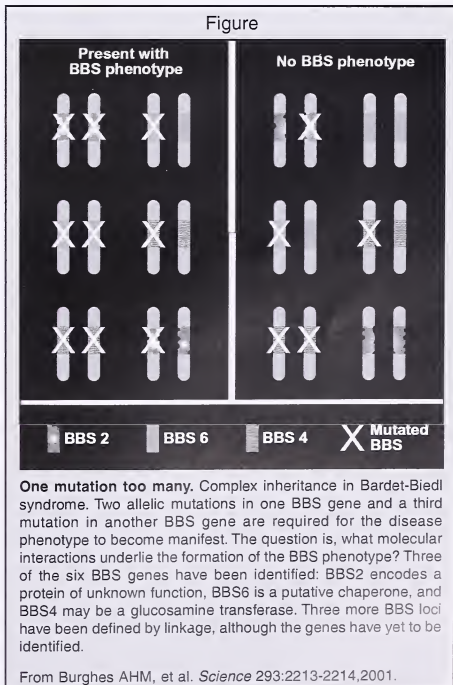
Katsanis et al<sup>2</sup> report that mutations in two genes, rather than one, cause Bardet-Biedl syndrome (BBS). Katsanis points out that six BBS loci exist in humans. Three of these have been identified (BBS2, 4, and 6); the other three have not, as yet. Mutated genes have been identified in BBS2, BBS4, and BBS6 genes. Katsanis et al describe 11 subjects, out of a group of 163, who were genetically characterized with heterozygous or compound heterozygous mutations in BBS2, and three families with normal individuals who had the same two mutated BBS2 alleles. In three pedigrees the affected BBS patient had mutations of both BBS2 alleles and a mutation in one BBS6 allele. In one family the affected BBS patient had a mutation of one BBS2 allele and mutations in two BBS6 alleles. Thus, in four families mutations in three BBS alleles were demonstrated and apparently necessary for expression of the disease phenotype. Katsanis proposed that BBS may not be a single gene recessive disease, but a complex trait requiring three mutant alleles to manifest the phenotype. The phenotype of BBS includes pigmentary retinopathy, polydactyly, obesity, developmental delay, and renal defects. The figure illustrates the complex inheritance in Bardet-Biedl syndrome.

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1. Burghes AHM, et al. *Science* 293:2213-2214, 2001.
2. Katsanis N, et al. Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 293:2256-2259, 2001.

**Editor's Comment:** The concept that mutations of genes on more than two alleles may be necessary for expression of a disorder is at odds with classical Mendelian transmission through dominant or recessive mechanisms, but is not incompatible with our understanding of diseases that appear to require multiple genetic and/or environmental factors for expression (e.g., diabetes mellitus, obesity, spinal muscular atrophy). Inasmuch as the majority of patients with BBS and mutations in BBS2 had normal BBS6, it is likely that these investigators will search for mutations in BBS4 (and BBS1 and 3 when they are identified) in this large group of BBS subjects. Since the phenotype of BBS is consistent despite the genotype, one suspects that the various BBS loci identified will be linked to one another in a metabolic process(es) that when interrupted leads to the disorder. Incidentally BBS6 is also mutated in patients with the McKusick-Kaufman syndrome of congenital heart disease, polydactyly, and transverse vaginal septum leading to hydrometrocolpos in females.

Allen Root, MD



**Volume 17, Number 1**

The Adult Consequences of Pediatric Endocrine Disease, II: Turner Syndrome  
Judith L. Ross, MD

**Abstracts**

Islet Transplantation in Seven Patients With Type I Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen

Hypoglycemia: A Complication of Diabetes Therapy in Children

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# GROWTH

## Genetics & Hormones

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## The Endocrine Function of Adipose Tissue

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### INTRODUCTION

The traditional view of the adipocyte as a passive receptacle for storage and combustion of triacylglycerol is undergoing rapid change. It is now recognized that a variety of adipocyte and adipose stromal cell derived proteins act both locally and distally through autocrine/paracrine and endocrine effects to regulate fat cell differentiation, and sense and adjust systemic energy balance.<sup>1</sup> These adipokines are molecules that were previously identified to be derived from immune cells, while others, cytokines produced by adipocytes, were known to be involved in hemostasis, inflammatory response, vasoregulation, and steroid metabolism (Figure 1). Many of these proteins increase as fat mass accumulates and, thus contribute to the multiple morbidities of obesity. Increased activity of three of these, tumor necrosis factor, interleukin 6, and resistin, play a role in the development of the insulin resistance present in obesity. In contrast, other adipokines, like adiponectin and leptin, are insulin sparing through stimulatory effects on the beta oxidation of fatty acids in skeletal muscle.

The concept of "lipotoxicity" postulates that the accumulation of excess lipids in hepatocytes and skeletal muscle cells interferes with insulin signaling,<sup>2</sup> and the increased lipolytic activity of visceral fat contributes to this process by shunting fatty acids through the portal vein to the liver. Local overproduction of glucocorticoids in visceral fat ("Cushing's disease of the omentum") is also pathogenic. Increased activity of 11 hydroxysteroid dehydrogenase (11 HSD-1) raises adipose tissue cortisol levels, adversely partitioning fat into visceral sites and stimulating release of metabolically harmful adipokines.<sup>2</sup> Many of these adipokines also act centrally. Leptin, tumor necrosis factor (TNF) and interleukin (IL-6) enter the hypothalamus where they affect sympathetic tone, feeding behavior, thermogenesis, reproduction, and the activity of various hypothalamic-pituitary axes. Adipocyte

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### Letter from the Editor:

The lead article in this issue covers a very current topic, one which pediatric endocrinologists may not be thoroughly familiar or are just beginning to incorporate into their sphere of interest (outline of article at [www.gghjournal.com](http://www.gghjournal.com)). However, it is a subject about which we all will be hearing a great deal more in the near future as pediatric endocrinologists become more involved in the care of obese patients. The epidemic of obesity is confronting our profession more than ever. Consequently, most readers of Growth Genetics and Hormones will benefit from having this article as a source for reference to broaden their knowledge about The Endocrine Function of Adipose Tissue. To serve this purpose the presentation of this article by necessity was very inclusive and written as an introduction to, and compilation about, the existence and known function of the many hormones outlined in the text. Dr. Diamond is to be commended for undertaking a difficult task and achieving the intended goal.

For the Editorial Board  
Robert M. Blizzard, MD  
Editor-in-Chief

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differentiation is controlled by the nuclear transcription factor, peroxisome proliferator activated receptor (PPAR)(Figure 2).<sup>3</sup> As energy surplus develops, adipocyte differentiation and lipid accumulation are inhibited through feedback loops of adipocyte-derived factors such as TNF, angiotensinogen (AGT), and resistin (for resistance to insulin). When energy deficit occurs, there is a decline in other adipocyte secreted proteins, such as adiponectin and leptin, and there is activation of trophic proteins such as acylation stimulating protein (ASP) and angiotensin II (AngII). These signal a drive to adipocyte formation and renewed triglyceride accumulation. Insulin is central to this process, promoting lipogenesis and energy storage. The development of insulin resistance which is concomitant with excessive accumulation of body fat may signify a physiologic counter regulation activated to maintain energy homeostasis of the adipocyte. As body fat accumulates beyond that needed for energy balance, and as adipose tissue is chronically exposed to excess dietary fatty acids and glucose, there are further maladaptive responses of adipokines, which result in insulin resistance, inflammation, hypertension, and endothelial disease.

A review of the function and regulation of adipokines is made in this paper to facilitate the understanding by which obesity may contribute to the pathogenesis of the complications of this disease and of the alterations associated with this condition.

## ADIPOKINES ASSOCIATED WITH INSULIN SENSITIVITY

### Adiponectin

Adiponectin [Adipocyte complement-related protein (ACRP)], a soluble defense collagen, which is a circulating matrix-like protein, is expressed abundantly and exclusively in white adipose tissue.<sup>4</sup> Adiponectin appears to be an endogenous anti-inflammatory and anti-atherogenic factor that is protective against insulin resistance and macroangiopathy.<sup>5</sup> Its serum concentrations are reduced in obese mice and humans and rise following weight loss. This suggests that adiponectin plays a negative feedback role in fat storage.<sup>6</sup> Levels are lower in men compared to women and in individuals with obesity, type II diabetes, and coronary artery disease as compared to healthy subjects.<sup>7</sup> Its concentrations correlate with the insulin sensitivity state and with steady state plasma glucose, and rise in response to insulin. The protein is not an insulin sensitizer, however, but protects insulin action by accelerating beta oxidation of free fatty acids in skeletal muscle.<sup>8</sup> Intravenous administration of the "fat burning" c-terminal globular region of AdipoQ, the mouse homologue of adiponectin, reduces circulating free fatty acids and diet induced weight gain and corrects both hyperglycemia and hyperinsulinemia in genetically obese animals.<sup>9</sup> Hypoadiponectinemia may also

Figure 1

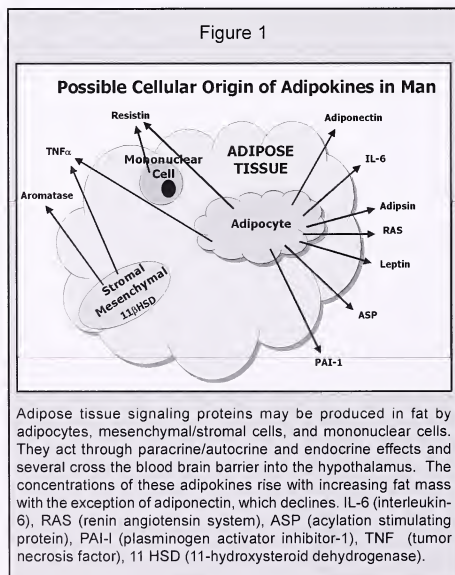
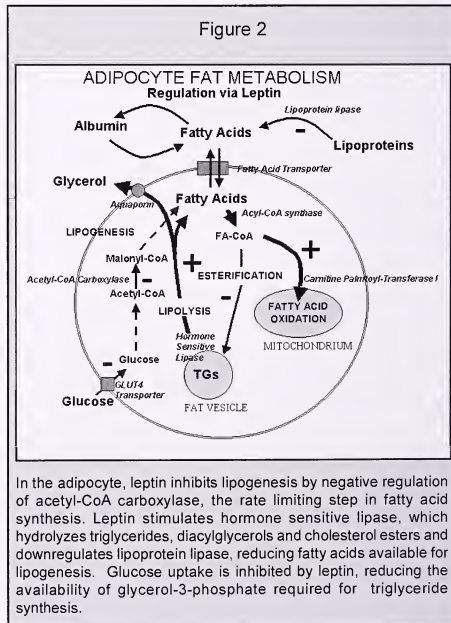


Figure 2



contribute to the insulin resistance of lipotrophic animals, explaining the apparent paradox of glucose intolerance in both obese and fat depleted models. Adiponectin is highly regulated during adipocyte differentiation and may mediate some of the insulin-sensitizing effects of thiazolidinedione (TZD) binding to PPAR. Clinically, treatment of insulin resistant human subjects with TZDs significantly increases plasma adiponectin concentrations without affecting body weight. Additionally, adiponectin suppresses phagocytic activity, macrophage release of  $\text{TNF}\alpha$ , and transformation of macrophages to foam cells in vitro. It also is deposited in vascular smooth muscle to protect vessel walls and thereby modulates the disease risks of coronary artery disease.<sup>10</sup>

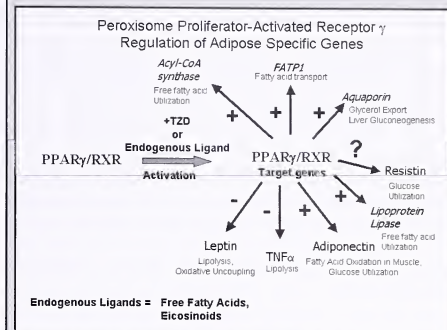
### Leptin

Leptin is a 16 kDa adipocyte-derived cytokine synthesized and released from fat cells in response to changes in energy stores and in systemic energy balance. Leptin's primary physiologic function is the defense of body fat. Declining levels in adipose tissue and serum signal the presence of energy deficit to the brain. Leptin circulates partially bound to plasma proteins and enters the CNS by diffusion through capillary junctures in the median eminence and by saturable receptor transport in the choroid plexus. In the hypothalamus leptin binds to long receptor isoforms which stimulate anorexigenic and inhibit orexigenic peptides.<sup>11,12</sup> Leptin also increases sympathetic nervous system activity and energy expenditure.<sup>13</sup> Adipocyte levels of leptin mRNA and protein correlate closely with both circulating leptin values and total body fat.

Leptin's lipolytic role in adipocyte metabolism is shown in Figure 3. Leptin reduces the levels of intracellular lipid in skeletal muscle, liver and pancreatic beta cells, thereby improving insulin sensitivity. In muscle this insulin sensitizing effect is achieved through inhibition of malonyl CoA, permitting increased transport of fatty acids into mitochondria for beta oxidation. These changes are partially mediated by central sympathetic activation of adrenergic receptors.<sup>2</sup>

Leptin synthesis is both constitutive and hormonally controlled. It is influenced by the state of energy reserve, and it is modulated by the sympathetic nervous system through an inhibitory feedback loop. Both adipocyte size and location dictate leptin production, although the mechanism(s) of these paracrine/autocrine modulated effects remain largely undefined. Larger fat cells contain more leptin than smaller ones and subcutaneous fat releases more leptin than visceral fat.<sup>14,15</sup> Several experimental findings suggest that glucose is an important regulator of adipocyte leptin release.<sup>16</sup> In cultured rat adipocytes, glucose inhibitors block leptin synthesis. In man, glucose infusion attenuates the rapid

Figure 3



Peroxisome proliferator-activated receptor (PPAR), a member of the nuclear hormone receptor superfamily, is a ligand-activated transcription factor. PPAR is central to adipocyte function, promoting differentiation of preadipocytes to mature fat cells. PPAR knockout (-/-) mice have no detectable adipose tissue. PPAR is endogenously regulated by heat shock proteins, fatty acids and prostaglandin J derivatives. Its DNA binding requires heterodimerization with 9 cis-retinoic acid receptor (RXR) followed by interaction with peroxisome proliferator response elements (PPRE) on adipocyte target genes. The insulin sensitizing thiazolidinedione (TZD) drugs are PPAR ligands whose effects on insulin signaling are mediated in part through PPAR stimulation or inhibition of intracellular adipokines.

fasting decline of leptin. The hexosamine biosynthetic pathway into which 2-3% of cellular glucose uptake enters may mediate this link. Exposure of isolated subcutaneous adipocytes to UDP-N-acetylglucosamine (an end product of hexosamine biosynthesis) increases leptin release. Its inhibition reduces glucose-stimulated leptin release and ob gene expression. UDP-N-acetylglucosamine levels in human subcutaneous adipose tissue correlate significantly with both body mass index (BMI) and serum leptin levels.<sup>17</sup>

Insulin stimulates the secretion of leptin when administered to human subjects for several days. In adipocytes from rat white adipose tissue, leptin is present in the endoplasmic reticulum in the absence of insulin, whereas it localizes into the plasma membrane following insulin treatment.<sup>18</sup> Glucocorticoids, whose effects may be primarily permissive, induce leptin synthesis in vitro and in vivo, with greater responsiveness in obese as compared to lean individuals.<sup>19,20</sup> Females produce more leptin than males when matched for age, weight and body fat. This is probably related to gender differences in fat depots and to the leptin-suppressive effects of testosterone. At birth, the leptin concentrations in umbilical cord blood from girls are double those present in boys.<sup>21</sup> Pulsatile

leptin secretion correlates with female sex hormones. However, there are conflicting data regarding the influence of ovarian sex steroids on leptin release.<sup>22,23</sup> Other controlling factors are listed in the addendum.<sup>24-26</sup>

The prevailing evidence of the physiologic role of leptin suggests that it is an anti-obesity hormone, but this concept must be reconciled with the inability of high endogenous leptin levels to prevent most obesity. It appears that in the majority of cases there may be leptin resistance mediated by inhibition of leptin signaling, thereby altering the dominant role of this hormone as a signal to switch between fed and fasted states.

## **ADIPOKINES ASSOCIATED WITH INSULIN RESISTANCE**

### **Resistin**

Resistin is a 12.5 kDa cysteine-rich adipocyte secreted protein which was identified during the screening for genes induced during adipocyte differentiation. This adipokine is down regulated by TZDs. It also is known as Fizz3 (for found in inflammatory zones). Worthy to note is that resistin is one of a family of similar molecules present in fat. Resistin administered to wild type animals induces insulin resistance, but in the obese-insulin resistant mouse it restores normal insulin sensitivity.<sup>27</sup> In morbidly obese humans, resistin mRNA from adipose tissue samples is increased as compared to that in lean controls.<sup>28</sup> However, a number of clinical and experimental observations suggest that resistin may not be the long sought major link between human obesity and insulin resistance.<sup>29</sup>

### **Tumor Necrosis Factor**

TNF $\alpha$  is a multi-potential cytokine with diverse immunologic functions. Initially it was described as a cause of tumor necrosis in septic animals and was associated with cachexia-inducing states, such as cancer and infection.<sup>30</sup> In obese humans TNF $\alpha$  and its receptors (TNFR1 and TNFR2) are synthesized and secreted in increased amounts by adipocytes and stromovascular cells. Their autocrine effects contribute to the insulin resistance of obesity and diabetes.<sup>31</sup> TNF $\alpha$  inhibits insulin action by down regulating GLUT4 mRNA in fat and muscle. It also reduces insulin receptor autophosphorylation and phosphorylation by decreasing insulin receptor substrate-1. Circulating free fatty acids (FFA) increase from the lipolytic effects of TNFR1.<sup>32</sup> TNF $\alpha$  induces lipolysis which is blocked by PPAR ligands in insulin resistant animals.<sup>33</sup> In man, TNF $\alpha$  concentrations decline with weight loss and treatment with TZDs. The administration of TNF $\alpha$  causes hyperinsulinemia without hypoglycemia.<sup>34</sup>

TNF $\alpha$  also has important effects on the hypothalamus. In rats, intravenous or intracerebroventricular injection of

TNF $\alpha$  stimulates ACTH secretion through eicosanoid cyclooxygenase mediated release of CRH and inhibits secretion of TSH.<sup>35</sup> Thus, TNF appears to have a net effect in prevention of obesity through the inhibition of lipogenesis and increased lipolysis with facilitation of adipocyte death via apoptosis.

### **Interleukin-6**

In man, ~30% of circulating IL-6 originates from adipose tissue.<sup>36</sup> Concentrations are higher in visceral fat as compared to subcutaneous fat. They increase with obesity and are stimulated by TNF and IL-1.<sup>37</sup> Elevated levels are associated with increased risk of coronary artery disease, athero-sclerosis, and unstable angina.<sup>38</sup> Acting on the liver, IL-6 is a primary stimulant of acute phase reactants, such as C-reactive protein, fibrinogen and haptoglobin, thus contributing to a hypercoagulable state. Importantly, IL-6 also promotes the release of endothelial adhesion molecules<sup>39</sup> and adversely affects insulin sensitivity by inhibiting GLUT-4, hepatic glycogenesis, and lipoprotein lipase. The resultant lipolysis increases non-esterified free fatty acids (NEFA) which impedes nitric oxide mediated endothelial vasodilation.<sup>40</sup>

IL-6 receptors are present in the hypothalamus where IL-6 stimulates thermogenesis and satiety by increasing prostaglandin synthesis and release of corticotrophin releasing hormone (CRH).<sup>41</sup> It remains to be determined whether IL-6 is a link between obesity and thromboembolic complications.

## **ADIPOCYTE PROTEINS AND LIPID METABOLISM**

### **Adipsin**

Adipsin (ADIPocyte-trypSIN) is a 24-kDa adipocyte secreted protease with close homology to human complement D. This protease is required for the synthesis of acylation stimulating protein (ASP) (vide infra), which is described below and which is an important mediator of lipogenesis. Although adipsin concentrations are reduced in rodent models of obesity, paradoxically they are increased in humans with excess adiposity;<sup>42</sup> for example in obese Pima Indians serum adipsin levels are 45% higher than in non-obese Pimas or other controls. In subjects with anorexia nervosa the adipsin levels are low and rise during refeeding. Insulin stimulated adipsin release is mediated by ADP-ribosylation factor 6 (ARF6) which acts on endocytotic and recycling pathways in the adipocyte; therefore being an important protein in fat metabolism.<sup>43</sup> Adrenalectomy of ob/ob mice raises circulating adipsin levels; and corticosterone replacement reverses these changes. Adipsin secretion also is stimulated in animals by sympathomimetic agents, but not by cold stress.<sup>44</sup>



### **Acylation Stimulating Protein (ASP)**

ASP is a 76-amino acid protein that stimulates fatty acid uptake and esterification into triglycerides. Retinoic acid (transported as retinyl ester by transthyretin and chylomicrons) stimulates the C3 gene leading to increased postprandial production of ASP.<sup>45</sup> Up to a quarter of patients with coronary artery disease have elevated concentrations of ASP. Hyperapobeta-lipoproteinemia, a familial dyslipidemia characterized by increased hepatic release of LDL and VLDL, may result from impaired adipose tissue actions of ASP.<sup>46</sup> In the ASP-knockout mouse, postprandial triglyceride clearance is delayed and weight gain decreased. Like insulin and additive to it, ASP promotes movement of glucose transporter vesicles in cell membranes in adipose tissue and muscle by activation of the diacylglycerol/protein kinase C pathway.<sup>47</sup> This provides glucose substrate for glycerol-3-phosphate synthesis of fatty acids and triglycerides. Thus a deficit of ASP results in increased post prandial fatty acids and decreased weight gain and triglyceride synthesis.

### **Aquaporin Adipose (AQPap)**

AQPap is an adipose specific glycerol channel gene abundantly and exclusively expressed in white adipose tissue. AQPap regulates glucose homeostasis by controlling the flux of glycerol into hepatic gluconeogenesis. In wild-type mice, AQPap expression increases during fasting, and declines with refeeding. This takes place through insulin action at the AQPap promoter's negative insulin response element (IRE).<sup>48</sup> AQPap is increased in adipose tissue from TZD treated mice and reduced in PPAR +/- heterozygous knock-out rodents.

### **ADIPOKINES & HEMOSTASIS**

#### **Plasminogen Activator Inhibitor-1 (PAI-1)**

PAI-I, which is synthesized in the liver and in adipose tissue regulates thrombus formation by inhibiting the activity of tissue-type plasminogen activator, an anti-clotting factor. PAI-I concentrations in serum increase in proportion to visceral adiposity and are entrained by adipocyte size and lipid content.<sup>49</sup> Omental tissue explants secrete significantly more PAI-I than subcutaneous tissue from the same subject.<sup>50</sup> Increased PAI-I levels are found in patients with coronary artery disease and following myocardial infarction, while levels decline with caloric restriction, exercise, weight loss, and treatment with metformin.<sup>51</sup>

### **THE ADIPOCYTE RENIN-ANGIOTENSIN SYSTEM (RAS)**

A renin-angiotensin system (RAS) located in the intra adipose tissue regulates fat cell mass and energy stores through paracrine/autocrine effects on adipocyte

differentiation and lipid storage. Angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE), angiotensin II (AngII) and its receptors (AT1, AT2), and the non-renin-angiotensin enzymes chymase, cathepsins D and G, and tonin, are all expressed by adipose tissue.<sup>52</sup> Plasma AGT, renin activity and ACE correlate positively with body mass index while adipose tissue AGT expression correlates significantly with waist-to-hip ratio in man.<sup>53</sup> Adipose tissue AngII controls terminal differentiation of preadipocytes to adipocytes through the action of prostacyclin (PGI<sub>2</sub>) and regulates adipose tissue blood supply. Adipose tissue AGT also influences adipocyte vascular resistance, but negatively regulates fat mass by decreasing lipogenesis. Ang II and AGT receptors are found in higher concentrations in visceral fat as compared to subcutaneous adipose tissue in both lean and obese individuals.<sup>54</sup> Glucocorticoids in the presence of insulin, and beta-adrenergic stimulation, and nutritional changes modulate adipocyte AGT gene expression.<sup>55</sup> In man, the role of the adipocyte RAS in the relationship between obesity and hypertension remains to be further defined.<sup>56</sup>

### **ADIPOSE AROMATASE AND INTRAADIPOSE GLUCOCORTICOIDS**

#### **Aromatase**

Sex steroids are not synthesized *de novo* in fat, but are formed by the action of stromal enzymes on adrenally derived precursors. In human adipose tissue aromatase activity is principally expressed in mesenchymal cells of undifferentiated preadipocyte phenotype.<sup>57</sup> P450arom, a heme protein product of the CYP 19 gene, converts androstenedione to estrone. Estrogen production in fat rises as body weight increases and as subjects age.<sup>58</sup> Importantly, adipose tissue-derived estrogens partition fat to subcutaneous and breast tissues, while androgens promote central or visceral fat accumulation.<sup>59</sup> Aromatase activity varies significantly by region, with greater expression in adipose tissue from buttocks and thighs compared to that from abdomen and breasts.<sup>60</sup> *In vitro*, aromatase expression is stimulated by glucocorticoids in the presence of serum, and by class I cytokines. TNF increases aromatase expression in adipose stromal cells exposed to dexamethasone; leptin has little effect.<sup>61</sup> In the aromatase deficient ArKO mouse which lacks a functional Cyp 19 gene, there is a progressive accumulation of intra-abdominal fat and reduced lean body mass.<sup>62</sup>

### **11- HYDROXYSTEROID DEHYDROGENASE**

11-hydroxysteroid dehydrogenase (11 HSD-1), which regenerates metabolically active cortisol from cortisone in man and corticosterone from 11 dehydrocorticosterone in mice, is increased in adipose tissue from obese



subjects. Adipose tissue corticosterone was overproduced by 30% in a transgenic (Tg) mouse that modestly over expresses 11 HSD in all its adipose tissues. The Tg male animals disproportionately accumulated visceral fat in adipocytes which were three times the size of those of control animals. The mice became hyperphagic, hyperglycemic, and hyperinsulinemic, had reduced levels of adiponectin and uncoupling protein-I, and had increased concentrations of leptin, TNF, angiotensinogen, lipoprotein lipase, and portal free fatty acids. This clinical and biochemical pattern mimics the human "metabolic syndrome".<sup>63</sup> In humans thiazolidinediones significantly reduce 11 HSD-I mRNA in vitro and in vivo, and preferentially reduce visceral fat.<sup>64</sup>

## OTHER ADIPOCYTE PROTEINS

Metallothionein is an adipocyte secreted low molecular weight metal binding and stress response protein which may function to protect fatty acids from oxidative damage.<sup>65</sup> The metallothionein genes (MT-I, MT-2) are expressed in adipocytes early in their differentiation process. In vitro, MT-I transcription is stimulated by dexamethasone, forskolin and bromo-cAMP, and to lesser extent by insulin and leptin. Fasting-induced adipose factor (FIAF), a circulating fibrinogen-angiopoietin-related protein, is an adipocyte derived protein which increases during caloric deprivation and interacts with PPAR.<sup>66</sup> Lipoprotein lipase, cholesteryl ester transferase, apolipoprotein E, and retinol binding protein are other adipocyte proteins important for lipid metabolism which are under study.

## CONCLUSION

The mechanisms by which obesity contributes to insulin resistance, hypertension, and endothelial disease are among the most important scientific questions facing medical investigators today. Research into the function and regulation of adipocyte signaling proteins, adipocyte differentiation, and the control of fat partitioning will likely result in further insight into these mechanisms and the discovery of targeted therapies for treatment of obesity and obesity related diseases.

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## Addendum (re Leptin)

Many regulatory sites for leptin are found within the ob gene promoter, including cyclic AMP and glucocorticoid response elements, as well as loci for CCATT/enhancer and SP-1 binding.<sup>24,25</sup> Thiazolidenediones reduce leptin mRNA in adipocyte 3T3-L1 cells through negative PPAR effect at the leptin promoter.<sup>26</sup> Peripheral leptin administration activates suppression of cytokine signaling-3 (SOCS-3) which is co-expressed in hypothalamic nuclei with long-form leptin receptors. Increased SOCS-3 expression in vitro has been shown to blunt leptin receptor signal transduction by inhibiting JAK activity. SH2-containing phosphatase 2 (SHP-2) also blocks STAT-3 mediated leptin transcription. Moreover leptin is negatively regulated by the sympathetic nervous system via beta-2 and beta-3 catecholaminergic input at the adipocyte. The increased sympathetic innervation in visceral fat may thus partly explain its reduced leptin content compared to subcutaneous fat tissue. Infusion of isoprenaline or epinephrine in man acutely suppresses leptin release, as does cold exposure. Growth hormone, thyroid hormone, and melatonin have also been shown to decrease leptin secretion.

## Abstracts from the Literature

### Celiac Disease in Children with Autoimmune Thyroid Disease

This study was designed to test for the presence of celiac disease among children with autoimmune thyroid disease (ATD). Ninety patients (78 females) ages 1.8 to 17.3 years with ATD were studied; 20 of them had Graves' disease, and 16 had other associated conditions i.e. alopecia (4), vitiligo (2), juvenile rheumatoid arthritis (2), autoimmune hepatitis (2), Down's syndrome (1) and other miscellaneous autoimmune alterations (5). Screening for IgA antiendomysium antibodies (EMA) and HLA typing for Class I and II DQA1 and DQA2 heterodimers were done. There were 7 patients with positive EMA; an intestinal biopsy in these patients revealed intestinal villi alterations, with partial or total atrophy, crypt hyperplasia and intraepithelial lymphocytes. Clinically, one of the celiac disease patients had iron deficiency, one had diarrhea, and one had short stature, while the others were asymptomatic. A significant positive correlation was present for celiac-susceptible heterodimers in the patients with celiac disease. The authors concluded that screening for celiac disease should be done on all patients with ATD.

*Nonetheless, it has been suggested that the presence of unidentified celiac disease could play a role in the development of autoimmune disorders, and the prompt diagnosis and treatment of this disease could prevent the onset of other alterations.<sup>5</sup> The availability of an accurate, sensitive and specific test (IgA antiendomysium antibodies) to screen for celiac disease should not be overlooked by Pediatric Endocrinologists who in my opinion should test all patients with autoimmune endocrine disorders regularly for antibodies reflecting the presence of celiac disease.*

Fima Lifshitz, MD

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Larizza D, et al. *J Pediatr* 2001;139:738-740.

**Editor's Comments:** *This report is one more in the recent literature documenting the presence of celiac disease among patients with endocrinopathies. The prevalence of celiac disease in patients with ATD was 7.7% which is higher than that observed in other studies of adults with ATD, and of course much higher than the 1% reported in normal populations.<sup>1-3</sup> In Vol 17 No 2 of Growth Genetics & Hormones, I abstracted and commented upon the article describing the presence of celiac disease in 4.6% of children with type I diabetes.<sup>4</sup> Celiac disease was a significant factor in the development of hypoglycemia complicating the course of the diabetic illness. The presence of celiac disease in the patients in this study, as well as those in other reports, was without clinical evidence of malabsorption and the patients were largely asymptomatic.*

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## The Role of Fetal-Maternal Microchimerism in Autoimmune Disease

Over the last 4 or 5 years, more and more diseases are described in which fetal cells are found at the site of autoimmune maternal disease and more recently maternal cells are being found at the site of newborn destructive ("graft-versus-host") diseases. Many diseases including systemic sclerosis and fetal dermatomyositis have now been attributed to fetal-maternal microchimerism. The report by Klinschar et al adds to the evidence that Hashimoto's thyroiditis includes fetal microchimerism in the fetal thyroid gland. These authors took thyroid gland specimens, extracted DNA, and then used Y probes to look for evidence of male cells in the maternal thyroids. They specifically used thyroid glands from women who had male children, and found evidence of male microchimerism in half the specimens. Among the controls (nodular goiter), only 1/25 specimens had evidence of Y chromosome microchimerism.

The importance of this observation is related to the question of whether the fetal cells can be a cause of autoimmune diseases since there is an excess of thyroid autoimmune disorders in females. The molecular

techniques presently used look for Y DNA probes in females and female cells in males. The new molecular techniques allow this sort of recognition. It seems likely that all of us carry some maternal stem cells and that women who have been pregnant carry fetal cells, which can respond to damage and stress. What is not clear is whether the fetal cells are the cause of auto immunity or simply represent a stem cell response to injury.

Klinschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.

**Editor's Comment:** *It will be important to look at multiple tissues for fetal cells. It appears that pregnancies which have been complicated are more likely to have fetal cells in circulation. Thus, pre-eclampsia and aneuploidy are known to have increased trafficking between mother and fetus. In addition, loss of co-twins can predispose to microchimerism. Keep your eyes open for more work in this area since it is highly likely that additional papers will try to discriminate the source of the cells, and determine the time at which they would have migrated to specific tissues.*

Judith G. Hall, OC, MD

Table  
Number of children, sons, and daughters in  
Hashimoto patients with and without detectable microchimerism

| Patient no. | No. of children | No. of daughters | No. of sons | Microchimerism |
|-------------|-----------------|------------------|-------------|----------------|
| 1           | 4               | 2                | 2           | Yes            |
| 2           | 1               | 0                | 1           | Yes            |
| 3           | 3               | 1                | 2           | Yes            |
| 4           | 2               | 1                | 1           | Yes            |
| 5           | 2               | 1                | 1           | Yes            |
| 6           | 2               | 1                | 1           | Yes            |
| 7           | 4               | 1                | 3           | Yes            |
| Mean        | 2.57            | 1                | 1.57        |                |
| 9           | 1               | 0                | 1           | No             |
| 10          | 2               | 1                | 1           | No             |
| 11          | 1               | 0                | 1           | No             |
| 12          | 1               | 0                | 1           | No             |
| 13          | 0               | 0                | 0           | No             |
| 14          | 0               | 0                | 0           | No             |
| Mean        | 0.83            | 0.17             | 0.67        |                |
| P value     | 0.009           | 0.013            | 0.035       |                |

Patients with microchimerism have significantly more children (sons and daughters) than patients without microchimerism, whereas no differences were found between the latter patients and controls.

Adapted from Klinschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.



## Mutations in *PTPN11*, Encoding the Protein Tyrosine Phosphatase SHP-2 Cause Noonan Syndrome

In approximately 50% of subjects with Noonan syndrome (NS is mapped to chromosome 12q24.1) the investigators identified mutations in the 15 exon gene (*PTPN11*) encoding the non-receptor protein [tyrosine phosphatase (PTP) - SHP-2]. This protein has two SH2 (Src homology docking) domains and a long enzymatic domain with the sites interacting to achieve an active or inactive state of function. Diverse missense mutations were found in the third exon encoding the amino-terminal SH2 (Src homology) domain and in three exons (7, 8, 13) encoding the PTP domain that apparently rendered the protein constitutively active. SHP-2 is a component of several intracellular signal transduction systems involved in embryonic development that modulate cell division, differentiation, and migration, including that mediated by the epidermal growth factor receptor. The latter pathway is important in the formation of the cardiac semilunar valves. The mutations associated with NS are in conserved amino acid sites in which the alteration leads to conformational changes that "lock" the protein in its enzymatically active state. The down-stream pathways that are affected by this "positive" change in enzyme activity have yet to be identified.

Tartaglia M, et al. *Nat Genet* 2001;29:465-468.

**Editor's Comment:** Noonan syndrome (OMIM 163950) is characterized by "Turner-like" facial features, short stature, webbed neck, cubitus valgus, pulmonic stenosis (rather than coarctation of the aorta which is frequent in Turner syndrome), developmental delay, and bleeding diathesis. Since the Noonan phenotype is genetically heterogeneous, other genetic errors may exist, including mutations in the non-coding regions of *PTPN11* that were not determined in the present report. The short stature and many of the skeletal abnormalities found in patients with Leri-Weill dyschondrosteosis and Turner

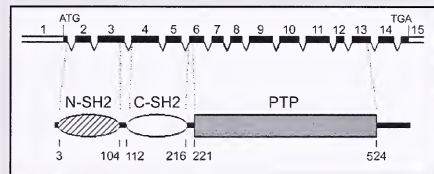
syndrome (TS) have been attributed to haploinsufficiency of *SHOX* (chromosome Xpter-p22.32) either due to its deletion or to loss-of-function missense or nonsense mutations.<sup>1,2</sup> Given the visual similarity of the NS and TS phenotype, it will be of interest to determine if the proteins regulated by *PTPN11* and *SHOX* interact. Might the product of *SHOX* be an inhibitor of SHP-2 generation or activity?

Allen W. Root, MD

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Figure



*PTPN11* organization and SHP-2 domain structure. The coding exons are shown at the top as numbered filled boxes, and the positions of the ATG and TGA codons are indicated. The functional domains of the SHP-2 protein, comprising two tandemly arranged SH2 domains at the N terminus (N-SH2 and C-SH2) followed by a protein tyrosine phosphatase (PTP) domain, are shown below. Numbers below the domain structure indicate the amino-acid boundaries of those domains.

Reprinted with permission from Tartaglia M, et al. *Nat Genet* 2001;29:465-468.

## Mothers with Congenital Adrenal Hyperplasia (CAH) and their Children: Outcome of Pregnancy, Birth and Childhood

The authors examined the gestational history of 122 women with 21-hydroxylase deficient CAH which was confirmed by genotyping in the majority. These women were born after 1948, followed in the investigators' clinic (University Children's Hospital, Munich) and were over 20 years of age at the time of study. Eighteen of the 122 women (15%) had delivered 31 children. The diagnosis of the 18 mothers was as follows: salt-losing, 1 of 48 total (2%); simple virilizing, 12 of 64 total (19%); and non-classical, 5 of 10 total (50%). The woman with

salt-losing CAH had two miscarriages before delivering her child. One woman with non-classical CAH had two tubal pregnancies.

Conception occurred between 18-36 years (mean 28 years). The pregnancies were uneventful with the women receiving hydrocortisone, prednisone, prednisolone, or dexamethasone during gestation. Sixteen pregnancies required cesarean sections, primarily in women not having nonclassical CAH. Five of the 31 offspring were <10th percentile for gestational



age. One developed an intracerebral hemorrhage. An additional patient was microcephalic at birth. None of the 18 female offspring had malformation of the external genitalia. Follow-up of the 31 offspring, 6 of whom were less than 5 years of age, 7 of whom were between 5-10 years, and 18 who were older than 10 years of age at the time of evaluation, revealed that all were growing, maturing, and developing normally.

Krone N, et al. *Clin Endocrinol* 2001;55:523-529.

**First Editor's Comment:** These data are encouraging in that women with simple virilizing and non-classical CAH are often able to conceive and deliver healthy children, thus confirming previous reports. More data on the degree of adrenal suppression during pregnancy, and knowing post-natal neonatal adrenal function would have been of interest.

That only one of 48 women with salt-losing CAH had an infant illustrates the difficulties still encountered in the management of many of these patients. As Krone et al discuss, the relative infertility of women with CAH may be due to hormonal (hyperandrogenism), anatomic (inadequate reconstruction of the vagina), or psychosocial factors (behavioral masculinization, low marriage rate, and/or sexual preference). It is anticipated that prenatal detection and treatment of females with CAH and establishing neonatal screening programs for this disorder will change substantially the "natural history" of pregnancy in females with CAH.

Regarding surgical reconstruction of the external genitalia in the virilized female, while clitoroplasty may be appropriate in the neonatal period, vaginoplasty

should be deferred until the peri menarchal period, as earlier reconstructive surgery is usually inadequate.<sup>1</sup> In 39 adolescent phenotypic females (20 with CAH) (mean age at examination 15 years) who underwent vaginal surgery in infancy at a median age of 10 months, Creighton et al found that approximately 60% had a good or satisfactory cosmetic appearance of the external genitalia, but almost all required further surgery to permit use of tampons during menses and, presumably, sexual relations in adulthood.

Allen W. Root, MD

**Second Editor's Comment:** Much is being discussed and written in 2002 regarding surgery on the genitalia of patients with enlarged clitorises. The current recommendation of many surgeons and pediatric endocrinologists is that surgery on the clitoris be delayed in most cases in the newborn period. For more details the reader is referred to references 1, 2, and 3 below. A lead article concerning the dilemmas of gender assignment and surgery will be published soon in GGH to provide up-to-date considerations for you our reader.

Robert M. Blizzard, MD

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## Growth Hormone Improves Clinical Status in Prepubertal Children with Cystic Fibrosis: Results of a Randomized Controlled Trial

Hardin and colleagues studied the effects of recombinant GH (0.3 mg/kg/wk) in 10 children with cystic fibrosis (CF) (ages 7-12, Tanner stage I) as compared to a control group of 9 similar children. All children recruited for the study were  $\leq 10^{\text{th}}$  percentile for both height and weight and had adequate caloric intake as determined on 2 evaluations. Only one had an abnormal growth hormone stimulation test. Children were excluded from the study if they had been hospitalized within 6 weeks or had been treated with systemic or oral steroids within 6 weeks. Evaluations were made of pulmonary functions including forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV<sub>1</sub>). In addition, peak expiratory pressure (PEP) and peak inspiratory pressure (PIP) were measured. Resting energy expenditure, was determined using indirect calorimetry, and lean body mass was determined by

whole body dual energy x-ray absorptiometry. Studies were made at baseline and every 3 months. Data were collected with regard to the number of hospitalizations and antibiotic therapy. All data for both the treatment group and the control group were similar at baseline.

The height and weight Z scores were significantly greater in the treatment group after one year than in the control group; furthermore the treatment group had a significant increase in lean body mass. Additionally, at 12 months the treatment group had a significant improvement in percent FVC, PIP, and PEP. There was no significant change in percent FEV<sub>1</sub>. The GH treated group had a significant decrease in the number of hospitalizations, although outpatient antibiotic therapy was not different between the two groups. There was no significant change in resting energy expenditure or nutritional intake during the study and carbohydrate

intolerance did not develop in either group. The advancement in bone age over the 12 months was not different between the two groups.

The authors conclude that growth hormone therapy is of significant benefit to pre-pubertal children with CF in terms of their height, weight, body composition, pulmonary function, and number of hospitalizations.

Hardin DS, et al. *J Pediatr* 2001;139:636-642.

**First Editor's Comment:** This study by Hardin and associates is the first randomized, controlled trial of growth hormone therapy in children with cystic fibrosis. The findings are highly significant, although they have only been collected for a single year. Many questions remain unresolved. It would be important for studies to be undertaken to determine whether or not the change in lean body mass was due to an improved use of ingested calories and protein as suggested by the authors. In addition, the long-term benefits of treatment need to be evaluated, and the optimal dose needs to be determined. Furthermore, it will be important to follow these children to determine whether or not they are at increased risk for glucose intolerance over time. Hardin and associates have provided the preliminary data necessary to undertake a much larger scale study of the use of growth hormone in these children.

William L. Clarke, MD

**Second Editor's Comment:** Growth Hormone treatment in patients with cystic fibrosis has been shown

to be of benefit in various short-term trials.<sup>1,2</sup> However this is the first randomized controlled trial of GH treatment in patients with this disease. Growth hormone resulted in improved clinical status and increased growth. In CF, malnutrition develops as a result of unfavorable energy balance caused by a combination of poor intake, malabsorption of nutrients, chronic pulmonary disease and increased energy expenditures. Malnutrition adversely affects the course of the disease as well as the survival of the patients. Therefore any means to improve the anabolic state of CF patients may be of benefit. In this study GH treatment also improved the quality of life. Nonetheless, detrimental effects of GH treatment could occur in patients with CF, as diabetes is prevalent among this population.<sup>3</sup> Although in this study no patient developed this problem, the data cannot be extended to other patients or to those who would undergo a longer-term treatment. It should also be kept in mind that improvements in growth and nutrition status of CF patients may be accomplished with aggressive nutritional supplementation without GH treatment.<sup>4</sup>

Fima Lifshitz, MD

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## Intake of Vitamin D and Risk of Type I Diabetes: A Birth-Cohort Study

To ascertain whether vitamin D supplementation or vitamin D deficiency in infancy could affect the development of type I diabetes, a birth-cohort study was done in Oulu and Lapland, Finland. All infants born in 1996 were studied (n = 12,055). Data were collected on vitamin D supplementation and on the presence of suspected rickets during the first year of life. The primary outcome measured was the diagnosis of type I diabetes by the end of 1997 (30 year follow-up). Of the 10,366 children included in the analysis, 81 were diagnosed with type I diabetes. Vitamin D supplementation was associated with a decreased frequency of this disease. Children who took the recommended 2000 IU of vitamin D on a daily basis had a rate ratio of 0.22 of developing the disease, as compared with those who received no vitamin D. The rate ratio in those who received a lesser amount of vitamin D supplementation was 0.12. Children suspected of having rickets during the first year of life had a rate ratio of 3.0 as compared with those without

such diagnosis. The authors concluded that vitamin D supplementation was associated with a reduced risk of type I diabetes.

Hypponen E, et al. *Lancet* 2001;358:1500-1503.

**First Editor's Comments:** This is a very provocative study implicating the deficiency of one hormone (vitamin D) on the development of another hormone deficiency (insulin). The mechanisms of such association were thought to be related to the triggering of an immune process resulting from the lack of vitamin D. This is consistent with data from animal studies, and with the observation that cod liver oil supplementation during pregnancy is associated with a reduced rate of type I diabetes in the offspring.<sup>1</sup> The Eurodiab study also showed that vitamin D supplementation in early childhood may prevent this disease.<sup>2</sup> However, only 0.3% of infants in the Eurodiab study were not given

vitamin D during the first year of life, thus the comparative population was rather small. The increased prevalence of this disease (3x) among children in this Finnish study, who were suspected of having rickets, is impressive. However the data are not very compelling since there was no radiologic or biochemical evidence of rickets presented.

The infants who took 2000 IU of vitamin D as a daily supplement had a 78% lower risk of developing diabetes. This dose of vitamin D, however, is high and not recommended by most authorities. (The Committee on Nutrition of the American Academy of Pediatrics, among others, state that an adequate intake of this vitamin is 200 IU per day.) Others have recommended dosages ranging from 400 to 1000u per day,<sup>3</sup> where there may be lack of sunlight exposure, particularly during the long winter months in the northern hemisphere. Although there is no single recommendation for the amount of vitamin D supplemented, exposure to the sun usually will satisfy the requirements to prevent rickets and vitamin D deficiency. As little as 1 minimal erythral dose (MED) of sunlight is equivalent to ingesting about 10,000 IU of vitamin D. Simple exposure of hands and face two or three times per week provides a third to a half of the MED (about 5 minutes for fair-skinned people) is more than adequate. Moreover, sunlight is without risk of hypervitaminosis D which may occur when large amounts of vitamin D supplements are ingested. Thus, caution should be exercised to the possible temptation of increasing vitamin D supplementation in an attempt to prevent type I diabetes. Further studies are needed

to assess if there are other factors to ascertain why there is a high prevalence of type I diabetes among populations who also are exposed to insufficient sunlight such as found in Finland.

Fima Lifshitz, MD

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**Second Editor's Comment:** In the early 19<sup>th</sup> Century, cod liver oil was given to prevent rickets. The classical role of vitamin D in the prevention of rickets is to assist absorption of calcium and phosphate. Vitamin D also appears to play a role in preventing some cancers and autoimmune diseases. Ideally, in a study such as the one reported here, evaluation would include plasma 25(OH) D or 1,25(OH) 2D<sub>3</sub> concentrations. When sun exposure is limited, as in northern Finland, supplementation or dietary intake is an important source of vitamin D. Breast milk does not contain enough vitamin D to cover an infant's needs. The role of vitamin D in the pathogenesis of type 1 diabetes certainly deserves follow-up. If vitamin D does impair the immune system functioning in infancy, there may be other long-term effects. Interesting as well, Finland has the highest incidence of type 1 diabetes in the world.

Judith G. Hall, OC, MD

## Beneficial Effects of Intensive Therapy of Diabetes during Adolescence: Outcomes after the Conclusion of the Diabetes Control and Complications Trial (DCCT)

The DCCT, in 1994, reported the results of intensive diabetes therapy of adolescents (age 13-17 years at the time of enrollment into the study). Those results demonstrated a significant reduction in the risk for the development, and progression of retinopathy and microalbuminuria. Since that time, subjects from both the intensive and conventional therapy groups have been offered the opportunity to participate in the epidemiologic study of diabetes interventions and complications (EDIC). EDIC is a long-term observational study of the DCCT cohort. In this manuscript the DCCT/EDIC research group presents their latest findings. Of the original 195 adolescents, 175 agreed to participate in the EDIC study. At the end of the DCCT all subjects returned to their health care providers in the community for continuing diabetes care, and all conventionally treated subjects were offered instruction in the use of

intensive therapy. Approximately 50% of the subjects continued to receive their care at a DCCT/EDIC site. Subjects were seen on a yearly basis for determination of HbA1c and the recording of severe hypoglycemic episodes. Retinopathy was assessed by stereoscopic fundus photography at year 4, and classified according to the criteria described in the DCCT trial. A 3-step or more progression was classified as significant. Renal function was determined every other year by measurement of albumin excretion.

At year 4, 1/3 of the subjects who were originally randomized to conventional therapy continued to use 1 or 2 injections a day. The rest switched to multiple daily injections or insulin pump therapy. Ninety percent of former intensive therapy subjects continued to use multiple daily insulin injections or pump therapy. Total insulin doses and frequency of blood glucose monitoring



were similar between the 2 treatment groups. The difference in HbA1c between treatment groups was highly significant at the closeout of the DCCT, but by the end of the first year of the EDIC study there were no significant differences in HbA1c levels between the two groups. This was the result of both an increase in HbA1c by the intensive therapy group, and a decrease by the conventionally controlled group. These HbA1c values remained stable over the next 3 years (8.38% vs. 8.45%, intensive vs. conventional). In addition, the relative risk of severe hypoglycemia for patients in the former intensive treatment group was  $< 1$ , which was a decrease from the rates during the DCCT, and an increase in hypoglycemic occurrence for the conventionally controlled group. There was no difference in body weight, BMI, or percentage of subjects overweight at year 4 of the EDIC study.

After 4 years of follow-up in the EDIC study, 65% of the original conventionally treated patients showed a 3-step or more progression in retinopathy as compared with 32% of the former intensive group patients. This represents an odds ratio reduction of 74% for those having been in the intensive control group. Thus, the benefits of intensive therapy persisted for an additional 4 years in a significant number despite increased levels of glucose control. Similar findings were observed for the progression of nephrological disease. There was an 85% reduction in the adjusted odds ratio for developing albuminuria in the former intensive treated patients vs. the former conventionally controlled group. Thus, the benefits of previous intensive therapy continued for another 4 years with regard to renal function.

The authors state that these results demonstrate conclusively that the benefits of intensive therapy outweigh any associated risks of hypoglycemia and weight gain, and persist for at least four years. In addition, the data suggest that less than optimal glycemic control during the early years of diabetes (in adolescence) has a long lasting, detrimental effect on

the development of complications even after better glycemic control is established. Thus the recommendation is that intensive therapy be the standard of care for adolescents with type 1 diabetes mellitus. The DCCT/EDIC study is planned to continue for at least 10 years.

The Diabetes Control and Complications Trial/Epidemiology of Diabetes of Interventions and Complications research group: *J Pediatr* 2001;139:804-812.

**Editor's Comment:** *The results of the DCCT/EDIC at year 4 in adolescents are not different from those presented for the entire group (New Engl J Med 2000;342:381-389). The findings are important and have significant implications for the treatment of adolescents starting at diagnosis, and perhaps pre-adolescent children with type 1 diabetes mellitus. Some have assumed that the intensive therapy achieved by the DCCT research group, while important in reducing complications, might not be a reasonable and cost-effective treatment regimen for all adolescents with diabetes. These data prove otherwise. Intensive therapy initiated early in the course of diabetes has prolonged and long-lasting effects of reducing the risks of microvascular complications. Alternatively, diabetes management resulting in poor glucose control during the early adolescent years may be associated with an increased risk of microvascular complications, even after intensive therapy and a reduction in HbA1c has been achieved. Thus, these data support the initiation of intensive diabetes therapy designed to achieve near normal glucose control as early as possible in newly diagnosed adolescents. This must be the standard of care. Patients, their parents, and third-party payers must be educated to understand, demand, and compensate for such treatment.*

William L. Clarke, MD

## Growth in Human Immunodeficiency Virus Type 1-Infected Children Treated with Protease Inhibitors

About 33% of children infected with HIV have impaired growth. The extent of such impairment may be regarded as a clinical criterion predicting progression to AIDS. The addition of protease inhibitors (PIs) has been demonstrated to frequently reduce plasma HIV RNA levels, to increase CD4 lymphocyte numbers, and to improve the general condition of children and adults with HIV retrovirus type 1 infections.

Steiner et al present data on the long-term (72 week) impact of PI treatment on growth of infected children.

Data are reported on 44 children between the ages of 0-17 years with confirmed infection. They were observed for 72 weeks prior to starting PI treatment. Zidovudine or zalcitabine were added to the previous treatment of two nucleoside analogue reverse transcriptase inhibitors. Growth, HIV-1 RNA plasma levels, and CD4 lymphocyte counts were determined at 0, 24, 48, and 72 weeks of treatment. Heights were reported in SD scores as determined from normal aged and gender individuals. Data from 44 children were analyzed in 3 age groups [6



children <3 years of age (group I), 23 children 3-10 years of age (group II), and 15 children >10 years of age (group III)]. All had completed 72 weeks of PI treatment. Multiple regression analyses were used to determine the relationship between parameters of growth and variables such as CD4 cell count and CDC HIV-1 categories. Children in group I were more frequently in the severe CDC clinical category "C" and had higher plasma HIV-1 RNA levels at baseline than those in groups II and III.

By 24 weeks of treatment, there was a significant decrease in mean plasma HIV-1 levels in children of group I vs. those in groups II and III. Twenty-seven of the 44 children showed a sustained reduction of HIV 1 RNA levels. In the 72 weeks before the initiation of PI therapy the differences between  $\Delta$ -Z scores at 24 week intervals indicated progressive growth retardation which was reversed with a significant increase in growth during the 72 weeks after the PIs were added. This increase was biphasic with a greater increase between weeks 0-24, and a second increase between 48-72 weeks. The greatest increase in growth was in the 6 children in group I, all of whom had significant growth retardation at baseline and in the 4 significantly retarded children in group II. The 19 other children in group II and all 15 in group III had growth rates maintained within 1 SDS of the mean. Growth while receiving PIs was negatively correlated with growth during the preceding period, and positively correlated with an increase in CD4 cells. No correlation was seen between the decrease in plasma HIV-1 levels. Thus, age categories and CDC clinical categories were significantly associated with catch-up growth, but multiple regression analysis revealed that only growth during the preceding period and the age

category were significantly associated with growth during PI therapy.

The authors note that previous studies have shown that stunting has been correlated with higher plasma HIV-1 RNA levels. Of note, the older children in the cohort were not as severely stunted as the younger children, and did not have as significant a growth response to PI therapy. The authors speculate that these findings may be the result of the older children having a slower progression of HIV infection than the younger children, since they survived infancy in the era prior to aggressive therapy. In addition, the authors point out that others have attributed stunting in HIV infected children to sub-clinical hypothyroidism, low IGF-1, or proteolysis of IGF BP3. The authors did not measure these hormone levels.

Steiner F, et al. *Eur J Pediatr* 2001;160: 611-616.

**Editor's Comment:** The data reported in this paper by Steiner, et al are important from two aspects. First, treatment with a protease inhibitor can improve growth rates in young HIV infected children. Secondly, those with the greatest catch-up growth are those who are the most stunted initially. Such information is similar to that which has been shown for treatment of nearly every chronic disease of childhood. Unfortunately the authors did not determine biochemical markers of growth, including IGF-1 and IGF BP3. They suggest this be done in future studies. These data might have been useful in helping decide which children could benefit the most from such therapy. The data presented, however, are clinically useful.

William L. Clarke, MD

## Paternal Contribution to Aneuploidy

The relationship of maternal age to chromosomal abnormalities is well established; however, there have been conflicting data with regard to paternal contribution. Of potential pertinence is that 10 – 30% of autosomal trisomies arise during paternal meiosis, 100% of XYYs and 50% of XXYs are paternal in origin, and 80% of Turner syndrome patients are missing the paternal X. Also, an increase in paternal age is associated with the development of uniparental disomy 15, and trisomy 18 is seen with increased paternal age. To further study the relationship of paternal age to diploidy and disomy of sperm, the authors of this paper screened human sperm using four-colour FISH probes. Chromosomes 6, 21, X, and Y were examined to determine the incidence of disomy in sperm related to paternal age where the normal usual sperm are haploid.

Almost 200,000 sperm were examined from 18 healthy donors, ages 24 to 74. The investigators found a significant increase in the level of autosomal disomy and a marginally significant increase in sex chromosome disomy with increasing male age. Significant individual variation was observed. The increase in disomy ranged from 0.3 to 17% for each 10-year period. This suggests that older men have a tendency to show synaptic abnormalities perhaps related to the deterioration of testicular environment with advancing age.

Bosch M, et al. *Euro J Hum Gen* 2001;9:533-538.

**Editor's Comment:** There is a growing interest in paternal contributions to congenital anomalies, both potential teratogens and the effect of aging itself. Although triploids are not usually viable, it is interesting

that paternal age would seem to lead to an increased contribution to triploid conceptions. This could also play some role in triploid-diploid mixaploid individuals. This article is an excellent review of current knowledge pertaining to diploidy, aneuploidy, and disomy in the

sperm of males of various ages and in various chromosomally determined clinical conditions.

Judith G. Hall, OC, MD

## New Syndrome of Hyperinsulinism and Hyperammonemia

Although there are many causes of hypoglycemia, a new syndrome associating hyperinsulinism with hyperammonemia was recently described (Zammarachi, et al. *Metabolism* 1996;45:957; Weinzimer, et al. *J Pediatr* 1997;130:661; Stanley, et al. *N Eng J Med* 1998;338:1352). This syndrome is identical or closely related to the leucine-sensitive hypoglycemia syndrome and is congenital in origin. Clinical manifestations are usually observed in neonates and/or infants. The diagnosis of patients with HSS is crucial as therapy differs radically, medical and not surgical, from that of other hyperinsulinemic patients. A positive response to diazoxide- and/or leucine-free diet is usually observed. All but one of the 12 patients in the article by De Lonlay had at least a partial response to diazoxide.

Genetically all 12 cases studied seem to be new mutations, as they occurred sporadically without family histories. This mutation results in a gain of function in the glutamate dehydrogenase gene (GLUD1). It also results in a decreased inhibitory effect of guanosine triphosphate on the enzyme. It has been suggested that the elevated oxidation of glutamate to  $\alpha$ -ketoglutarate stimulates insulin secretion by increasing the ATP/ADP ratio in the pancreatic Beta cell, although this is unproven. All 12 patients studied had mutations located within or outside the GTP binding site, without

any correlation between phenotype and genotype. The mutations in the GLUD1 gene are found in exons 6, 10, 11, and 12, which includes the antenna region of the enzyme and the GDP binding domain.

In a review of hyperinsulinemic patients by the authors in their institution over the past 20 years, plasma ammonia concentrations were measured in 71 (45 neonates and 26 infants) and hyperammonemia was found in 12 of the 71. The incidence of this type of hypoglycemia is significant. The authors conclude that ammonia concentrations should be measured in every patient investigated for hyperinsulinism and that, conversely, hypoglycemia should be looked for in all patients with unexplained hyperammonemia.

De Lonlay P, et al. *Pediatr Res* 2001;50:353-357.

**Editor's Comment:** *Heterogeneity is the name of the game, and molecular techniques allow us to recognize many of the reasons for heterogeneity. Within heterogeneity, many new biochemical pathways and mechanisms of disease are being identified. As in the case of this syndrome, different types of therapy become most appropriate.*

Judith G. Hall, OC, MD

## 15 Years After Chernobyl: New Evidence of Thyroid Cancer

A striking increase in childhood thyroid cancer was reported after the Chernobyl accident. Because proper dosimetry was not done at the time, the exact amount of exposure to children was not clear. The children who attended school within a 150 km radius of Chernobyl have been carefully screened over the ensuing 14 years. The nuclear power plant accident happened on April 26, 1986. One case of thyroid cancer was recorded per 2,409 children born between April 27, 1986 and December 31, 1986, (intrauterine exposure). A much higher rate, with 31 thyroid cancers among 9,720 children (ages 1 day – 4 years), was seen in those born in the 4 years prior to the accident. Over 20,000 children have been followed and repeatedly examined using ultrasound, as well as measurements of TSH, free thyroxine and thyroid peroxidase antibodies. An increase in thyroid cancer has not been seen in children

born since 1987 (post Chernobyl conceived). All of the cancers were papillary adenocarcinomas.

Shibata Y, et al. *Lancet* 2001;358:1965-1966.

**Editor's Comment:** *The conclusion of this follow-up study is that children at a young age and probably up until 10 years of age are at particularly high risk for developing thyroid cancer after exposure to radioactive fallout. Hopefully, there will never be another Chernobyl. If there is, careful dosimetry to know the amount of exposure and the rapidity of decay will be important. However, it is clear that children, particularly young children, are at the greatest risk and need to be followed carefully.*

Judith G. Hall, OC, MD

## Letter to the Editor

I read the commentaries and the review of the paper by Zucchini et al on SCA final height after growth hormone treatment from *Arch Dis Child*, in the October 2001 issue of GGH. I would like to add the following points.

As the reviewer states, the treatment had begun late (approximately 10.8 years). What the reviewer does not state clearly is that the GH dose was too low. I calculated the dose to be about 0.22 mg/kg/week. The FDA approved GH for SGA at a recommended dose of 0.48 mg/kg/week. It is not surprising therefore that less than 50% of the recommended dose gives disappointing results. de Zegher et al presented near final height at the joint meeting in Montreal and the robust height SDS gains appeared to be sustained.

In essence then, the disappointing results of the Zucchini paper can be summarized as "too little, too late". That conclusion did not come across in the comments.

Paul Saenger, MD

**First Editor's Comments:** We appreciated the remarks of Dr. Saenger with regard to the abstract of the article by Zucchini, etc, *Arch Dis Child* 2001 84:340. Although, as Dr. Saenger pointed out, the dosage of GH used in the study was significantly less than that approved by the FDA for treating short SGA children, the children treated in this study were classified as growth hormone deficient based on stimulation tests. Thus one might argue that the magnitude of the difference between recommended and actual GH dose was not as different for these GH deficient children as it might have been had they been GH sufficient. Indeed, the presentation by de Zegher in Montreal last summer was very encouraging. Long-term studies, treating SGA children from an early age, at the recommended dose are necessary to answer the question of the overall benefit on adult height of GH treatment of SGA children.

William L. Clarke, MD

**Second Editor's Comment:** The Reviewer thanks Dr. Saenger for his helpful comments about the manuscript of Zucchini et al<sup>1</sup> concerning the effect of rhGH in short children born small for gestational age (SGA). The dose of rhGH utilized by these investigators (calculated to be 0.27 mg/kg/week) was indeed less than that employed by de Zegher et al<sup>2-3</sup> (ranging between 0.23 and 0.7 mg/kg/week). In addition, these investigators began treatment with rhGH between 2-8 years of age, thus affording longer treatment periods. The adult heights of their patients have not been reported as yet, although through 6 years of therapy there was an increase in height of +2 SDS. However, treatment with high doses of rhGH resulted in insulin resistance that may not be completely reversible<sup>4</sup> and in high levels of IGF-I during treatment.<sup>5</sup>

Even if rhGH is able to increase adult stature to a statistically significant extent, there are no data indicating that greater height is meaningful in terms of improved psychosocial well-being, educational attainment, or economic success. Given the potential hazards of insulin resistance, elevated levels of IGF-I (if only temporary), and lack of documented enhancement in the quality of life (QoL), treatment of SCA children with rhGH, particularly at the dose that has been approved by the FDA, seems hazardous to this reviewer and should only be employed in an investigative setting until its staturel and QoL efficacies and safety have been well documented.

Allen W. Root, MD

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# GROWTH

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### Phallic Construction 2002: Current Concepts and Future Directions

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#### INTRODUCTION

The penis is anatomically complex, being involved with both voiding and sexual activity. Both have significant psychosexual implications for affected patients. While the functions of sperm and urine transport may be bypassed using modern technology; we are as yet unable to replicate the unique anatomic and biomechanical properties of the penis. Therefore, current attempts at replacement of an absent or inadequate penis are designed to create an acceptable phallus or penis-like structure. These reconstructive efforts are referred to as phallic construction or phalloplasty.

The optimal phallus should provide all of the following: 1) both tactile and erogenous sensibility, 2) a neourethra which allows voiding while standing, 3) the capability to permit prosthetic insertion which permits successful vaginal intromission, 4) cosmetically aesthetic acceptability of both the phallus and proposed donor sites, and 5) acceptable phallic growth to adult size in the case of pediatric phalloplasty. Optimally the surgery should be accomplished in a reproducible single stage with acceptable morbidity. Modern reconstructive and microsurgical techniques permit us to achieve these aims much of the time. However, single stage reconstruction eludes us in most cases.

Phallic construction is one of the most challenging procedures in reconstructive surgery. At our center we use a multi-disciplinary approach which includes urologists, plastic surgeons, gynecologists, endocrinologists and other experts. The purpose of this review is to discuss the history of phallic construction that has led to current techniques of phalloplasty. These will be briefly outlined in order to address some of the most recent indications for phallic construction, which

include the procedure's use in trauma patients, in patients with congenital anomalies, and in transgender patients. Discussed are our results in each patient subcategory.

#### HISTORY

The evolution of phallic construction techniques has paralleled advances in reconstructive surgery. Initially, random tubed skin flaps were used, which were transferred in tubed delay fashion. These techniques were supplanted by the use of island and/or musculocutaneous flaps. With the advent of modern microsurgical techniques, microvascular free transfer flaps have become the state-of-the-art for phallic construction.<sup>1</sup>

Bogoraz<sup>2</sup> reported the first successful phallic construction in 1936. He employed an abdominal tubed flap to construct a phallus, in a case of post-traumatic penile amputation. This patient ultimately had successful intercourse using a segment of rib cartilage implanted into the phallus as a stiffener, and fathered children after the reconstruction.

Maltz<sup>3</sup> and Gillies and Harrison<sup>4</sup> are credited with developing the tube within a tube concept which permits a second inner tube to function as a urethra within the outer phallic shaft. Because the urethra was fashioned from hair-bearing abdominal midline skin, urethral

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strictures and fistulas were the rule. Also the unreliable blood supply of the lower abdomen often compromised the flap's overall viability. Despite its aesthetic and functional limitations, variations of this abdominal flap remained popular throughout the 1950s and 1960s. In some cases, the inner tube was used for baculum placement to induce rigidity and not for voiding function.

A major step forward in phallic construction was achieved when Noe et al<sup>5</sup> used the reliable abdominal branch of the external pudendal artery to vascularize the phallus. Using more reliable vascularity, musculocutaneous flaps were successfully constructed by Orticochea,<sup>6</sup> Horton et al,<sup>7</sup> and others. Although these flaps were more aesthetically pleasing and more reliable, they remained insensate, and often required multiple "touch up" surgeries to achieve an acceptable result.

Puckett and Montie<sup>8</sup> performed the first microvascular free transfer flap phalloplasty in 1978. The seminal work of Gilbert et al<sup>9</sup> provided erogenous sensation to the phallus via anastomosis of a sensory nerve within the flap to the patient's pudendal nerve and the radial forearm flap single stage phalloplasty described in 1984 by Chang and Hwang<sup>10</sup> brought this evolving field to the current position. Additionally, Lovie et al<sup>11,12</sup> described the use of the ulnar forearm flap for head and neck reconstruction and Gilbert et al<sup>13</sup> used this flap for phallic construction, which became this center's procedure of choice.

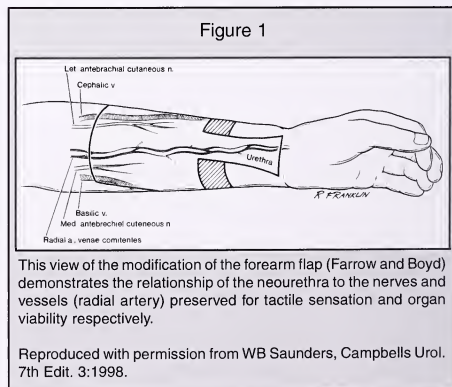
## SURGICAL ADVANCEMENTS

The free forearm flap is the gold standard for the modern phallic construction. These flaps are ideal from a technical standpoint, as they are malleable, and they remain relatively hairless, thus improving the aesthetic result. All of the currently employed forearm flap designs share certain common features, including arterial inflow from either the radial or ulnar artery (and venous drainage via basilic, cephalic veins and/or vena comitans), and erogenous sensation provided by either the medial or lateral antebrachial cutaneous nerves (Figure 1). A drawback to this flap is the post-operative appearance of the donor site. While functional or sensory problems are rare to non-existent within the forearm or hand, the cosmetic appearance may be disturbing to some patients. The appearance of this site can be improved by resurfacing the forearm with a full thickness skin graft from the groin. Other phallic construction options have been employed in patients who refuse forearm scars including fibula osseocutaneous flaps<sup>14,15</sup> and metatidoioplasty (plastic surgery to convert a clitoris to a penis),<sup>16</sup> but these are, we feel, clearly sub-optimal choices.

The original Chang & Hwang flap centered the phallic shaft around the radial artery, with the neo-urethra somewhat distant to the principal blood supply. The Blemer modification of this design centers the neo-urethra over the central portion of the flap, with the phallic shaft created by two skin islands separated from the neo-urethra by de-epithelialized strips. This modification results in less ischemic injury in the area of the neo-urethra, and allows for extension of the neo-urethra both proximally and distally along the length of the shaft. This extra length may be critical for a reliable anastomosis to an often foreshortened native urethra. The main disadvantages of this modification, when based on the radial artery, are that the urethra is centered over the hairiest portion of the forearm and two suture lines result from closure of the skin island around the neo-urethra.

Classically, the forearm flap was based upon the radial artery but in our hands it is based upon the ulnar artery,<sup>12</sup> since the increased caliber and length of the ulnar artery makes the anastomosis of the vascular pedicle technically more straight forward. Furthermore, the relatively hairless skin overlying the ulnar aspect of the forearm usually is best suited for urethral and phallic construction. Over the last 10 years, this center has adopted the ulnar forearm flap which also provides for construction of an integral neoglans (Figure 2).

Preoperative evaluation focuses upon the patient's general health, particularly from a cardiovascular standpoint. Heavy smoking with its associated vascular disease is an absolute contraindication to this type of microsurgery. The vascularity of the non-dominant forearm is assessed with the Allen test, followed by selective upper extremity Doppler sonography or angiography as needed. To date, we have not had upper extremity complications related to diversion of the ulnar arterial blood flow.



The flap is carefully designed with dimensions specific to the patient's requirements for phallic and urethral length. Dissection is carried out superficial to the deep antebrachial fascia, allowing for an extra tissue layer overlying the nerves and muscle tendons of the forearm. The ulnar artery, basilic and cephalic veins, and medial and lateral antebrachial cutaneous nerves are each meticulously dissected through the forearm and elevated with the flap. After the flap has been elevated, it is tubularized while still perfused on the forearm. The central skin island (neo-urethra) is tubularized, after which the outer phallic islands are tubularized. Finally, the newly constructed glans is transposed over the distal shaft.

The phallus is transferred to the anatomic area of the penis. The ulnar artery is typically anastomosed to the deep inferior epigastric artery, and the veins are anastomosed to either the deep inferior epigastric vena comitans, or to the saphenous veins. The urethral anastomosis is performed after vascularity has been restored. The sensory nerves of the flap are coapted to the dorsal nerves of the penis or clitoris; or in some cases, to the deep internal pudendal nerve. At the end of the procedure, the patient has a natural appearing phallus (Figure 3), and this appearance is further enhanced by scar remodeling in the subsequent year. The final step is forearm donor site coverage with thick full-thickness skin grafts – usually harvested from the groins.

## SEXUAL FUNCTION OF THE NEOPHALLUS

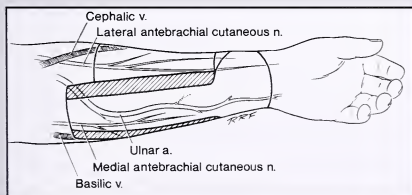
The goal of achieving reliable phallic rigidity has remained a challenge in the field of phallic construction. Many options have been attempted with variable results. Occasionally, the neophallus may possess enough intrinsic stiffness to allow intromission without a prosthetic stiffening device. The original technique of

Bogoraz<sup>2</sup> involved implantation of rib cartilage in the phallus, and for several years thereafter cartilage or nonvascularized bone were the standard approaches to obtaining phallic rigidity. The disadvantages of these techniques included warping and resorption of the cartilage/bone with time. Others<sup>17</sup> used vascularized bone segments incorporated in the phallus to provide rigidity. Another option has been to create a separate tube for a removable baculum.<sup>17</sup>

Prosthetic implants also have been inserted successfully.<sup>18</sup> The phallus usually develops tactile sensitivity between 4 and 9 months postoperatively. Such sensitivity must be present to protect against pressure necrosis prior to implanting a prosthesis. Also, the neourethra must have proven to be durable and infection free by this point. Unlike patients who have suffered traumatic penile amputation, congenital aphallic patients and female to male transgender patients lack corporal bodies in which to seat and anchor the prosthetic device to the pelvis.

In order to circumvent this problem, we have created the "neotunica," which is a Gore-Tex (polytetrafluoroethylene) graft, which acts as a sleeve surrounding the actual implant.<sup>18,19</sup> In a transgender patient without corporal remnants, the cylinder is ensheathed in the Gore-Tex sleeve, and the sleeve is then anchored to the periosteum of the ischial tuberosity (inferior pubic ramus) as well as to the pubic symphysis. If a hydraulic prosthesis is used, the pump is placed in the scrotum. If corporal remnants are present proximally,

Figure 2



The forearm flap utilizing the ulnar artery as the source of blood supply and the antebrachial cutaneous nerves for tactile sensitivity are shown in this diagram.

Reproduced with permission from WB Saunders, Ehrlich/Alter *Reconstructive and Plastic Surgery of the Genitalia: Adult and Pediatric*, 1999.

Figure 3



The phalloplasty should, and often does, result in a favorable cosmetic result. Rigidity may need to be enhanced by utilizing one of the available prostheses (see text).

they may be opened and used to seat the cylinders. The neotunica is then used to surround the distal ends of the prosthesis.

The category of prosthesis used is partially dependent on patient preference. Articulated as well as hydraulic implants have been employed. At this center we have had good results with the Duraphase® prosthesis and the AMS 700CX® prosthesis. Early in our experience, we tended to place single "rods," however we now place dual "rods" in the majority of cases. Two rods provide better rigidity, and are felt to have less potential for erosion.

## INDICATIONS FOR PHALLIC CONSTRUCTION

### Trauma

Penile amputation injuries have devastating psychological consequences that usually persist throughout the victim's lifetime. In North America, these injuries are fortunately rare. If the patient presents with the amputated tip of his penis, replantation offers excellent results and can be reviewed further.<sup>19</sup> In many cases the patient does not present with the severed part, and other reconstructive options – including phallic construction– must be entertained.

### Pediatric Phallic Construction

Phallic construction in the prepubertal population continues to be a controversial topic, but should be considered for two broad categories of children. The first and less controversial category consists of boys who have sustained trauma to the penis. These boys have already been assigned the male gender. These patients usually are not candidates for gender reassignment, and phallic construction permits these boys to maintain their male gender identity.

The second category of patients who may be considered, consists of genetic XY babies who have a congenitally anomalous penis and often genital ambiguity. These babies may have classic micropenis, aphallia, partial androgen insensitivity or an enzymatic defect such as 5 alpha reductase deficiency. Also boys with cloacal exstrophy may fall into this category, although cloacal exstrophy is not classified as an intersex condition. While boys with classic exstrophy/epispadias complex are typically able to function after epispadias repair and chordee correction, the rare patient may have corporal bodies that are inadequate to reconstruct even the most rudimentary penis. Phallic construction has been successful in some of these patients.<sup>20</sup>

In prior years, many of the patients with micropenis, aphallia and exstrophy were gender-converted in early

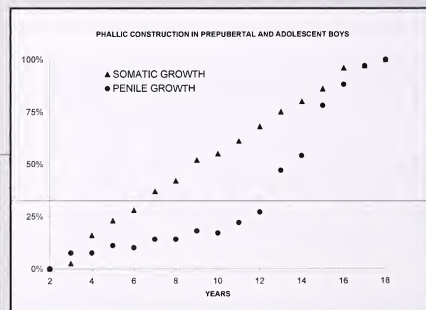
childhood and reared as girls. The fact that many such patients have experienced gender dysphoria later attests to the validity of the hypothesis that the genetically male brain is "masculinized" in utero. The advent and success of modern phallic construction techniques now permits these males to retain their genetic sex, and in rare patients potentially procreate later in life.

The timing of pediatric phallic construction remains of paramount importance. The key issue is construction of a phallus which is of appropriate size for a child, but which will reach adult dimensions post-pubertally. The normal penis is an androgen sensitive organ which grows to adult size during puberty under the influence of dihydrotestosterone. A forearm flap phallus is not androgen sensitive, and will grow at the rate of other somatic tissues. Therefore the somatic and genital growth rate must be factored into the equation when calculating relative flap size at any age (Figure 4).<sup>21</sup> We recommend construction of the neophallus between the ages of 6 and 8 years of age for patients in the pediatric subgroup.

### Female-To-Male Transsexualism

Gender dysphoria is a widely recognized psychological condition wherein the patient is of normal phenotype but feels "trapped" in the body of the wrong sex. The incidence of this condition in the United States is approximately 1:50,000, with a male:female ratio of approximately 6-8:1.<sup>22</sup> Most psychiatrists believe that conversion of adult transsexual patients via psychotherapy back to their biologic sex is nearly impossible. Many of these patients benefit from hormonal and surgical gender reassignment.

Figure 4



Penile growth normally has an adolescent growth spurt. Phalluses constructed from forearm flaps have growth more in accord with somatic growth although of more limited nature.

Reproduced with permission from David Gilbert, previously unpublished.



Transgender surgery should be performed only at centers devoted to the complete care of these patients, as psychologic and medical needs require integrated assistance.

Our center utilizes a multi-disciplinary approach to these patients, utilizing the combined skills of two clinical psychologists, a gynecologist, two urologists and a plastic surgeon. Patients are evaluated by all members of the committee before acceptance for transgender surgery. (The Harry Benjamin criteria).<sup>23</sup>

Transsexual patients qualifying for phallic construction at our center undergo surgery in multiple stages. The first stage consists of hysterectomy and oophorectomy (usually via a vaginal approach), vaginectomy, colpcleisis, and urethral lengthening. The second stage, phallic construction, is as already discussed. Prosthetic placement is done at a third stage in those select patients that request it.

## RESULTS OF PHALLIC CONSTRUCTION

Between 1986 and 1994, 40 patients underwent phallic construction at this center. Another 34 patients have undergone phallic construction between 1994 and 2001. Of the first 40 patients, 22 were female to male transgender patients. As previously mentioned, the introduction of the staged approach, with urethral lengthening, has reduced the incidence of difficult fistulas in this group. Thirty-four of 40 patients were available for follow-up. Stricture at the neourethral anastomotic site occurred in 68%, and urethrocutaneous fistulas at the penoscrotal junction in 32%. At the time of that review in 1993,<sup>24,25</sup> 68% of the series were symptom free or required only self-dilation. The modification of staged reconstruction, along with anastomotic covering with a muscle or fascial flap has reduced the overall urethral complication rate to about 30%.<sup>25</sup>

The results of recent penile prosthesis implantation have been more encouraging than previously reported by this center and others. We reported 8 patients in whom prosthetic implantation was attempted, 6 (75%) still have prostheses in place.<sup>26</sup> Infection necessitated prosthesis removal in 4 patients, of whom two were successfully reimplanted. Seven of 8 patients have been sexually active using their prostheses. The infection rate has declined in the past several years secondary to the introduction of perioperative closed suction drains and broad spectrum antibiotics. We currently have reported approximately 40 patients with only 2 explants in the last 20 patients.<sup>27</sup> One was performed for delayed erosion, and the second for vascular compromise of the flap in the immediate post-implant timeframe.

We have performed phallic construction in the pediatric population,<sup>21</sup> in 7 prepubertal and 4 adolescent boys, as well as in 5 older boys who had reached 18-24 years of age. Only one flap failed in the childhood/adolescent group (91% success rate). All patients who underwent flap nerve coaptation to the pudendal nerve reported return of protective sensation. All of the adolescents/young adults who underwent phallic construction noted erogenous sensation and the ability to orgasm. The question of flap growth in the pediatric subgroup is currently under review.

## CONCLUSION

While phallic construction remains a challenging aspect of reconstructive surgery, it has evolved tremendously since its inauspicious beginnings in Russia in the 1930s. Modern phalluses are mostly aesthetically acceptable, durable, and in many cases very satisfying for the patient. Urethral reconstruction in the neophallus also has improved considerably, with reduction in the number of recorded fistulas and strictures. The search for an autogenous tissue source to facilitate rigidity continues, but we and others have had significant success thus far with the use of prosthetics in carefully selected patients. The prepubertal phallic construction continues to stir debate; but we believe that for genetic males, it presents an alternative to gender conversion, and patients must be so counseled.

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## Future Articles

*The Current Frontiers of In Vitro Fertilization  
Molecular Genetics of Peripheral Precocious Puberty  
Controversies in the Treatment of Intersex  
Agonadial, Germ Cell Failure & Other Multiple  
Malformation Syndromes Associated with Gonadal  
Failure*

# SOMATOMEDIN HYPOTHESIS: TIME FOR REEXAMINATION

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The following article is a slightly modified article from *The Endocrinologist* 2001:470-473 and is reproduced by permission. The editors suggest you read The Letter to the Editor on page 48 before proceeding.

In 1957, Salmon and Daughaday<sup>1</sup> observed that incorporation of radioactive precursors of cartilage acid mucopolysaccharides could be stimulated in vitro by serum from hypophysectomized rats that had received growth hormone (GH) in vivo. Addition of GH directly to the medium, however, did not enhance precursor incorporation. The authors inferred that GH did not act directly on cartilage; instead, it did so by generation of a factor in the serum that enhanced the incorporation. The serum factor was originally named "sulfation factor", because radioactive sulfate was used as the precursor. The magnitude of the effect was proportional to the volume of serum used, and the factor was originally used as a bioassay for GH activity.<sup>2</sup> The in vitro incorporation test was discarded when radioimmunoassays of GH became available.<sup>3</sup> Subsequently, the sulfation factor was renamed "somatomedin", because it seemed to be the effector by which GH stimulated somatic growth.<sup>4</sup> Several somatomedins were identified, and the components of the system were designated by letters of the alphabet, as somatomedin A, B, and C.<sup>5</sup>

Before the development of a radioimmunoassay for insulin,<sup>6</sup> its activity in serum was measured by bioassay of its effects, such as glucose uptake by isolated tissues in vitro.<sup>7</sup> Radioimmunoassays of serum from fasting animals, however, showed that as little as 10% of the effect on serum glucose was caused by insulin itself.<sup>8</sup> Furthermore, the insulin-like activities were minimally suppressed by the addition of anti-insulin antibodies to the serum.<sup>9</sup> The "noninsulin" effects were attributed to the presence in the serum of nonsuppressible insulin-like activities, and a nomenclature was subsequently adopted designating them as insulin-like growth factors (IGF).<sup>10</sup>

The amino acid sequences of two nonsuppressible insulin-like activities (IGF-1 and IGF-2) were elucidated by Rinderknecht and Humbel,<sup>11</sup> and their tertiary structures were subsequently determined by Blundell et al.<sup>12</sup> They consist of A-domains homologous to the A-chain of insulin, B-domains homologous to the B-chain, C-domains homologous to the C-chain of proinsulin, and D-domains that extend from the C-terminals of the A-chains. Analysis of somatomedin-C, the principal growth factor of the somatomedin family, showed that it had the same amino acid sequence as IGF-1, and the two were considered to be identical.<sup>13</sup> Because the largest fraction of IGF-1 in the circulation is derived from the liver, where the expression of the gene is regulated by GH,<sup>14</sup> the *somatomedin hypothesis* was developed. It stated that the anabolic effects of GH on cartilage and other tissues were mediated through IGF-1 synthesized in the liver and not by direct action of GH on these original target tissues.<sup>4</sup> Although the hypothesis has gained widespread acceptance, there is mounting evidence that it may have to be modified or even abandoned. A priori, it would seem unlikely that a factor that exerts hypoglycemic effects<sup>15</sup> should be the effector of GH action.<sup>16</sup> Since GH is an insulin counter-regulatory hormone,<sup>17</sup> it seems paradoxical that it should exert its effects through a factor that produces hypoglycemia.

Isaksson et al.<sup>18</sup> summarized evidence available in 1985 that GH acts directly on prechondrocytes, epiphyseal plate cartilage, cloned preadipose cells, and myoblasts without the intervention of a mediating factor. GH also has been found to act directly on other tissues in vitro, such as stimulating erythropoiesis in vitro.<sup>19</sup>

More recently, additional evidence doubting the somatomedin hypothesis has accumulated. The evidence comes from three different sources. First, Salmon and Burkhalter<sup>20</sup> revisited the experiments originally conducted by Salmon and Daughaday<sup>1</sup> that formed the basis for the hypothesis. In these newer studies, they found that in contrast to their earlier experiments, GH added directly to cartilage from hypophysectomized rats did stimulate incorporation of radioactive sulfate into proteoglycans and radioactive

thymidine into DNA. They ascribed their newer findings to the use of a different medium in the more recent experiments; HEPES-buffered amino acid-glucose solution with a low concentration of bovine serum albumin. Amino acids were not added to the medium used in the original experiments, and the authors also speculate that a nondialyzable component of hypophysectomized rat serum may have inhibited the incorporation of sulfate into cartilage.

Secondly, a series of observations that cast doubt on the hypothesis was reported by Yakar et al<sup>21</sup> who devised an elegant set of experiments to determine if hepatically derived IGF-1 is the circulating mediator of GH effects on postnatal growth and development. Using the Cre/loxP recombination system, they deleted the IGF-1 gene exclusively in the livers of mice. Their finding of a 75% reduction in the concentrations of IGF-1 in the serum confirmed that the liver is the primary source of circulating IGF-1. Despite this reduction in circulating IGF-1, there was no evidence of growth impairment when the liver IGF-1-deficient mice were compared with their wild-type litter mates. These experiments have been confirmed by Sjogren et al<sup>22</sup> using the model devised by Yakar et al.<sup>21</sup>

A third observation casts doubt on the hypothesis. This concerns the issue of the lipogenic properties of IGF-1. In a report of long-term treatment of European patients with GH insensitivity syndrome, IGF-1 treatment led to accelerated growth, but there was also a substantial gain in fat mass that correlated significantly with the increase in height.<sup>23</sup> Ecuadorian patients with the same syndrome experienced a significant increase in growth rate when treated with IGF-1.<sup>24</sup> They also experienced a relative increase in mean body weight for height when they were treated with the higher of two doses of IGF-1.<sup>24</sup> It should be noted that not all investigators have reported an increase in fat mass.<sup>25</sup> Increased lipogenesis has also been shown to occur in a subject with an IGF-1 deletion who was treated with IGF-1.<sup>26</sup> The authors inferred that the lipogenic effects could be ascribed to the reduced concentrations of GH in the serum after IGF-1 treatment. This explanation is untenable, however, because increased lipogenesis was also found in the subjects with GH insensitivity syndrome.<sup>23,24</sup>

Increased fat mass is inconsistent with the hypothesis that IGF-1 mediates the effects of GH, which is a lipolytic and anabolic hormone.<sup>27</sup> It is more in keeping with an insulin-like action, such as that seen in infants of mothers with diabetes in whom hypoglycemia is prevented by placental exchange of glucose despite high concentrations of insulin in the fetal circulation.<sup>28</sup> The increased length and fat content of these infants is evidently because of the anabolic and lipogenic effects of insulin secreted by the fetal pancreas.<sup>29,30</sup> In

considering the role of IGF-1 in growth promotion, distinguishing between the effects of circulating IGF-1 and IGF-1 produced by autocrine/paracrine mechanisms is important. In their experiments, Yakar et al<sup>21</sup> found that growth was severely restricted in IGF-1 knockout mice in which the gene was deleted from all tissues. There can be little doubt, therefore, that the IGF and their binding proteins are important growth factors when produced locally by autocrine/paracrine mechanisms. Moreover, as pointed out previously, expression of the hepatic gene for IGF-1 is regulated by GH,<sup>14</sup> and plasma concentrations of IGF-1 are uniformly increased in adults with acromegaly and children with gigantism.<sup>31</sup> Despite earlier findings that plasma IGF-1 and IGF-binding protein-3 concentrations might be useful in the diagnosis of GH deficiency, there are substantial disagreements on this issue.<sup>32-34</sup>

It is time to take note of the deficiencies in the hypothesis and possibly to abandon it completely. There is a strong body of evidence that liver-generated IGF-1 is unlikely to be responsible for the linear growth effects of GH and that the actions of GH on its target tissues do not require mediation by this factor in the circulation. It is also unlikely that measurement of these growth factors and their binding proteins in the plasma will be useful in assessing the role of GH in growth retardation.

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## Abstracts from the Literature

### Leptin-Replacement Therapy for Lipodystrophy

Severe lipodystrophy is known to be associated with leptin deficiency, insulin resistance, hypertriglyceridemia and hepatic steatosis. Thus, the authors assessed whether leptin-replacement would ameliorate this condition and its complications. Nine female patients (ages 15 to 42 years; 8 with diabetes mellitus) with lipodystrophy of various types, with serum leptin levels of less than 4 mg/ml, and with high insulin levels received recombinant methionyl human leptin subcutaneously twice a day for four months in escalating dosages (0.03 mg to 0 – 0.4 mg/kg/day) to obtain low, intermediate, and high physiologic serum levels of leptin. During the treatment, the serum leptin levels increased from a mean of 1.3 +/- 0.3 mg per ml to 11.1 +/- 2.5 mg per ml.

The glycosylated hemoglobin values in the diabetic patients decreased, a mean reduction of 1.9%. After four months of therapy, the average triglyceride levels decreased by 60% and the liver volume diminished in size by an average of 28% in all patients. Leptin also led to a discontinuation or a large reduction in the anti-diabetes therapy. The self-reported daily caloric intake also decreased significantly. No major problems or side effects occurred. The authors concluded that leptin replacement improved glycemic control and decreased triglyceride levels in patients with lipodystrophy and leptin deficiency.

Elif AO, et al. *N Engl J Med* 2002;346:570-578.

**Editor's Comment:** These investigators demonstrated that leptin deficiency contributes to insulin resistance and other metabolic abnormalities associated with severe lipodystrophy. The reduction of glycosylated hemoglobin associated with leptin therapy is important, reflecting improved diabetic control. This could lead, if the effect persists, to a decrease in the relative risk of retinopathy and/or nephropathy in the diabetic population. The decreased triglyceride levels may reflect a reduced relative risk of adverse cardiovascular events. The alterations that characterize lipodystrophy are known to be refractory to other treatments, and, therefore, this paper reports a novel action of this hormone in addition to its known role in the control of energy homeostasis.

For those readers wishing more information regarding leptin, consult the article in the last issue (GGH 2002 Vol 18:2), which is entitled "The Endocrine Function of Adipose Tissue" and the article entitled "Molecular Physiology of Leptin and Its Receptor" (GGH 1998 Vol 14:2). Several articles from the literature concerning leptin have been abstracted in GGH since 1998.

Fima Lifshitz, MD

### Effect of Growth Hormone Therapy on Height in Children with Idiopathic Short Stature: A Meta-Analysis

The authors reviewed all published (English language) manuscripts and manually searched all issues of the *JAMA*, *Journal of Pediatrics*, *Pediatrics*, and *Acta Paediatrica*, and the meeting abstract books of the *Lawson Wilkins Pediatric Endocrine Society* and the *Endocrine Society* between 1985-2000 for publications (N=761) that reported primary effects of recombinant human growth hormone (rhGH) on the growth of children. From this group, the authors culled those

papers reporting adult stature in more than 5 healthy children with "idiopathic" short stature treated with rhGH whose heights were below the 10th percentile at the initiation of treatment and who had "normal" GH secretion ( $\geq 10$  ng/mL during provocative testing) and in which more than 50% of the starting population completed the study. From this pool, 19 articles describing 10 controlled studies (N=434) and 34 articles reporting 28 uncontrolled studies (N=655) were selected



for more thorough analysis. In both groups the mean age at the beginning of treatment was 10-11 years, baseline growth rates were approximately 4.3 cm/year, and therapy with rhGH was maintained for approximately 5 years.

In the *controlled* studies, adult stature of rhGH-treated children exceeded that of the control group by 0.84 SD (5-6 cm) with the treated group achieving an adult stature of -1.51 SDs and the control group -2.29 SDs. The adult stature of the rhGH-treated group exceeded their pretreatment predicted adult height by 0.54-0.65 SDs (+3.6-4.6 cm). In the *uncontrolled* studies, the adult stature of the rhGH-treated group exceeded their pretreatment predicted adult height by 0.56-0.63 SDs (+3.8-4.5 cm). The authors concluded that administration of rhGH can modestly increase the adult stature of children with idiopathic short stature. They estimated the cost of treatment to be approximately \$14,170/cm (\$35,000/in). The authors discuss the limitations of this meta-analysis (such as the heterogeneity of the populations treated; absence of data on those children who did not complete the course of treatment with rhGH) and point out that there are no data demonstrating any beneficial effect of treatment on psychological well-being, educational achievement, or vocational advancement.

Finkelstein BS, et al. 2002 Arch *Pediatr Adolesc Med* 156:230-240.

**Editor's Comment:** The authors are to be complimented on the completion of an arduous task. Of concern to this reviewer is the inclusion criterion for short stature of height below the 10th percentile. This reviewer cannot imagine that there are any pediatric endocrinologists who prescribe rhGH for otherwise normal children with heights between the 3rd-10th percentiles. One would very much like to see the data reanalyzed to include only children with heights below the 3rd percentile (or -2 SD) at the initiation of therapy. Among the questions

that would be of interest to answer are: 1) Was the growth promoting effect of rhGH more apparent in those with the shortest stature? 2) Did the children with familial (intrinsic/genetic) short stature respond more/less favorably than did those with non-familial short stature? 3) Did pre-treatment skeletal maturation influence the linear growth response to rhGH?

In addition to the data analyzed by Finkelstein et al, two additional reports of the effect of rhGH on adult stature in children with idiopathic short stature have been published. Lopez-Siguero et al<sup>1</sup> observed a mean gain in adult height of 4.5 cm in 30 boys treated with rhGH compared to an historical control group of 42 lads. Wit and Rekiers-Mombarg<sup>2</sup> reported that treatment with rhGH (0.17-0.32 mg/kg/week for approximately 7 years) resulted in a gain in adult height SD score of 1.3 versus baseline height in 53 patients with idiopathic short stature (12 born small for gestational age) as compared to a gain of 0.7 SD in an historical control group of 64 subjects. There was an increment of 4 cm in adult height over pretreatment predicted adult height in those children receiving rhGH (+0.8 cm in controls). In children who received the highest dose of rhGH (0.32 mg/kg/week) throughout the study, the increment in adult stature over pretreatment predicted adult height was 7 cm. These authors concluded that higher doses of rhGH led to greater increments in gain in adult height. However, they also concluded that in the absence of proven benefit of greater stature on well-being, the ethical controversy about the administration of rhGH to healthy children, and the high cost of rhGH treatment, "rhGH treatment for (idiopathic short stature) cannot be advised in general." This reviewer would agree with this conclusion.

Allen W. Root, MD

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## Centers for Disease Control and Prevention 2000 Growth Charts for the US: Improvements to the 1977 National Center for Health Statistics Version

The childhood growth charts used by most centers in North America are the charts produced by the National Center for Health Statistics (NCHS) in 1977. There are a number of problems with those charts that have been overcome in the newly produced charts from CDC. Specifically, the 1977 charts did not fully represent a cross-section of children living in the U.S. They were also deficient in including breast-fed infants. They did not make the transition well, using the recumbent lengths

on the infant charts and standing heights on the childrens-adolescents growth charts, and only heights up to 18 years of age were utilized. The new charts follow adolescents up to 20 years of age. The new charts also allow both percentiles and z-scores to be determined and provide body mass index for age charts and smooth the percentile curves.

The national data collection in a series of five surveys between 1963 and 1977 were used to develop the 2000



CDC charts. Other sources of data were also included. There were two important exclusions. Very low birth rate infants were excluded from the infant growth charts and, secondly, all infants excluded from the NHANES III study were also excluded.

The growth charts are not presented here as they are available on the internet (<http://www.cdc.gov/growthcharts>). They should be very helpful for all physicians and nurses caring for children.

Ogden CL, et al. *Pediatrics* 2002;109:45-60.

**Editor's Comment:** We certainly agree that the new growth charts are an improvement over previous charts available for monitoring growth in children in the United States. The editorial on childhood growth charts written in the same journal as an accompaniment to the publication of the growth charts should be carefully read. Careful measurements of children for both height and weight, and the plotting of the data on an appropriate growth chart **MUST BE** a routine in all pediatric practices.

Fima Lifshitz, MD  
Judith G. Hall, OC, MD

## Reduction in the Incidence of Type II Diabetes with Lifestyle Intervention or Metformin

The Diabetes Prevention Research Group, a consortium of 27 clinical centers, conducted a randomized clinical trial involving adults in the U.S. who were at high risk for the development of T2DM. The study was designed to answer three questions: (1) does a lifestyle intervention or treatment with Metformin delay or prevent the onset of diabetes; (2) do the two interventions differ in effectiveness; and (3) does the effectiveness differ according to age, sex, race, or ethnic group. To answer these questions, 3,234 individuals were randomized to one of three treatment groups: (1) standard lifestyle recommendation plus metformin, (850 mg twice daily); (2) standard lifestyle recommendation plus placebo twice daily; or (3) an intensive program of lifestyle modification.

The standard lifestyle recommendation included written information and an annual individual session of 20-30 minutes emphasizing the importance of a healthy lifestyle. The participants in growth 1 and 2 were told to reduce their weight, to increase their physical activity, to follow the Food Pyramid Guide, and to follow a diet the equivalent of a National Cholesterol Diabetes Education Program Step 1. The participants in group 3, the intensive lifestyle intervention group, were to achieve and maintain a weight reduction of at least 7% by following a low fat diet and by performing moderate physical activity such as brisk walking for at least 150 minutes per week. In addition, these subjects participated in a 16-week curriculum promoting dietary education, exercise, and behavior modification.

The primary outcome variable was diabetes as diagnosed by an annual oral glucose tolerance test or a semi-annual fasting plasma glucose test. The blinded treatment phase was terminated one year early, because by that time there was evidence of efficacy on the basis of 65% of the planned person-years of observation.

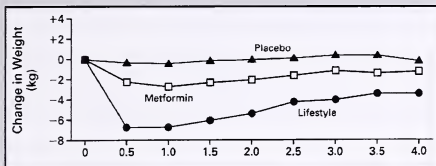
Approximately two-thirds of the subjects in the study were female, 54% were Caucasian, 20% African-

American, 16% Hispanic, 5% American-Indian, and 4% Asian. Seventy percent had a positive family history of diabetes. The mean age for the entire group was 50.6,  $\pm 10.7$  years, the mean weight 94.2  $\pm 20.3$  kg; the mean BMI 34  $\pm 6.7$ , the mean plasma glucose 106.5  $\pm 8.3$  mg/dl, and the mean glycated hemoglobin was 5.9%. The mean baseline data were similar in the 3 groups.

In the lifestyle intervention group, 50% achieved the goal of a 7% weight loss by the end of the first 24 weeks and 38% had maintained that weight loss at the last visit. Seventy-five percent participated in 150 minutes of physical activity per week at the end of 24 weeks and 58% maintained that level. Daily caloric intake decreased by a mean of 450 kcal in the lifestyle intervention group, 249 kcal in the placebo group, and 296 kcal in the metformin group. The average fat intake (34.1% of total at baseline) decreased by 6.6  $\pm 0.2\%$  in the lifestyle intervention group and by 0.8  $\pm 0.2\%$  in the placebo and metformin groups. Participants in the lifestyle intervention group had a much greater weight loss and greater increase in physical activity, than did the subjects in the other groups. The average weight loss was 5.6 kg in the lifestyle intervention group, and 2.1 kg and 0.1 kg in group 2 and 1. (Figure 1)

The incidence of diabetes was 4.8, 7.8, and 11.0 cases/hundred patient years for groups 3, 2, and 1 respectively. The incidence of diabetes was 58% lower in the lifestyle intervention group (group 3) than in the placebo group (group 2) and 31% lower in the metformin group than in the placebo group. (Figure 2) These results were statistically significant and the estimated cumulative incidence of diabetes at 3 years was 28.9%, 21.7%, and 14.4% in groups one, two, and three, respectively. Unfortunately, the study had inadequate power to access the significance of the effects within ethnic groups, but effects did not differ significantly according to sex, race, or ethnic group.

Figure 1



Changes in body weight according to study group. Each data point represents the mean value for all participants examined at that time. The number of participants decreased over time because of the variable length of time that persons were in the study. For example, data on weight were available for 3085 persons at 0.5 year, 3064 at 1 year, 2887 at 2 years, and 1510 at 3 years. Changes in weight and leisure physical activity over time differed significantly among the treatment groups ( $P < 0.001$  for each comparison).

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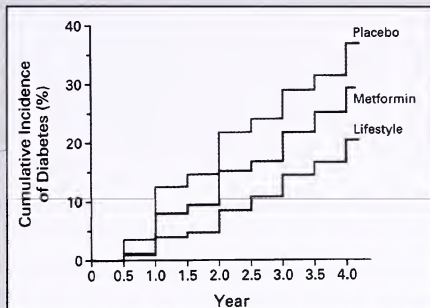
The authors state the hypothesis that Type II diabetes can be prevented or delayed in persons at high risk for diabetes was proven, and the effects were similar in men and women and in all racial and ethnic groups, regardless of age. The authors point out that their results show a risk reduction associated with lifestyle intervention that is similar to a previous test study conducted in Finland. The current study however, was not designed to test the relative contribution of dietary changes, increase in physical activity and/or weight loss. This is the first study, however, to demonstrate the efficacy of drug therapy in reducing the risk of developing Type II diabetes in high risk individuals.

Diabetes Prevention Group *N Engl J Med* 2002;346:393-403.

**Editor's Comment:** This is an exceedingly important publication, as was another significant paper published last year in the *New England Journal of Medicine* on the prevention of Type II diabetes mellitus by making alterations in lifestyle among subjects with impaired glucose tolerance (*N Engl J Med* 2001;344:1343-1350). The current study conducted in an older group of subjects has similar implications for children at high risk of developing Type II diabetes. In addition, the current study suggests that metformin, at a relatively modest dose (850 mg bid), can reduce the risk by 31%.

Most pediatric endocrinologists are faced with increasing numbers of overweight children coming to

Figure 2



Cumulative incidence of diabetes according to study group. The diagnosis of diabetes was based on the criteria of the American Diabetes Association. The incidence of diabetes differed significantly among the three groups ( $P < 0.001$  for each comparison).

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their clinics for evaluation. Many of these children are at very high risk for the development of Type II diabetes. The clinical armamentarium remains limited. Clearly, studies are needed to confirm the effectiveness of metformin in preventing the onset of Type II diabetes in the pediatric age group. However, previous experiences amongst pharmaceutical companies attempting to recruit and retain children with Type II diabetes for clinical trials suggest that this will be a very difficult task. Such a clinical trial may require nearly as much effort as the clinical treatment of Type II diabetes. Although, most physicians would recommend a change in lifestyle modification for overweight children, the execution of changes in dietary intake and physical activity within the context of a family with varying degrees of motivation remains extremely difficult.

William L. Clarke, MD

**Second Editor's Comment:** This editor must conclude that we may succeed in changing the lifestyle of some obese adults but only in a few obese children, but we should keep trying. With children and adolescents, gentle persuasion will be more effective than parental demand.

Robert M. Blizzard, MD

## Hypospadias and Early Gestation Growth Restriction in Infants

Reports from Europe and the United States have indicated that there is an increasing incidence of hypospadias. This study by Hussain et al involved two tertiary care neonatal intensive care units in Connecticut. It was a retrospective study of 14 years of admissions. It showed a 10-fold increase in hypospadias over the 14 years, from 0.4% of admissions in 1987 to 4% in the year 2000. The increased occurrence of hypospadias among premature infants was associated with intrauterine growth retardation. An increased frequency of hypospadias was also noted among the infants born in the lower percentiles (3<sup>rd</sup> to 25<sup>th</sup>).

An association of hypospadias with the smaller quartiles of head circumference (3<sup>rd</sup> to 25<sup>th</sup>) was also present. The frequency was highest in first-born infants and those born to older mothers. No association was noted with race, maternal diabetes, hypertension, or pre-eclampsia. No specific teratogens were identified. There

does not seem to be an increase of a particular recognizable syndrome in spite of the association with intrauterine growth restriction. The consistent involvement of all growth parameters, i.e., weight, length, and head circumference suggested that hypospadias is related to overall poor intrauterine growth.

Hussain N et al. *Pediatrics* 2002;109:473-478.

**Editor's Comment:** *A specific etiology for the observed increase in hypospadias does not seem to be forthcoming. These are obviously real concerns with such a striking change over the last decade. The question of endocrine disruptors and the association of advancing maternal age are important, but no real clarity exists as to their real role at this time.*

Judith G. Hall, OC, MD

## Growth, Developmental Milestones, and Health Problems in the First Two Years in Very Preterm Infants Compared with Term Infants: A Population Based Study

Bucher et al report the results of a questionnaire sent to parents of Swiss infants born before 32 weeks of completed gestation. The parents were asked to answer questions concerning weight, body length, head circumference at 24 months of age, developmental milestones, eye and ear problems, long-term medications, fever, cough, and infectious diseases during the last 12 months. Information regarding developmental milestones is recorded in the Swiss Health Card given to each parent of a newborn infant. A comparison group for this study included two control infants for each index infant. The second was contacted if the first did not respond. Infants of multiple births or with severe malformations or syndromes were excluded. The control infants had to have been born in the same hospital, at term (after 37 weeks), and within 14 days of the expected date of birth of the index infant, and of the same gender as the index infant.

Three hundred nine infants born between January 1, 1996 and December 31, 1996 were included. Index infants had significantly lower body weight, body length, and smaller head circumference at 24 months *corrected* age as compared to their matched control. The mean weight difference at the age of 2 years (*corrected* for the very preterm infants) was 1.2 kg for boys, and 1.2 kg for girls. The mean difference in body length was 3.5 cm for girls and 3.3 cm for boys. Thirty-three percent of index infants were below the third percentile for length

at 24 months *corrected*. The difference in head circumference was small (0.7 cm), but statistically significant ( $p < 0.001$ ). Height and weight parameters were similar in the parents of pre-term and term infants, and in agreement with normal growth standards for adults. In the very preterm infants, there was significant motor delay, increase in eye problems and in use of long-term medications, but no difference in infectious diseases during the prior 12 months. Sitting was not delayed, but walking (mean of 14.5 months vs 13.5 months in controls ( $p=0.4$ ) and drinking out of a cup (50% of each group at 16.5 vs 13.5 months;  $p<0.001$ ) were delayed. Of the very preterm infants, 16% were unable to walk independent at 18 months *corrected* age. These infants are at increased risk for developing cerebral palsy. The authors state that such a retrospective study can include much bias, but that has been accounted for by utilizing a significantly large control group. The cause of significant growth delay remains unclear. Suggested causes include: (1) decreased length of gestation; (2) insufficient supply of nutrients over prolonged periods of time after birth; or (3) intercurrent illnesses in the first year, such as chronic lung disease which may increase energy requirements and interfere with nutrient intake.

Bucher HU, et al. *Eur J Pediatr* 2002;161:151-156.



**Editor's Comment:** The authors recall several studies in which catch-up growth in pre-term infants has been stated to occur up until adolescence, and note that the patients in this study should be followed at least through school age. The data are intriguing however, for several other reasons. First, it is possible that these very young children (less than 30 weeks gestation) may respond with accelerated growth to recombinant growth hormone therapy in much the same way as do children with intrauterine growth retardation. Initiation of such therapy at a young age might significantly improve not only final

height, but developmental milestones as well. The discrepancy in head circumference in the very pre-term infant, although minimal, is nonetheless of considerable concern. Thus as the authors point out, it would be important to carefully record growth patterns, and developmental milestones over time in the attempt to define those children who might benefit most from earlier hormonal investigation and intervention. It would appear that the Swiss Minimal Neonatal Data Set is an excellent resource for the collection and analysis on such data.

William L. Clarke, MD

## Adult Height in Advanced Puberty with or without Gonadotropin Hormone Releasing Hormone Analog Treatment

The authors define "advanced puberty" as "the onset of puberty in girls between 8 and 10 years and in boys between 9 and 11 years." (Others might also use the term "early puberty" for such subjects.) In a retrospective assessment of the effect of a gonadotropin releasing hormone agonist (GnRHa - D-Trp<sup>6</sup>-GnRH) upon adult stature in children with "advanced puberty," the authors administered GnRHa for 2-2.4 years to 9 adolescent girls with serum estradiol concentrations in excess of 20 pg/mL, and 8 pubertal boys with testosterone values greater than 100 ng/dL who had a pubertal gonadotropin secretory response to GnRH. Mean adult height of treated subjects was compared to that of a control group of untreated subjects. In treated girls, mean adult stature (155.3 cm) was insignificantly different from pretreatment predicted height (151.9 cm). In control females (N=31), mean adult and predicted heights were also similar (157 cm and 156.7 cm, respectively). In both groups, adult heights were close to their target heights. In treated boys, mean adult height (164.1 cm) was less than mean predicted height (173.2 cm) and mean target height (170.4 cm). In untreated boys (N=9), adult height, predicted, and target heights were similar (169.1, 170.8, and 170.2 cm, respectively). The authors concluded: "These data suggest that advanced puberty decreases the growth potential by about 5 cm, and that GnRHa treatment does not prevent this."

Couto-Silva AC, et al. *J Pediatr Endocrinol Metab* 2002;15:297-305.

**Editor's Comment:** Luckily, GnRHa did not increase adult stature in girls with "advanced puberty" and may even have led to decreased stature in boys. While under specific and individual circumstances (such as major behavioral problems, disabling physical handicaps, or significant developmental delay), one might consider interruption of pubertal development in subjects of normal adolescent age, to do so for the purpose of achieving a greater adult stature is an unjustified use of agents such as GnRHa. Similarly, the use of recombinant human growth hormone (rhGH) to increase to a minimal extent adult stature in normal but short children is unjustified medically, psychosocially, or financially. Unfortunately, we may shortly expect to read a manuscript in which both GnRHa and rhGH have been administered to children with "advanced puberty."<sup>2,3</sup> At what point did the pediatric endocrinologist cease being a physician-scientist and become a physician-cosmetologist?

Allen W. Root, MD

### References

1. Finkelstein BS, et al. *Arch Pediatr Adolesc Med* 2002;156:230-240.
2. Kamp GA, et al. *J Clin Endocrinol Metab* 2001;86:2969-2975.
3. Kaplowitz PB. *J Clin Endocrinol Metab* 2001;86:2965-2968.

## GH Anabolic Effects of GC-Dependent Children with IBD

This pilot study utilizing 6 boys and 4 girls was designed to determine whether rhGH could overcome some of the catabolic effects of chronic glucocorticoid (CG) treatment (24 months) of IBD. Subcutaneous rhGH (0.05 mg/kg/d) was given for a minimum of 6 months. Seven patients continued for 12 months. Body composition

changed favorably with increased fat free mass and decreased fat mass. Linear growth velocity increased from  $3.5 \pm 0.4$  cm/yr pre-rhGH to  $7.7 \pm 0.9$  cm/yr after 6 months. The GV persisted for the next 6 months in all 7 treated. Bone calcium accretion increased as did alkaline phosphate specific for bone [(a measure of bone



formation)  $p = .03$ ). Fasting and 2 hour post prandial glucose levels, fasting insulin levels, and HbA1C remained in the normal range. The authors concluded that treatment with rhGH at the doses used has beneficial effects in prednisone-dependent growing children, on body composition without detrimental effects in carbohydrate metabolism or the intermediate metabolism of substrates. Larger studies will be needed to assess long term safety and efficacy.

Mauras N, et al. *Metabolism* 2002;51:127-135.

**Editor's Comment:** This well designed study provides encouraging data that rhGH can overcome the anti-anabolic effects of prednisone, enhance the growth rate, and do so without measurable toxicity over 6-12 months. Of particular interest was the disappointing observation that there was no change in the disease activity as determined by the Crohn's Disease Activity Scale adapted for pediatric subjects. There were significant increases in serum levels of IGF-1 and IGF-BP3. The authors suggest that a state of "functional" GH deficiency caused by chronic steroids may be overcome with rhGH administration. It is important to remember that rhGH has not been effective in treating patients with IBD who are not on glucocorticoid treatment. Also of importance is to recall the reports of Rivkees et al and Allen et al who reported the acceleration of growth in glucocorticoid treated children with significant growth retardation who

were treated with rhGH. Allen et al reviewed the data of the Genentech National Growth Study in which 83 children with extreme glucocorticoid induced short stature were treated for at least 12 months with rhGH. The authors concluded: (1) growth suppressing effects of chronic GC are counter-balanced by GH therapy; the mean response being a doubling of baseline growth rate, (2) responsiveness to GH is negatively correlated with GC doses, and (3) glycosylated hemoglobin levels increased slightly, but glucose and insulin levels were not altered by GH therapy. These authors summarized: "In a cohort of 83 poorly growing GC-dependent children, we suggest that the growth suppressing effects of GC can be variably overcome by GH. The short term risks of combined GH and GC treatment appear low; potential long term effects require further surveillance and study. Treatment of GC-dependent children with GH remains experimental; children considered for such treatment should be enrolled in studies that facilitate careful monitoring and data analysis." Dr. Mauras and her co-investigators have heeded the suggestion and extended the data. Rivkees et al, Allen et al, and Mauras et al are to be commended for clinical investigation that significantly enhances patient care.

Rivkees SA, et al. *J Pediatr* 1994;125:322-325.

Allen DB, et al. *J Clin Endocrinol Metab* 1998;83:2824-29.

Robert M. Blizzard, MD

## Inadequate Leptin Level Negatively Affects Body Fat Loss During a Weight Reduction Program for Childhood Obesity

These authors report findings of body fat loss in 37 female and 45 male overweight children, ages  $10.9 \pm 3.5$  years, during a weight reduction program and correlated the weight loss with plasma leptin levels. The authors note that a large proportion (40-80%) of the variance in BMI can be ascribed to genetic factors; leptin appears to signal adiposity and leptin levels have not been shown to be predictive of successful weight loss. Leptin levels, although found to correlate positively with indices of general obesity, have not been found to be predictive for the success of weight loss in observational, longitudinal studies of dietary intervention. Some studies have shown that low serum leptin at baseline is associated with greater weight loss. Others have shown, in adolescents, that a greater baseline of leptin concentration correlates with weight reduction.

In the current study, fasting plasma leptin levels were determined and subjects were stratified on their leptin Z-score into low leptin ( $< -2$  SD), high leptin ( $\geq +2$  SD), or appropriate leptin ( $\geq -2$  to  $\leq +2$  SD), prior to their weight loss. Body fat was determined by BMI and skin

fold thicknesses. All subjects participated in a nutritionally balanced meal plan at 60% of the recommended energy allowances for age and sex. Physical activity was monitored, but no attempt was made to alter it. There were no significant differences in physical activity amongst the 3 groups of children stratified by fasting plasma leptin levels. Data was collected at 3 and 6 months which showed that 20 children had high leptin levels, 20 had relatively low leptin levels, and 42 fell in the appropriate leptin level range. There were no statistical differences among the three groups of children at baseline. Mean BMI and skinfold thickness at the end of 6 months were significantly lower than baseline data. BMI reduction was more evident in the subjects with adequate leptin levels but the differences were not statistically significant. Reduction in triceps and subscapular skin folds was also more pronounced in the appropriate leptin production group. The differences in the average of these changes were statistically significant after both 3 and 6 months.

The authors suggest that children with relatively high or low leptin levels are less likely to lose body fat, as determined by skinfold thickness, during a 6 month hypocaloric diet, and that the ability to lose fat may be strictly dependent on genetic and environmental factors. Therefore, when environmental factors are altered, those with hyper or hypo-leptinaemia are less likely to respond to those changes.

Miraglia del Giudice E, et al. *Acta Paediatr* 2002;91:132-135.

**Editor's Comment:** This is an interesting and important manuscript even though some of the data do not reach statistical significance. Researchers have been unable

to show that fasting plasma leptin levels are indicators of the probable success or failure of weight-loss programs. Recent data suggest that, in adults, lifestyle changes including weight loss, and increased physical activity can significantly reduce the risk of Type II Diabetes in high-risk adults. The information in groups of patients who might be more amenable to weight loss programs is therefore very important. Further studies are required in order to better understand the etiology of the differences in leptin levels in the 3 groups of children studied by del Giudice. Confirmation of these data would be of great importance.

William L. Clarke, MD

## Preterm Infants Born at Less Than 31 Weeks Gestation have Improved Growth in Cycled Light Compared with Continuous Near Darkness

The neonatal intensive care unit environment cannot possibly replicate the womb for all preterm infants. The purpose of this study was to evaluate the effects of cycled light versus near darkness on health and growth of preterm infants. The study was set up as a randomized interventional study comparing infants receiving cycled light from birth, cycled light at 32 weeks post-conceptual age, and cycled light at 36 weeks post-conceptual age. Infants receiving cycled light at birth and at 32 weeks post-conceptual age gained weight faster than infants not receiving cycled light until 36 weeks (Fig 1). There was no difference among the groups in length of hospitalization stay, or number of ventilator days, but the power was low for these variables. The authors concluded that cycled light had significant weight gain benefits over near darkness in preterm infants.

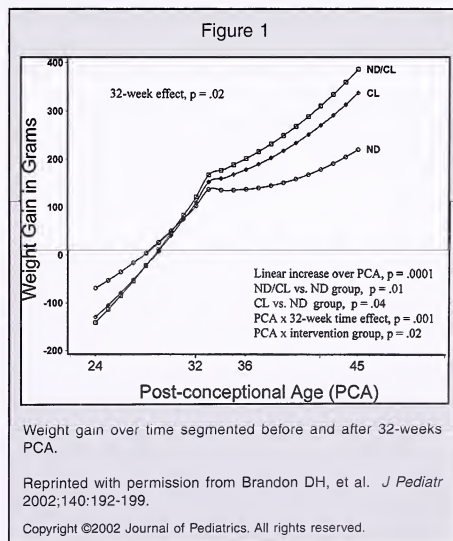
Brandon DH, et al. *J Pediatr* 2002;140:192-199.

**Editor's Comment:** The findings of this study confirm the observations of others who reported that cycled light from birth or beginning at 32 weeks post-conception positively influenced weight gain in preterm infants. The positive effects of weight gain in preterm infants were first reported by Mann et al *BMJ* 1986;293:1265-7. However, there have been other reports that suggested that continued bright light is detrimental to the health of preterm infants (*J Perinat Neonat Nurs* 1991;4:47-54 and *Infant Behav Dev* 1995;18:87-95). Since near-darkness has become the standard of care in nurseries, these findings are important. The presence of significant circadian rhythms provided by maternal cycles even while the fetus is in the intrauterine environment suggest that replicating them after birth may be of benefit. *Growth, Genetics and Hormones* published an excellent

review of circadian rhythms written by Dr. Rivkees in Vol 18, No.1, 2002.

Cycled light could be important for human development, in addition to the demonstrated benefits in growth. The effects on weight gain, though significant, might only be one part of the benefit of cycled stimulation mimicking intrauterine life for the preterm infant. Potentially, cycled light may also have a major impact on retinal development and other functions.

Fima Lifshitz, MD



# Letter to the Editor

Dr. Blizzard & Members of the Editorial Board:

I am writing to you because I continue to be disturbed by the fact that many pediatric endocrinologists, including several leaders in the field, continue to ignore published papers casting serious doubts on the validity of the somatomedin hypothesis. The more recent publications of Salmon (whose experiments with Dr. Daughaday half a century ago led to the origins of the hypothesis) have essentially refuted the findings of those original publications, but many pediatric endocrinologists seem to have decided that they do not exist.

I enclose a brief article that I recently wrote summarizing the evidence against the hypothesis: the recent experiments of Salmon and Burkhalter, the experiments done by Derek LeRoith's group at the NIH showing that deletion of the hepatic gene for IGF-I did not impair growth in mice despite a 75% reduction in circulating concentrations of IGF-I; the demonstration that virtually all tissues have growth hormone receptors and do not depend on a circulating messenger to mediate its actions; and the fact that somatomedin is an insulin-like growth factor despite the fact that growth hormone is a counter-regulatory factor that opposes the actions of insulin.

Writings and oral presentations by prominent pediatric endocrinologists continue to cite as gospel the original Salmon and Daughaday papers as though they are unaware of the refutation of those experiments by Salmon and Burkhalter even though they have appeared in peer reviewed journals.

Perhaps *Growth, Genetics & Hormones*, one of the most respected pediatric endocrine publications, might be able to do something about calling the attention of those in the field who need to reexamine the validity of the hypothesis.

Solomon A. Kaplan, MD

## Letter from the Editor

The Editorial Board is pleased to respond to Dr. Kaplan's letter of March 27, 2002. His article in *The Endocrinologist* has been updated and is published in response to his letter to the Editorial Board. We believe Dr. Kaplan has succinctly summarized the current knowledge in respect to the inter-relationships between IGF-I, hGH, and growth. Thank you, Dr. Kaplan.

Robert M. Blizzard, MD  
Editor-in-Chief

See article on page 38, *Somatomedin Hypothesis: Time for Reexamination*.

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# GROWTH

## Genetics & Hormones

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### THE CURRENT FRONTIERS OF IN VITRO FERTILIZATION

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*Eastern Norfolk Medical School*

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*Baltimore, Maryland*

#### INTRODUCTION

In the early 1980s when *in vitro* fertilization (IVF) became a clinical reality it was considered therapy for diseased fallopian tubes. However, its effectiveness soon made it applicable to other causes of infertility, such as endometriosis unresponsive to other therapy, oligospermia with at least a million sperm identified in the ejaculate, and in other possible indications such as infertility of unidentified etiology, and infertility thought to be due to immunological factors.

Improvements in both clinical and laboratory technology at the turn of the millennium made IVF the treatment of choice for all forms of tubal disease (except perhaps iatrogenic sterilization), for endometriosis if infertility was the principal complaint, and for oligospermia regardless of the sperm count, and even for cases of azoospermia in which sperm could be obtained directly from the testis and intracytoplasmic sperm injection (ICSI) used for a single sperm to cause fertilization and pregnancy. It should be said up front, that it appears as if the majority of cases of oligozoospermia are due to genetic causes with the gene primarily carried on the Y chromosome. Therefore, with the use of ICSI, there is a greater transmission of genetic disorders to the next generation since the Y sperm fertilizes the egg. In spite of this, few patients reject this therapy. Occasionally, IVF therapy is used in infertility of undetermined origin and in less frequent conditions, such as the female whose mucous destroys sperm before they can ascend into the uterus.

While the above are the best possible therapeutic options, in current practice, many patients do not receive contemporary therapy. There are numerous reasons for this, but primary among them is that when IVF came into use, the health insurance industry declined coverage on the basis that it was "experimental therapy".

Although IVF is the best possible therapy for several causes of infertility, the insurers continue to deny coverage, resulting in the application of obsolescent therapy for countless patients. For example, diseased fallopian tubes which prevent pregnancy are often surgically repaired because it is covered by insurance. There is reason to believe that contemporary therapy, i.e. IVF, used when medically indicated would be less costly and less risky than the obsolescent therapy supported by the insurance carriers. While some states now have mandated insurance coverage, this is suboptimal because of the restrictions and fixed prices which are often built into the legislation. On a population basis, the United States is now far behind other countries in utilizing IVF. In a study by Collins,<sup>1</sup> it was shown that many other nations are far more frequent users of IVF than the US (Figure 1).

#### EXPECTATION OF PREGNANCY

The 1998 official IVF Registry Report published in January 2002<sup>2</sup> showed that in the US there were 58,937 cycles involving IVF with a delivery rate per retrieval of 29.1% or 17,150 deliveries. There were 5,273 fresh donor oocyte cycles with a delivery rate for transfer of 41.2% (2,179 deliveries) and 11,228 frozen embryo transfer procedures with a delivery rate per transfer of 19.3% (2,167 deliveries). These percentages are as expected, as fresh donor procedures unequivocally are more successful than frozen embryo procedures. The Registry data are more than three years out-of-date and

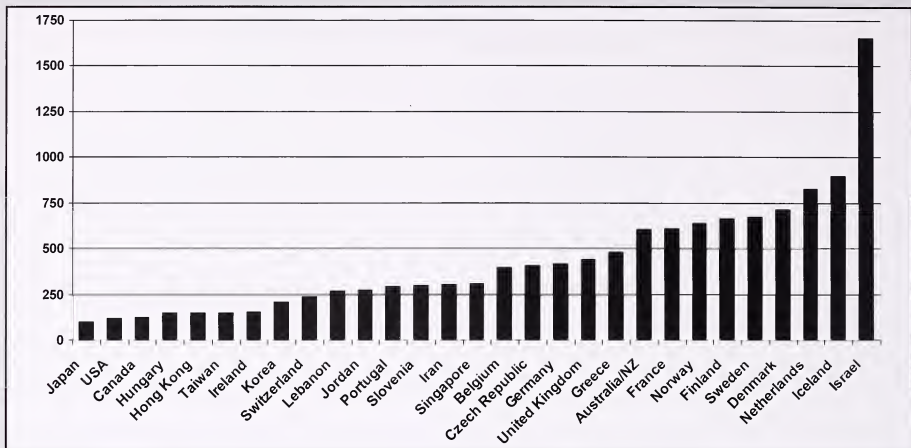
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Figure 1

IVF/ICSI Cycles per Million Population

Adapted from Collins J. Cost-effectiveness of in vitro fertilization. *Seminars in Reprod Med* 2001;279-289.

for a variety of reasons can indeed be misleading to the unwary reader as different assisted reproductive technology (ART) programs have different performance guidelines and different methods of pooling the data.

It has long been known that fecundity, i.e. the probability of pregnancy per month of exposure, declines with the age of the female partner. This age factor cannot be overcome by the use of IVF; thus, therapeutic results reported in the ASRM/SART Registry<sup>2</sup> show a marked age related effect (Table 1). The therapeutic significance is that patients must be further educated about the eroding effect of age on the reproductive process and pregnancy should be undertaken as early as possible.

Multiple pregnancies have been a troublesome problem with IVF. Since the initiation of IVF and of ovulation induction (which also started around 1980) the multiple pregnancy rate in the US as reported by the Bureau of Vital Statistics (Figure 2) has increased each year through 2000, the last date for which data are available. Although the triplet and higher rate decreased slightly in 1999 and 2000, the increase in the rate for twins more than made up for this decrease so that the overall multiple pregnancy rate has increased each year. Examination of the 1998 ASRM/SART Registry reveals that of all deliveries 61.8% were single births, 31.7% of the deliveries were twins, 6.2% were triplets, and 0.3% were quadruplets or more. This is unacceptable and is caused by pressure from both patients and programs alike. They wish to have a high pregnancy rate which

can be accomplished with multiple transfers, but at the expense of multiple pregnancies which are undesirable. The goal should be to have a reasonable pregnancy rate with no more than 1% triplets.

Taking all these considerations into account, in 2002 a female who is a good responder, i.e. one who produces at least 5-6 mature oocytes to the required gonadal stimulation, is not over 38 years old, has both ovaries, and has a sperm producing partner, should expect to have a pregnancy 50% of the time with fresh transfer with a risk of less than 1% of having triplets and less than 4% of having twins.

## CRYOPRESERVATION

No program in IVF can be considered "full service" unless it offers cryopreservation which can hold frozen excess preembryos for future use. Indeed, in expressing the pregnancy rate for a particular IVF program, a misleading figure is given, unless the pregnancy potential from the frozen material is included. We have published<sup>3</sup> a theoretical model in which a true expression of pregnancy rate resulting from stimulated cycles can be calculated. The interested reader is referred to this publication for full details. Briefly, it is quite impossible to properly evaluate the pregnancy outcome of a particular stimulation cycle unless supplementary pregnancies, if any, from cryopreservation are considered as part of the pregnancy rate of that particular stimulation cycle. This can be done by adding

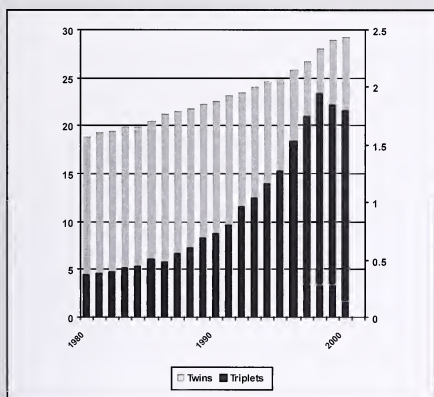
Table 1  
IVF procedures (with and without ICSI) by age group and cause of infertility.

| 1998 IVF procedures               | No. of retrievals | Canceled cycles (%) | Transfers Per retrieval (%) | No. of pregnancies | No. of deliveries | Deliveries Per retrieval (%) | Multiple Births per Delivery (%) |
|-----------------------------------|-------------------|---------------------|-----------------------------|--------------------|-------------------|------------------------------|----------------------------------|
| <b>No male factor infertility</b> |                   |                     |                             |                    |                   |                              |                                  |
| Women <35 years of age            | 16,648            | 10.0                | 93.4                        | 6,878              | 5,948             | 35.7                         | 43.4                             |
| Women 35-37 years of age          | 8,524             | 14.7                | 94.2                        | 3,109              | 2,543             | 29.8                         | 37.9                             |
| Women 38-40 years of age          | 7,063             | 19.5                | 92.7                        | 2,006              | 1,498             | 21.2                         | 29.0                             |
| Women >40 years of age            | 4,348             | 24.6                | 89.9                        | 721                | 446               | 10.3                         | 20.2                             |
| <b>Male factor infertility</b>    |                   |                     |                             |                    |                   |                              |                                  |
| Women <35 years of age            | 7,546             | 7.7                 | 94.7                        | 3,042              | 2,647             | 35.1                         | 40.3                             |
| Women 35-37 years of age          | 3,147             | 11.6                | 94.8                        | 1,206              | 1,000             | 31.8                         | 35.5                             |
| Women 38-40 years of age          | 2,366             | 14.6                | 92.9                        | 750                | 563               | 23.8                         | 31.8                             |
| Women >40 years of age            | 1,129             | 19.1                | 91.9                        | 231                | 144               | 12.8                         | 13.9                             |
| 1998 totals                       | 50,771            | 13.9                | 93.6                        | 17,943             | 14,789            | 29.1                         | 38.2                             |
| 1997 totals                       | 44,170            | 14.0                | 93.4                        | 15,047             | 12,302            | 27.9                         | 39.0                             |

SART/ASRM. ASRM/SART registry: 1998 results. Fertil Steril 2002.

Figure 2

Multiple Pregnancy Rate with IVF and Ovulation Induction



The rating of twins and triplets and more from the Bureau of Vital Statistics, U.S. Public Health Service.

all cryopregnancies to fresh pregnancies, or can be patient specific (ie., considering cryopreservation as augmentation only among patients without a pregnancy from pre-embryos transferred fresh, or from previously transferred frozen material from the same harvest). For the patient-specific concept, cryopregnancies occurring among patients with a previous fresh or frozen pregnancy from the same harvest would be considered additive to the multiple pregnancy rate, i.e. twins, etc., but would be considered as 'delayed' multiple

pregnancies. Published results have not reflected the real purpose of cryopreservation; this is shown by the methods of presentation of cryopreservation in the publications of collecting agencies, such as the US Society for Assisted Reproductive Technology, the Great Britain Human Fertilization and Embryology Authority, the Australia-New Zealand Agency, and others. In general these publications report cryopreservation results as unrelated to a particular oocyte harvest or treat a cryopreservation as an additional transfer from the same cohort of prezygotes/pre-embryos, thus diluting the fresh pregnancy rate, as cryoresults are often not as good as fresh results.<sup>3</sup>

Generally speaking, expectation of a pregnancy from cryopreserved material is not as great as from fresh. Although the data are not exactly comparable, the ASRM/SART Registry for 1998 gave an overall pregnancy rate per transfer for fresh oocytes in IVF of 37.8% and 24.3% for cryopreserved material. With careful selection of fertilized eggs prior to cryopreservation, the pregnancy expectation from cryopreserved material approaches that of fresh material.

## PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD has been available since about 1990.<sup>4</sup> By this technique, one or two blastomeres are removed from the preembryos of the 6-10 cell stage and examined for single gene defects by the polymer chain reaction (PCR) or by fluorescent in situ hybridization (FISH) for gross chromosomal defects. Preembryos with defects are discarded and those found to be normal are transferred or frozen for future transfer.

**Table 2**  
PGD referrals (n) according to indication

|                       |     |
|-----------------------|-----|
| Chromosomal           | 647 |
| X-linked              | 294 |
| Autosomal recessive   | 290 |
| Autosomal dominant    | 254 |
| Mitochondrial         | 6   |
| Two indications       | 9   |
| Y-chromosome deletion | 2   |
| Social sexing         | 30  |
| Unknown               | 29  |

ESHRE PGD Consortium Steering Committee (May 2001) Hum Reprod 17:235, 2002.

Diagnostic ability with PGD is precisely that of amniocentesis which is done at 15-18 weeks of pregnancy or chorionic villus sampling which is done at 10-14 weeks of pregnancy. PGD appeals to those who cannot morally terminate an affected fetus but who do not feel morally bound to implanting an in vitro affected preembryo. It also appeals to those who are prepared to undergo the requirements and expense of PGD and IVF simply to avoid the possibility of an elected termination, even though they may have no moral conflict in aborting an affected fetus.

The opportunity to use PGD is not offered by all centers, but its use is gradually increasing. According to data collected by the ESHRE,<sup>6</sup> in 2001 there were 1,561 PGD procedures reported. The most common cause for referral was concern about chromosomal abnormalities. Specific gene disorders accounted for slightly over one-third of the cases (Table 2). Cystic fibrosis was the most common monogenic disorder.

PGD is not without an occasional error, and its efficiency in relation to fertility factors is somewhat less than IVF because of the limited number of preembryos that can be selected for transfer resulting from the screening out of affected fertilized eggs.

## DONOR GAMETES

Donor *sperm* have long been used when infertility was due to sperm deficiencies. Currently, the use of donor *sperm* and *oocytes* can be considered standard practice for those who are prepared to accept nonfamilial genetic material. In some circumstances, donor gametes are used to replace gametes which are likely to or are known to harbor a mutant disease-causing gene. This is particularly valuable when the affected gene is not amenable to preimplantation genetic diagnosis.

When donor *sperm* are used either with or without IVF, the donors are vigorously screened. Requirements differ from center to center. At the Jones Institute the donors

must be 18 to 39 years of age, have a semen volume of 2 mL with a sperm count of at least 60 million, with sperm motility greater than 60%, and at least 7% of the sperm must be of normal form by strict criteria. There can be no excess of WBCs. More than 50% of the sperm must survive the cryo-survival test. The family history of the donor must be free of genetic disease. A physical examination must reveal no urethral discharge or genital warts or ulcers. Laboratory screening includes a serological test for syphilis, cytomegalovirus, hepatitis B and C, HIV-1 and HIV-2, and T-cell lymphotropic virus I and II. Serum tests must be negative for herpes, chlamydia and gonorrhea, and donors must pass a urine test for drug screening. In addition, donors must be free of cystic fibrosis and, if Jewish, tested for Hexosaminidase-A which causes Tay-Sachs disease. Black donors must be free of the sickle-cell trait. Potential Asian or Mediterranean donors with a positive hemoglobin electrophoresis for thalassemia are eliminated.

Semen quarantine is usually carried out for 6 months at which time the donor is checked for HIV and other possible potential problems before semen is released for use. All this is in accordance with the recommendations of the American Society for Reproductive Medicine (ASRM). Clinical pregnancy rates with donor *sperm*, with or without IVF, are consistent with a normal fecundity rate if there is no impediment to pregnancy on the part of the female.

When donor *eggs* are supplied, the donor has a similar historical review for genetic problems, as well as laboratory studies. However, it is impractical to quarantine an *egg* for six months, as the *eggs* do not freeze nearly as well as the *sperm*. Therefore *egg* quarantine is essentially never done. HIV testing in the *egg* donor is done by the antigen test rather than the antibody test, as a prompt answer can be obtained, although there is some uncertainty as to the time required for the appearance of the antigen. Clinical pregnancy rates for donor *eggs* in IVF are a cut above that obtained by IVF in general - due to the younger age of the donor. The pregnancy rate with donor *eggs* is consistent with the age of the donor and unrelated to the age of the recipient. There is great uncertainty about an upper age limit for the use of donor *eggs*.

ASRM has issued a guideline indicating that donor *eggs* should not be used in a recipient at an age above a woman's normal reproductive life. This guideline probably has been left purposely vague. The guidelines must have been violated as there are accounts of recipient mothers 60 years of age and over. Each program must adopt its own standard in regard to age limit. Some variations in the standard donor *egg* scenario have occurred. For example, there have been



menopausal grandmothers who were prepared to receive an anonymous donor egg for their daughter - such an egg, of course, fertilized by the daughter's husband. There are no guidelines for these offbeat situations, thus each program must handle them on an individual basis. Calling for assistance might be appropriate, such as the utilization of sociologists, and/or an ethics committee, or other outside resources to establish guidelines and share responsibility for these decisions.

Suffice it to say, when donor eggs are used, and especially if the recipient's age is 40 or above, a preconception medical evaluation is in order. Such an evaluation would look for those conditions which might cause complications during pregnancy or those which might be aggravated by pregnancy, such as obesity, hypertension, and diabetes. Only those women who are totally medically fit should be considered as recipients.

An upper age limit for a prospective father is sometimes an issue *with or without* donor sperm. This seems to arise when a prospective father is 60 or above and marries a much younger wife. One must ask, "Does the program have a responsibility in this circumstance to consider the welfare of the child; specifically, is there any reason to be concerned about how a man of 60, 70 or 80 years of age can function responsibly, mentally and physically, with teenage children?" A program probably has no responsibility here, but the issue is thought provoking.

## CONCLUSION AND A FINAL WORD

Prior to IVF it was common for physicians who treated infertility patients to tell them that everything had been tried, and it was now time to consider adoption or a childless future. Basic IVF technology changed much of that, as did the addition of donor gametes for those prepared to accept alien genetic material; the physician is now able to offer an option to essentially all couples. The era of IVF also has made it possible to go beyond

the mere solution of the problem of infertility. Preimplantation genetic diagnosis now makes it possible to eliminate disease-causing mutant genes. Thus, we are beginning to diminish the number of children born with handicaps. Such children previously were thought to represent an intrinsic risk of bearing children.

If the era of IVF has written a new chapter in the treatment of infertility, are there additional chapters to be written? To be sure! The aging oocytes represent a challenge. Can they be rejuvenated? I think it will be possible. IVF is inefficient, but changing this represents a problem. With eight fertilized two-cell zygotes in the dish, experience tells us that on average only two or three of these have the potential to progress to a term fetus. We are far from perfect in identifying which ones are the two or three. Can our selection potential be improved? I think it *will* be possible. Cryopreservation is very efficient for *sperm* but very inefficient for the *egg* due to its size. Can cryopreservation of the egg be achieved? I think it will be possible.

These are only examples. There are several other possibilities - some of which may be considered by some in the realm of science fiction, but all aimed at improving the human condition. Reproductive medicine and its developing technology have placed us in the midst of a reproductive revolution.

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## Abstracts from the Literature

### Genetic Screening for Maternal Uniparental Disomy of Chromosome 7 in Prenatal and Postnatal Growth Retardation of Unknown Cause

This very enlightening paper from Finland is worth reading by all pediatric subspecialists for its wealth of information. The authors first relate that uniparental disomy (UPD) associated with growth retardation has been found in at least 9 chromosomes (2,6,7,9,14,16,17,20 & 22) and concluded that UPD thus may provide explanations for some cases of growth retardation of unknown cause. Inheritance of *both*

parental genomes is essential for normal growth and development.

In their study, these authors focused on UPD of chromosome 7 and particularly on maternal or matUPD7. The study was prompted as matUPD7 has been reported in approximately 10% of patients with Russell Silver syndrome (RSS) and in a few patients with intrauterine growth retardation (IUGR) without RSS.



Basically 2 groups of patients were studied: (1) 39 patients with unequivocal RSS and, (2) 166 patients with unexplained growth retardation but who did not have RSS. The latter group was divided into 2 subgroups: (2a) those with IUGR and postnatal growth retardation (PNGR) and, (2b) those with only PNGR. For final analysis, the RSS patients were separated into 2 subgroups also: (1a) RSS with matUPD7, and (1b) those without mat-7-UPD.

Only 6 of the 205 patients studied had matUPD7 and all had RSS. Thirty-three of the 39 in the RSS group did not have UPD. Comparison of these two groups revealed that RSS infants (with or without matUPD7) were significantly shorter at birth than infants in group 2a and 2b. The birth weights and lengths of RSS patients with or without matUPD7 were equally small. However, birth weights did not differ between groups 1a, 1b, and 2a. Notable difference of parental age at birth was observed between group 1a and the other 3 groups. MatUPD7 patients had significantly higher ( $p < .05$ ) maternal age (38 years) and paternal age (40 years) than those in the other 3 groups.

Midparental heights were near average for all groups. Maternal obstetrical complications known to possibly restrict fetal growth (e.g. toxemia, high blood pressure, and alcohol or tobacco use) were reported in 5 (15%) of 33 of group 1b, 24 (26%) of 91 in group 2a, and only in 5 (7%) of the 75 mothers of the PNGR (group 2b).

The authors point out that matUPD7 and growth hormone deficiency (GHD) can occur together as can

GHD and other causes of IUGR and PNGR, and emphasize that other metabolic disorders do not exclude matUPD7. MatUPD7 has been reported in 3 patients with cystic fibrosis, all of which were exceedingly short. Consequently the authors advise screening for matUPD7 if abnormally short stature occurs conjointly with cystic fibrosis or other recessive disorders mapped to chromosome 7. However, because matUPD7 is rare among IUGR and PNGR patients, except in RSS, screening will be primarily helpful in this group of RSS patients.

Hannula K, et al. *Pediatrics* 2002;109:441-448.

**Editor's Comment:** *The long-term natural history of matUPD7 is not yet clear. Fertility and possible transmission of UPD has not been evaluated. For these reasons, and others such as responsiveness to various therapies, screening in appropriate instances is important. All RSS patients should be screened and those RSS patients with and without matUPD7 should be further evaluated to determine the molecular biological differences between the two groups. The authors discuss some possibilities in their manuscript. The entire manuscript is very enlightening and is recommended both for theoretical considerations and factual data.*

Judith G. Hall, OC, MD

## Quality of Life and Self-Esteem in Children Treated for Idiopathic Short Stature

This study from Leiden University in the Netherlands dealt with changes in health-related quality of life (HRQOL) and self-esteem in children with idiopathic short stature (ISS) participating in a study on the effects of growth hormone (GH) treatment. There were 36 prepubertal children who were randomly assigned to a treatment or to a control group. Children, their parents and their pediatricians completed a HRQOL and a self-esteem questionnaire, 3 times in 2 years. The data indicated that children with ISS did not have lower scores at the start as compared with the normal population, except for social functioning. The pediatricians noticed an improvement in HRQOL in the children in the treatment group. Those in the treatment group did grow significantly more than those in the control group. However, the parents and the children being treated reported no change in HRQOL. Indeed, in some instances they reported being worse than before. The child's satisfaction with height was more related to HRQOL than was measured height. The authors

concluded that the assumption that growth hormone treatment improves HRQOL or self-esteem in children with short stature could not be supported by this study.

Theunissen NCM, et al. *J Pediatr* 2002;140:507-515.

**First Editor's Comments:** *It is widely assumed that short stature may be a handicap and that this condition may result in psychosocial problems, such as ridicule, and mascotism. Indeed, short people might be victims of discrimination and prejudice, often referred to as "heightism". For that reason, many have opted to receive GH with the intent to accelerate growth and improve the final adult height, and in that way improve their psychosocial status. The response to GH treatment in these children appears to be modest, resulting in a possible gain in final height of 5-9 cm, after many years of treatment. However, few studies have approached the concept of HRQOL as an outcome measure of this treatment. In this study, children with a height of more*

than two standard deviations below the mean for age and sex, who were not GH deficient, were found to have appropriate HRQOL and self-esteem, and did not show improvements after GH treatment. The parent's opinion about their social competence after treatment was also not changed. Of interest was the lack of agreement between the informants, who were the patients and parents, with the pediatrician's perception of the effects on quality of life after GH. The relationship between stature, growth, HRQOL and self-esteem might be determined by the expectations of the participants rather than by the improvements in growth. These children, as well as their parents, might have had unrealistic expectations and, therefore, not be satisfied with the treatment, despite improved standard deviation scores for height. Therefore, when we undertake treatment of a non-growth hormone deficient short child, we should consider aspects other than height. GH treatment should not be initiated just because the child is short. An interesting editorial accompanied this article and was written by Basil J. Zitelli in the same issue of the journal, and the reader is encouraged to review that as well. (*Journal of Pediatrics* 2002;140:493-495).

Fima Lifshitz, MD

**Second Editor's Comment:** Dr. Zitelli in his commentary points out with emphasis that offering

children and parents therapy for short stature raises expectations of success. Motivation to be included in GH trials frequently involved the hope of gaining height, yet if expectations were not met through therapy, poor self-esteem and parental anxiety and disappointment were acutely felt by the child. With the variability and unpredictability of results for any particular child, GH therapy becomes an intervention that may be more detrimental than the original complaint of short stature.

Investigators have added another layer of therapy to enhance growth. To delay epiphyseal fusion, gonadotropin releasing hormone agonists have been added to GH treatment regimens. This may potentially compound the iatrogenically introduced fear in the normal short child of being abnormal or affected with a disease that requires 2 medications to treat.

The last issue (GGH 2002 Vol 18:3) has an abstract and commentary regarding the use of LHRHa in advanced puberty. The conclusion of the authors was "these data suggest that advanced puberty (as differentiated from sexual precocity defined as sexual development in girls before the age of 8 years and boys below 9 years) decreases the growth potential by about 5 cm and that GnRHa therapy does not prevent this".

Robert M. Blizzard, MD

## A Gene as a Major Cause of Sotos Syndrome has been Identified

Sotos syndrome is a relatively common neurologic disorder characterized by prenatal and postnatal overgrowth, advanced bone maturation, large skull with acromegalic features, and significant developmental delay. Most cases are sporadic, but autosomal dominant inheritance has been suggested in some instances and autosomal inheritance in a few rare instances. Reports of balanced translocations have pointed to several chromosomal sites as the location of a gene responsible for the syndrome. One of these has led to the identification of mutations of a nuclear hormone receptor cofactor as a major cause of this syndrome.

Kurotaki et al analyzed DNA from a patient with a de novo translocation 46,XX,t(5;8)(q35;q24.1) that had been reported previously by Imaizumi et al. From analysis of a series of overlapping clones, a contig, that covered the break point, they identified a partial sequence that corresponded to a gene originally cloned in mice, *NSD1*. They then isolated and characterized the human *NSD1* showing that it encoded a protein of 2,696 amino acids that is expressed in many tissues including fetal brain, skeletal muscle and kidney, and that the 5q35 breakpoint is located within *NSD1*.

The group next analyzed DNA from 38 patients with the clinical diagnosis of Sotos syndrome. De novo point mutations that would predict truncated gene products with loss of function were identified in four individuals. Fluorescent in situ hybridization (FISH) analysis revealed a common 2.2 Mb deletion in 18 and a smaller deletion in one of 30 patients in whom a suitable chromosomal spread was available. These deletions included the entire *NSD1* gene. In total, a loss of function mutation or a deletion of *NSD1* was found in 77% of patients implicating haploinsufficiency of *NSD1* as a cause of Sotos syndrome.

*NSD1* is thought to act as a co-activator or co-repressor of nuclear hormone receptors, such as the androgen receptor, depending on the promoter context of the target gene and the cellular context. In other words, in one cell type *NSD1* may interact with a combination of regulatory factors unique to the cell type to activate a target gene, whereas it may interact with another set of factors to inhibit expression of target genes in another cell type. The mutations thus alter expression of target genes in relevant tissues.

Clinically, the authors state that the identification of a deletion or mutation of this mutated gene on

chromosome 5 will sometimes help in the diagnosis of Sotos syndrome. Investigatively, the knowledge reported in this article will eventually shed light on some of the underlying mechanisms producing human mental retardation and physical growth.

Imaizumi K, et al. *Am J Med Genet* 2002;107:58-60.  
Kurotaki N, et al. *Nat Gen* 2002;30:365-366.

**First Editor's Comments:** Sotos syndrome has been considered to be a relatively heterogeneous entity. The identification of the responsible gene(s) will undoubtedly lead to a better definition of the syndrome and a better understanding of the features observed. Sotos syndrome can now be added to the growing list of disorders with microdeletions in which fluorescent probes are available to identify affected individuals.

In the last few years, identification of individuals with translocations has been instrumental in identifying the genes responsible for many genetic disorders. Sotos syndrome has been considered to be sporadic, even though there were a few reports of parent/child involvement. This discovery clearly confirms that an abnormality in only one allele leads to the syndrome.

As in other microdeletions, the size of the deletion may indicate how severely an individual is affected.

Judith G. Hall, OC, MD

**Second Editor's Comment:** The results reported in this paper argue strongly that Sotos syndrome is caused by a partial loss of NSD1 function. The range of nuclear receptors whose action is affected by NSD1 is not known, nor are the target genes whose level of expression are influenced by NSD1. Given the overgrowth features of Sotos syndrome, one would conclude that the relevant genes are involved in controlling growth and maturation, probably at a very basic level. Moreover, one would expect that the mutations lead to loss of co-activation of growth inhibiting genes, loss of repression of growth promoting genes, or some combination of the two. Questions still remain regarding which cell types are involved. NSD1 is known to be expressed in the fetal brain, which presumably explains the CNS manifestations, but the cells responsible for the skeletal features are still not known.

William A. Horton, MD

## β-Cell-Specific Deletion of the IGF-I Receptor Leads to Hyperinsulinemia and Glucose Intolerance but does not Alter β-Cell Mass

Global deficiency of IGF-I receptors result in hypoplasia of pancreatic islet β-cells. In order to examine the role of the IGF-I receptor in an individual tissue, the investigators from the Joslin Clinic and elsewhere developed a mouse model in which there is "knock-out" of the IGF-I receptor on only the pancreatic islet β-cells. All other tissues continue to express the IGF-I receptor normally, and circulating IGF-I concentrations are comparable to values in controls, indicating no generalized absence of IGF-I presence or action. The investigators did so by breeding animals with conditional *Igf1r* targeting by a neomycin selection cassette for exon 3 flanked by *loxP* sites that was subsequently excised with mice expressing *cre* linked to the rat insulin promoter.

β-cell-specific IGF-I receptor "knock-out" mice (KO) survived normally *in utero* and after birth. β-cell mass, insulin, and glucagon content were normal in control and KO animals at 6 months. *In vitro*, islets from KO mice failed to release insulin in response to glucose in a normal manner and basal insulin secretion was not suppressed by IGF-I added to the incubation medium. *In vivo*, fasting glucose levels were similar, but basal insulin and C-peptide concentrations were higher in KO than in control mice. There was impaired glucose tolerance following intraperitoneal glucose. The

immediate first phase of insulin secretion was absent, and the second phase was blunted in KO animals while the insulin secretory response to L-arginine was comparable in KO and control mice. KO mice had reduced islet cell expression of the genes encoding important glucose-sensing proteins, including the GLUT-2 glucose transporter, and glucokinase which is the enzyme necessary for glucose phosphorylation. Thus, the β-cell IGF-I receptor is not necessary for β-cell growth, but it is needed for the selective β-cell insulin secretory response to glucose.

Kulkarni RN, et al. *Nature Genet* 2002;31:111-115.

**Editor's Comment:** Present technology has opened the portal to the investigation of the function of cell-specific proteins. One wonders if patients with impaired glucose tolerance, paradoxically increased basal insulin values, and subnormal insulin glucose-specific insulin secretion, present a loss-of-function defect in β-cell IGF-I receptors. This article and the one on page 62 (β-cell Expression...) are related and have potential importance in the future treatment of diabetes mellitus.

Allen Root, MD



## Leptin Acts as a Growth Factor on the Chondrocytes of Skeletal Growth Centers

In order to examine the mechanism(s) by which obesity might lead to enhanced linear growth and advanced skeletal maturation relative to chronologic age, these investigators studied the effects of leptin, a 16-kDa protein product of adipocytes with anorexigenic properties, upon cartilage cell growth and function *in vitro*. They employed mandibular condyles from 6-day-old mice in organ culture for their model of endochondral ossification. Leptin-specific receptors were identified in chondrocytes in the cartilage growth plate; the molecular weight (148 kDa) of these receptors suggested that they were likely to be the intact, biologically active isoform of this class I cytokine receptor. Addition of leptin (0.5 and 1.0  $\mu\text{g/mL}$ ) to the organ culture stimulated chondrocyte division in a dose dependent manner, thereby increasing the width of the proliferative zone and the size of the mandibular condyle. Enhanced functional chondrocyte maturation was demonstrated by increased production of chondroitin sulfate and collagen type II after incubation with leptin. The authors also found that leptin increased expression of the IGF-I receptor in chondrocyte precursors and that immunoneutralization of IGF-I prevented the growth and functional effects of leptin, thus suggesting that leptin's actions are mediated by the IGF-I/IGF-I receptor unit. The authors concluded that leptin has direct effects upon cartilage growth and differentiated function.

Maor G, et al. *J Bone Miner Res*;17:1034-1043.

**Editor's Comment:** *It has been previously reported that leptin stimulates osteoblast differentiation and maturation. However, leptin levels do not correlate with bone mineral density, an index of bone strength that is more closely related to lean body mass than to body fat content or total body weight. Indeed, experimentally central administration of leptin actually reduces bone mass by an as yet unrecognized mechanism. Of concern and consideration in evaluating this study is the need to employ very high concentrations of leptin to demonstrate biological effects, levels far greater than those achieved in vivo even in the most obese subject. Furthermore, there was a biphasic effect of leptin in this system in that, when incubated with 1.5  $\mu\text{g/mL}$ , most of the reported effects were attenuated. Nevertheless, the data are of interest in furthering our understanding of how obesity might mediate its effects on linear growth and cartilage maturation - particularly in the interesting patients who grow despite complete GH deficiency as after neurosurgical removal of a craniopharyngioma or those with septo-optic dysplasia.*

Root AW, Diamond FB Jr. *Pediatric Endocrinology* 2nd ed, Saunders, Philadelphia, 2002, p 65-95.

Allen W. Root, MD

## Effect of Supplemental Zinc on the Growth and Serum Zinc Concentrations of Prepubertal Children: A Meta-Analysis of Randomized Controlled Trials

This study performed meta-analyses of all randomized controlled intervention trials that completed the assessment of the effects of zinc supplementation on the serum zinc concentrations and physical growth of pre-pubertal children. A total of 33 acceptable studies with appropriate data were identified by MEDLINE searches and other methods. Weighted mean effect sizes were calculated for changes in height, weight, weight-for-height, and serum zinc concentrations. The authors used random-effects models, extrapolated by meta-regression techniques.

Zinc supplementation produced highly significant, positive responses in height (+0.35 SDS) and weight (+0.39 SDS) increments. Zinc supplementation caused a large increase in the children's serum zinc concentrations (+0.82). Growth responses were greater in children with low initial weight-for-age z scores, and in those aged more than 6 months with low initial height-for-age z scores.

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The authors concluded that interventions to improve the zinc nutriture of children should be considered in populations at risk of zinc deficiency, especially and particularly in those where there are elevated rates of children who are underweight or experience stunting.

Brown KH, et al. *Am J Clin Nutr* 75:1062-1071.

**Editor's Comments:** The benefits of zinc supplementation for children's growth have been debated for many years. This meta-analysis conducted by Brown et al showed that zinc supplements probably are of benefit for children in developing countries. It is not surprising that in such populations there are nutrient deficits which can be corrected by specific nutrient supplementation. Underlining the potential nutritional deficiency status of the population studied and reported, there was a higher significant aggregate zinc effect on children's growth in those who exhibited deficits of body weight for height. It might also be inferred that children who do not exhibit growth retardation or body weight-for-height deficits might not be nutrient-deficient, and may, therefore, not benefit from zinc supplementation. It should also be kept in mind that zinc deficiency is difficult to document, and that zinc supplementation, either alone or in combination with other nutrients, is

not easily accomplished nor tolerated by children. Zinc supplements are also expensive where they might be needed the most, namely in developing countries. The foods richest in zinc are from animal sources which are also often not accessible in these countries. Children in the United States and other developed countries who ingest a wide variety of meat products are highly unlikely to be zinc deficient.

I agree with the authors who state in the last paragraph of this article "Because of the important functional consequences of zinc deficiency for children's growth and other health outcomes, interventions to improve zinc nutriture should be considered in those populations at particularly high risk of zinc deficiency. Additional research will be needed to determine whether the mean serum zinc concentration of a population is a useful predictor of response to zinc supplementation. On the other hand, the population mean serum zinc concentration does increase after supplementation, so this measure can be used to indicate whether public health interventions to promote increased zinc intakes are successful." For those interested in this topic, reviewing the original manuscript and its excellent and extensive graphic expression of data will be appreciated.

Fima Lifshitz, MD

## Placental-Specific IGF-II is a Major Modulator of Placental and Fetal Growth

A substantial proportion of imprinted genes, i.e., genes expressed from only one parental chromosome, are involved in placental development and fetal growth in mammals. In the mouse for example, *Igf2* is expressed paternally in the placenta and fetus, while its receptor is expressed maternally. Imprinted genes can act directly on the fetus by influencing cellular proliferation and apoptosis; they can also affect fetal growth by influencing placental structure and physiology and the supply of maternal nutrients. Debate over the evolutionary significance of imprinting in mammals has led to the so-called genetic conflict hypothesis or theory of imprinting. It predicts that paternally expressed genes act on the placenta to promote extraction of resources from the mother to enhance fetal growth while maternally expressed genes act to restrain fetal growth to conserve maternal resources for long-term reproductive fitness of the mother. Testing this hypothesis has been difficult because the relevant genes are expressed in both placenta and fetus and their tissue-specific inactivation has not been achieved.

Recently, it has been shown that the mouse *Igf2* has four promoters, one of which, designated P0, directs paternal expression of *Igf2* in the labyrinthine trophoblasts of the placenta. Deleting this promoter

through gene targeting enabled Constância and colleagues to study the impact of paternally-directed placental IGF-II on fetal growth. The P0 knockout for *Igf2* was confirmed by in situ hybridization that revealed a marked reduction of *Igf2* expression specifically in the labyrinthine trophoblasts. Expression of *Igf2* from its other promoters was normal in mutant placentas and fetal tissues as were levels of IGF-II in the fetal circulation.

Lack of the P0 *Igf2* transcripts with paternal transmission primarily resulted in placental growth restriction, which was detected early in gestation at embryonic day 12 (E12) of the 19-day mouse gestation. The impaired growth of the mutant placentas remained relatively constant throughout the remainder of the pregnancy (weight of mutant placentas 76%, 82%, 68%, 68% of normal at E12, E14, E16, E18, respectively) suggesting that the paternally-directed, labyrinthine trophoblast-specific *Igf2* transcripts are required to sustain normal growth of the placenta.

In contrast to the early decrease in placenta size, the indirectly affected fetuses became growth restricted only toward the end of gestation. Their weight was 96% of normal at E16, but dropped to about 70% at birth. The ratio of fetal to placental weight increased as

gestation proceeded and was significantly higher for mutant compared to normal pregnancies reflecting the small placenta size.

To address the discrepancy between placental and fetal growth, the authors compared normal and mutant placentas structurally and functionally. Other than size, no obvious differences in tissue organization or cell morphology were detected. They next compared maternal-fetal transport of different radiolabelled compounds, one transferred by passive diffusion and the other by active transport. Their results showed that passive diffusion declines proportionate to the relative reduction in placental size. Active or system A transport, however, increases during mid gestation, apparently compensating for the loss of passive transfer until near the end of gestation when this compensation is insufficient to meet the needs of the fetus and fetal growth drops off. Importantly, the system A transporter has been shown to be a determinant of fetal growth.

In summary, deletion of a placental-specific imprinted transcript results in fetal growth restriction, primarily through a decrease in total nutrient transfer across the placenta. This example of a morphologically normal but small placenta affecting fetal growth supports the genetic conflict theory of imprinting, in which a placental-specific gene expressed from the paternal allele regulates the supply of nutritional resources to the fetus. On the other hand, fetal demand for nutrients is genetically regulated by the level of growth factors such as IGF-I and IGF-II. Increasing fetal size therefore requires a higher level of demand (for example, higher fetal IGF-II) as well as a higher level of supply (by increasing, for example, placental surface area). Reduced fetal size can be the outcome of reduced supply (as in the P0 mutant described here) or of reduced demand (for example *Igf1* knockout, which reduces fetal but not placental size). The mouse *Igf2* gene is remarkable in combining the

control of both the supply and the genetic demand for maternal nutrients in a single gene.

Constância M, et al. *Nature* 2002;417:945-948.

**First Editor's Comment:** *This work supports the genetic conflict theory of imprinting showing that placental-specific genes expressed from the paternal allele contribute substantially to the supply of nutrients a fetus receives from its mother. It also shows that the placenta can partially compensate at least for the loss of this paternal effect. It will be interesting to learn more about the nature of the compensation, which represents a potential mechanism to exploit in treating intrauterine growth retardation. It is important to acknowledge, that the relationship between mother and fetus differs substantially between mice and humans, especially with regard to size and duration.*

William A. Horton, MD

**Second Editor's Comment:** *As a pediatric endocrinologist who has had a special interest in IUGR for many years, I found the reading of the original article most informative. Not mentioned in the abstract or First Editorial comment was the following brief statement, "At birth, P0 mutant pups were 69% of normal birth weight. This was followed by postnatal catch-up growth which was complete by three months of age." While, as Dr. Horton stated above that mice and humans (may) differ substantially, there is a corollary between the catch up growth in these IUGR mice and the catch up growth that is seen in most IUGR human neonates (primarily those without associated dysmorphology) in the first two years of life. Subsequent studies dealing with the genetic conflict theory in humans should be very informative and intriguing.*

Robert M. Blizzard, MD

## Insulin-like Growth Factor I and Leptin in Umbilical Cord Plasma and Infant Birth Size at Term

Umbilical cord blood samples were collected from 12,804 consecutive deliveries, and cord plasma samples were collected from 585 singleton infants born in Norway at term after uncomplicated pregnancies. These were analyzed for plasma leptin, IGF-I, IGFBP-1 and IGFBP-3. Data were analyzed following log transformation of IGFBP-1 and leptin values. Linear regression analysis was used to determine the contribution of maternal and infant factors to umbilical levels of these hormones. The mean age of the mothers of these infants was 28 years. Seven percent had smoked at the beginning of the pregnancy, and 36 percent were primiparous. Male

infants had a higher birth weight and length than girls, but girls had a higher ponderal index. Leptin and IGF-I levels were higher in the cord blood of female infants than in males. None of the maternal factors which were analyzed, including pre-pregnancy weights, smoking, or number of previous pregnancies were significantly associated with levels of cord leptin. IGF-I, IGFBP-3, and leptin increased proportionately with increasing birth weight. Levels of IGF-I and leptin were the strongest predictors of both birth weight and birth length, and were independent of length of gestation, maternal age, parity, pre-pregnancy weight, smoking and offspring sex.

The authors conclude that their data suggest that the sexual dimorphism in the regulation of leptin and IGF concentrations, which previously was demonstrated in later childhood, may already be established at birth. They also suggest a possible role for leptin and/or the IGF-I system in relation to birth size and to the risk of diseases such as non-insulin dependent diabetes and cardiovascular disease which have been shown to be frequent in low birth weight infants.

Vatten LJ, et al. *Pediatrics* 109:1131-1135.

**Editor's Comment:** *These findings have important implications for understanding the relationship between low birth weight and adult morbidity - especially*

*cardiovascular disease, hypertension, and type 2 diabetes. It would appear that leptin, IGF-I, and IGFBP-I, which have been shown to be important factors in growth in utero, may be important in understanding the risk of developing these adult diseases. It would be very important to follow a cohort of children from birth through adulthood with serial measurements of IGF-I, IGFBP-3, and leptin in order to better understand how these factors change over time and how they might contribute to the development of serious adult disorders. Studies such as those by Vatten et al in Norway support the importance of conducting such difficult epidemiological studies.*

William L. Clarke, MD

## A Longitudinal Study of the Effects of a Gluten-Free Diet on Glycemic Control and Weight Gain in Subjects With Type 1 Diabetes and Celiac Disease

Amin et al from Oxford reported their findings of longitudinal growth characteristics and glycemic control in children with type 1 diabetes along with celiac disease (CD). Annually, from 1994 and 1998, 230 children with type 1 diabetes were screened starting in the first year after the onset for the presence of IgA and anti-endomysial antibodies (EMA). A total of 10 children were EMA positive and another one was AGA positive, which was 4.8% of the clinic population. Only one patient demonstrated symptoms typical of CD, including failure to thrive and steatorrhea; four complained of some mild abdominal discomfort. Jejunal biopsy showed classical histopathology of CD in all eleven patients. These subjects were matched for age, sex, and diabetes duration with two control diabetic children who were negative for EMA. Height, weight, and HbA<sub>1c</sub> were measured at the time of diagnosis of CD and every 3 months. Antibody levels were tested every 3 months until negative, and then yearly. The ANOVA model was used to determine the influence of CD on both HbA<sub>1c</sub> and BMI SDS. The data are presented as mean  $\pm$  SEM.

Mean BMI SDS in the CD group was significantly lower ( $-1.2 \pm 0.1$  vs.  $-0.1 \pm 0.1$ ,  $P=0.005$ ), as was mean weight SDS ( $-0.7 \pm 0.3$  vs.  $0.5 \pm 0.3$ ,  $P=0.002$ ) than in those without CD. However, there was no difference between the two groups mean height or C-peptide level. Mean age of diagnosis of CD was 11.2 years (2.2-17.3). The mean duration of diabetes at diagnosis was 3.8 years (0.9-7.2). Mean HbA<sub>1c</sub> was significantly lower at diagnosis in the children with CD ( $8.9\% \pm 0.3\%$  vs.  $9.8\% \pm 0.3\%$ ,  $P=0.002$ ), but there was no difference in the mean daily insulin dose in the two groups. The difference in mean BMI SDS between the subjects and the controls was eliminated by 12 months of gluten-free diet ( $1.1 \pm 0.13$  vs.  $1.0 \pm 0.1$ ,  $P=0.11$ ). HbA<sub>1c</sub> levels were lower

than in the controls during the period of gluten-free diet ( $8.3 \pm 0.2$  vs.  $10.0 \pm 0.2$ ,  $P=0.002$ ). Insulin requirements increased in both groups, but no difference in those requirements developed between the two groups. Using a general factorial linear model, CD was associated with lower BMI SDS and lower HbA<sub>1c</sub> across time, independent of other factors such as insulin dose and regime. Also, while on a gluten-free diet, the children with CD had lower HbA<sub>1c</sub> which was independent of BMI SDS or the insulin dose or regimen. The EMA antibodies tended to disappear while the patients were on the gluten-free diets.

The authors reviewed recent reports regarding the association in children between type 1 diabetes and CD. Prevalence rates range between 1.7 to 10%. However the data on whether intervention with gluten-free diet would be of benefit remain controversial. This is, in part, because there are few longitudinal follow-up data and few age and sex matched controlled studies. The authors note that their findings could have been influenced by the small sample size or the increased input by dieticians which was received by case subjects. They stress, that because the long-term complications of CD include gastrointestinal malignancy, lymphoma, infertility, and osteoporosis, the screening of children with type 1 diabetes at a young age may be cost effective and warranted.

Amin R, et al. *Diabetes Care* 25:1117-1122.

**Editor's Comment:** *These findings are very intriguing. Many pediatric endocrine clinics are now screening children with type 1 diabetes for EMA or tissue transglutaminase IGA to identify CD. There is controversy as to whether or not children who are*



asymptomatic with their CD will benefit from a gluten-free diet, and whether or not there is any effect of a gluten-free diet on the management of their diabetes. Amin and co-workers have demonstrated that indeed children with CD and type 1 diabetes are anthropometrically different from those children without CD, and that treatment reverses this finding. In addition, there appears to be a treatment benefit on overall glucose control. The authors noted that their data could

have been influenced by the frequent visits to the dietician by case subjects. It will be important to determine whether gluten-free diet is of benefit in all children with diabetes, and or whether similar nutritional input to all type 1 diabetic children could improve HbA<sub>1c</sub> to the extent observed in this study.

William L. Clarke, MD

## Risk for Abnormal Outcomes is Increased with Assisted Reproductive Technology

The advent of assisted reproductive technologies (ART) has increased the complexity of care in newborn nurseries. An increased number of premature infants and multiple births are among a variety of risks that occur with the increased frequency of ART. These risks should be shared with all prospective parents (patients).

An article by Schieve et al studied 42,463 infants who were born between 1996 and 1997, and who had been conceived utilizing ART. These infants were compared to the three million plus infants born in the United States during that period. Among singleton births conceived by ART, and born at 37 weeks or after, the risk for low birth weight was 2.6 times that in the general population. The use of ART was also associated with an increased rate of multiple births which also increases the rate of IUGR births and many other complications.

Hansen et al reported on 301 infants conceived by intracytoplasmic sperm injection and 837 infants conceived with in vitro fertilization (IVF). These were compared to naturally conceived infants from the same region. The infants conceived with ART had an increase of birth defects which was greater than double the occurrence among the naturally conceived. The abnormalities involved a broad spectrum of congenital anomalies. The etiology for the increased risk was unclear. However, advanced maternal age, the usual underlying causes of infertility, medications used to induce ovulation and maintain pregnancy, factors associated with procedures such as freezing and thawing of embryos, and delayed fertilization of the oocyte individually or collectively, contributed to this increased risk.

Strömberg et al studied the neurologic sequelae of children born after IVF. Through a population based retrospective cohort assessment, they compared the neurologic outcome of 5,680 children born after IVF against the neurological outcome of 11,360 matched controls. For each of the 2,060 twins born after IVF, a second set of twin controls was used. Children born after IVF demonstrated an odds ratio of 1.7 of needing habilitation services. Among singletons born after IVF,

the risk was 1.4. The most common neurologic disorder was cerebral palsy, with a relative risk of 3.7 for all children born after IVF and 2.8 for singletons. Data concerning twins born after IVF was essentially the same as control twins in respect to neurologic sequelae. Twins with low birth rate and prematurity were more likely to require habilitation services. Maternal age did not seem to be a factor in this study.

Multiple births have an increased risk factor for neurologic sequelae and, consequently, Ozturk et al. strongly recommend that no more than two embryos be placed in the uterus while performing IVF.

Hansen, et al. *N Engl J Med* 2002;346:725-730.

Ozturk, et al. *Lancet* 2002;359:232.

Schieve, et al. *N Engl J Med* 2002;346:731-737.

Strömberg, et al. *Lancet* 2002;359:461-465.

**First Editor's Comment:** Information regarding the increased risk of problems associated with ART must be shared with the families who are considering using them. Healthcare providers must also be aware of these risks. The increased expenditures associated with ART are not just the cost of the procedure, but also involve the long-term health care costs. Healthcare costs have become more expensive because of these complications, and these are not usually considered when assessing the expenditures of ART.

Judith G. Hall, OC, MD

**Second Editor's Comment:** A dictum of physics is only rarely violated. Specifically every positive force has a negative force and vice versa. Chances are what we take daily. There are no positive assurances about anything except death. Therefore, we should expect that every technology will not be perfect – either in construction of the technology itself, or carrying out of a procedure with the technology and in the results thereof. Thus, we should not be disturbed by some imperfections of the system, although we should continue to try to make it perfect.



*Human error as well as errors of nature also complicate life, including life related to IVF. The Associated Press on July 10<sup>th</sup> released in newspapers around the world a report entitled "Test Tube Baby Mix-Up Causes Alarm: Birth of Black Babies to White Couple Raises Questions About Reliability of the Program". This*

*occurrence was in England. Such occurrences of error undoubtedly are very rare, but inevitably occur.*

*Life goes on, but not always without error. The positivities of what IVF has, does, and will accomplish, far outweigh the negativity of the errors of nature and man.*

Robert M. Blizzard, MD

## Hypovitaminosis D Prevalence and Determinants Among African American and White Women of Reproductive Age: Third National Health and Nutrition Examination Survey, 1988-1994

This study addressed the issue of the prevalence and the determinants of hypovitaminosis D among 1,546 African American and 1,426 white women of reproductive age (15-49). These women were not pregnant and participated in the Third National Health and Nutrition Examination Survey (1988 – 1994). Hypovitaminosis D was defined as serum 25-hydroxyvitamin D concentrations of < 37.5 nmol/L. The prevalence of hypovitaminosis D was 42.4% among African American women as compared to only 4.2% among white women. The presence of hypovitaminosis D was independently associated with low consumption of milk or cereal, less than ideal use of vitamin D supplements, cold seasons, urban residence, low body mass index, and use of oral contraceptives. Even among the 243 African Americans who consumed an adequate intake of vitamin D from supplements (>200 IU/d), 28.2% had hypovitaminosis D. The authors concluded that the high prevalence of hypovitaminosis D among African American women warrants further examination of the vitamin D recommendations for these women. The determinants of hypovitaminosis D among women should be considered when these women are advised regarding dietary intake and supplement use.

Nesby-O'Dell S, et al. *Am J Clin Nutr* 2002;76:187-192.

**Editor's Comments:** *The report by this group of investigators provided compelling data with irrefutable evidence that vitamin D deficiency constitutes a major unrecognized epidemic in many young black adult women and in 5% of white women of childbearing age. This survey might have shown a much higher prevalence of hypovitaminosis D if it had been performed in the winter. We may also assume that vitamin D deficiency*

*might be equally prevalent among males of the same age and race, although this was not studied. This article clearly documents it is still currently possible to frequently find vitamin D deficiency in the United States, which plagued our ancestors during the 19<sup>th</sup> century. There are vulnerable populations, such as those who are not exposed to the benefits of sunlight irradiation, and in those who are dark skinned. The latter may not be able to synthesize sufficient vitamin D from the skin to prevent vitamin D deficiency, and may be in need of higher levels of vitamin D intake as compared to their white counterparts. Therefore, the recommendation to examine the dietary recommendations for young black women and men should be quickly undertaken. Since the black population has a high incidence of lactase deficiency and, therefore, not able to tolerate milk, oral vitamin D supplements may be needed.*

*In this study there were no measurements of parathyroid hormone levels or the active metabolic vitamin D (25-D hydroxy vitamin D), both of which are very sensitive indicators of calcium homeostasis and vitamin D deficiency. The high prevalence of hypovitaminosis D among "healthy young female adults" is important as vitamin D deficiency is associated with osteomalacia, bone pain, muscle aches, muscle weakness, and fibromyalgia. It also causes secondary hyperparathyroidism, which can precipitate and exacerbate osteoporosis by increasing mobilization of mineral and matrix from the skeleton. Therefore, there is reason for each of us to pay attention to an easily remedied medical problem that affects many of our patients whether they are adults or children.*

Fima Lifshitz, MD

## β-Cell Expression of IGF-I Leads to Recovery from Type 1 Diabetes

A method by which to reverse the process that leads to destruction of pancreatic islet cells and type 1 diabetes mellitus is the "Holy Grail" that all diabetologists seek.

In the present report from Barcelona, the investigators of the School of Veterinary Medicine and Gene Therapy Center succeeded in doing just that in an animal model

in which the key is selective overexpression of IGF-I in  $\beta$ -cells.

Transgenic mice were developed in which mouse IGF-I was linked to the rat insulin promoter and thus targeted to the  $\beta$ -cell, where IGF-I expression was many fold greater than in control animals. In these mice, at 6 months of age there was a 1.5 fold increase in  $\beta$ -cell mass but normal pancreatic insulin content. Circulating concentrations of IGF-I were comparable in control and transgenic animals. The latter did not develop hypoglycemia, hyperinsulinemia, or neoplasms and had normal life span and reproduction.

At two months of age, administration of streptozotocin (STZ) led to the development of insulinitis, hyperglycemia, hypoinsulinemia, and death at four months of age in the control groups from two strains of mice (C57BL and CD-1) utilized. In the C57BL mice which overexpressed IGF-I only in the  $\beta$ -cell, STZ lead to transient modest hyperglycemia, impaired insulin secretion, mild but reversible insulinitis, and subsequent normal life span. In the CD-1 transgenic mice, hyperglycemia and hypoinsulinemia following STZ were extreme, but again transient with long term survival (Figure). After recovery from hyperglycemia, the growth was normal in the  $\beta$ -cell-targeted IGF-I transgenic animals.

Histological examination in C57BL mice revealed a mild decrease in islet b-cells and budding of insulin containing cells from pancreatic ductal epithelium. Thus, IGF-I appeared to at least partially protect  $\beta$ -cells from destruction while also increasing generation of new  $\beta$ -cell precursors. Since the  $\beta$ -cell IGF-I receptor is found on the  $\beta$ -cell membrane, the high levels of IGF-I synthesized by the  $\beta$ -cell specific IGF-I transgenic mice must be acting in a paracrine or autocrine manner to protect  $\beta$ -cells insulted by STZ.

Histological examination in the CD-1 mice revealed much less severe insulinitis in the transgenic STZ treated mice than in the control STZ treated animals. There was slow recovery from insulinitis, but with  $\beta$ -cell proliferation and neogenesis, blood sugar and insulin serum levels were restored to normal.

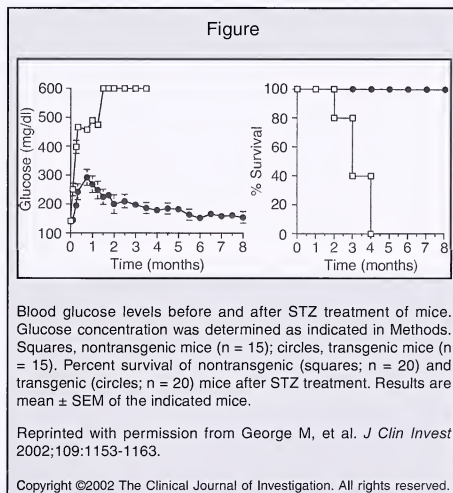
The authors concluded that co-expression of IGF-I and insulin in  $\beta$ -cells protected these cells from permanent destruction by STZ by increasing resistance to the inflammatory insult itself, augmenting  $\beta$ -cell division, and encouraging differentiation of new  $\beta$ -cells. They suggest that IGF-I may be a candidate gene for

transfer to pancreatic  $\beta$ -cells in the gene therapy of patients developing type 1 diabetes mellitus.

George M, et al. *J Clin Invest* 2002;109:1153-1163.

**Editor's Comment:** This exciting paper raises the possibility that IGF-I might be capable of halting the progression of  $\beta$ -cell loss in patients developing type 1 diabetes mellitus if a method can be found to target this growth factor to the insulted  $\beta$ -cell in the intact patient. Perhaps equally feasible, and possibly even more beneficial, might be the insertion of IGF-I into the  $\beta$ -cells of patients at risk for development of type 1 diabetes mellitus to "protect" or to help them recover from the anticipated insults in the future that will lead to insulinitis. The latter objective may be more useful because the present experiments, which were successful, were conducted in animals that had high IGF-I pancreatic islet contact before the STZ insult. Such an approach would, hopefully, simulate the successful experiment recorded in this article.

Allen Root, MD



## Growth and Maturation in Marfan Syndrome

The Marfanoid habitus is well known to pediatric clinicians; it is characterized by tall, asthenic habitus. In Marfan Syndrome (MFS), there is multi-organ involvement including eye, heart and muscular/skeletal abnormalities. Erkula et al, largely from Johns Hopkins

data, have retrospectively compiled growth pattern data on 180 clinically diagnosed MFS patients. They have generated growth charts and growth velocity charts for infant, children and adolescent males and females. Not unexpectedly, males and females with MFS are larger

at birth, grow at a greater velocity, and end up taller than average. Interestingly, skeletal maturation is also advanced and puberty is earlier when compared to the general population.

These data are extremely important and very helpful for those caring for children with MFS to determine whether a child is outside the expected range for MFS. This and further accumulated data will be very important in respect to the management of the spinal deformities common in MFS, as well as considering either surgical or hormonal therapies to decrease ultimate height.

The study was done using retrospective measurements, primarily from familial cases where the diagnosis had been made on a clinical basis. The authors express some concern about precision of height and weight measurements since they were collected by non-auxologists and because longitudinal data early in life were very limited. Nevertheless, the data are extremely useful in defining the overall natural history of growth in MFS. The authors point out that the excessive linear growth seen in MFS begins prenatally. The growth velocity is consistently higher than that observed in the general population, although body mass does not exceed that in the general population. This combination leads to the slender habitus in MFS.

An important consideration in MFS is the development of idiopathic scoliosis. On average, it develops earlier in children with MFS than in children in the general population. Since it is a common occurrence in MFS, it needs to be screened early and treated aggressively.

The study also documented that skeletal maturation occurs earlier in MFS than in the average population. This is an important consideration when thinking about various therapeutic modalities such as the timing of

surgical epiphyseodesis or hormonal therapy to produce cessation of growth and for considering utilizing braces to treat scoliosis.

Erkula G, et al. *Am J Med Genet* 2002;109: 100-115.

**Editor's Comment:** *This manuscript should be prime reading for those taking care of MFS patients. Space limits the presentation of the multiple figures presented in the manuscript. These growth charts are available in the original manuscript. These types of growth data are extremely important for relatively rare genetic syndromes and can only be accumulated in centers with enormous experience. Not only is the natural history important to elucidate, but understanding how and when to apply various therapies is extremely important.*

*Interestingly, the authors point out that some individuals with MFS are taller than others and, surprisingly, that some MFS patients are obese. Secondary genes or other mutations that affect height and weight are being sought. Such studies may be revealing in better understanding the variations of normal stature as well. It is the careful study of rare genetic disorders that helps to provide better therapy of diseased states and better understanding of normal development. We should be very grateful to this group, which has collected these data over many years. I cannot help but note and be dismayed that it is very difficult to find funding for this type of research and, yet, it is so extremely important. Therefore, we should be even more grateful to the authors and hope that they will be reporting similar data obtained in the studies of other rare genetic growth disorders.*

Judith G. Hall, OC, MD

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# GROWTH

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### MANAGEMENT OF CHILDREN WITH INTERSEX CONDITIONS: PSYCHOLOGICAL AND METHODOLOGICAL PERSPECTIVES

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Pediatric medicine has undergone considerable upheaval in the past few years over the treatment of children with disorders of sexual differentiation. There have been challenges to all aspects of traditional practice, including sex assignment, genital surgery, the role of the patient and parents in decision-making, disclosure of medical details, the composition of the treatment team, and nomenclature. These challenges have been met with serious attention by pediatricians and other health professionals involved in the care of these children, and there has been considerable discussion of the merits of changes to current practice.<sup>1-8</sup> This report considers the status of the evidence relevant to treating children with intersex conditions, with particular emphasis on psychological and methodological issues.

#### BACKGROUND

For 50 years, treatment of children with intersex conditions was guided by the belief that gender identity results from social rearing rather than biological factors, provided that gender-confirming genital surgery is done early in life.<sup>9,10</sup> Although there have always been questions about this policy, anecdotal evidence generally suggested that it produced good outcome.<sup>11,12</sup> The policy and the evidence used to support it have recently been subject to detailed scrutiny because of several well-publicized reports. This includes a case of ablatio penis raised female who was unhappy with the assigned sex,<sup>13,14</sup> conference reports of XY males with absent or malformed penis due to cloacal exstrophy reared as females who declare themselves to be boys,<sup>15</sup> and reports of adverse outcomes from intersex patients.<sup>16,17</sup>

Several issues have emerged from recent discussions (Table 1). The focus has been on sex assignment and genital surgery, with traditional treatment and challenges often seen in polar terms (Table 2). Discussions have often been acrimonious, and recommendations based

on personal beliefs or anecdotes, although it is clear that the interests of patients are best served by careful application of evidence.

#### EVIDENCE REGARDING SEX ASSIGNMENT

##### Determinants of Gender Identity

Decisions regarding sex assignment require recognition of the complexity of gender identity. Gender identity cannot be simply predicted from any single factor; neither is it always consistent with sex of rearing, nor is it simply related to extent of prenatal hormone exposure. The publicized individual with ablatio penis<sup>14</sup> was reared as a boy early in life and it is unclear how this contributed to his gender identity. Another individual with a similar history but with earlier female reassignment had a different outcome, particularly female gender identity.<sup>18</sup> To date, there have been no published systematic studies of individuals with cloacal exstrophy, and case reports indicate variations in gender identity, with no clear indication of the percentage who identify as males or are unhappy as females.<sup>19,20</sup>

The most systematic evidence regarding gender identity comes from two conditions. Females with congenital adrenal hyperplasia (CAH) overwhelmingly identify as female.<sup>21-23</sup> The very small minority of females with CAH who are unhappy as females or live as males are not necessarily those with the greatest genital virilization or

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Table 1

### Controversies in Treatment of Children with Intersex Conditions

#### Sex assignment

What criteria should be used?  
What determines gender identity?  
When (if ever) is gender identity fixed?

#### Genital reconstructive surgery

Is it necessary? (Why?)  
When should it be done?  
What are its benefits and risks?

#### Decision-making

Who makes the decisions?  
When should decisions be made?  
What information is used to make the decisions?  
What support is available?

#### Information-sharing

What are the parents told at the time of diagnosis and decision-making?  
What does the child learn and when?  
What support is available?  
What is the best way to share information?

#### Involvement of mental health professionals

Should psychologists or psychiatrists be part of the diagnosis and treatment team?  
Does counseling to families facilitate decision-making?  
Does routine and continuing counseling to patients and families improve outcome?

the most prenatal androgen excess. Males with micropenis have not been studied as extensively as females with CAH, but they identify as males when reared that way and appear to function well.<sup>24,25</sup>

There is little systematic evidence to guide decisions about sex assignment in other intersex conditions.<sup>20</sup> Recent studies of individuals with micropenis and those with ambiguous genitalia with perineoscrotal hypospadias of varying etiology suggest that gender identity is generally consistent with sex of rearing.<sup>26,27</sup> But, for several reasons, caution is necessary when generalizing from these studies. First, a substantial proportion of participants (about 25%) were dissatisfied or questioned their sex of rearing. Second, as is typical of retrospective studies, patients who were dissatisfied or atypical were probably underrepresented: 30% of eligible patients did not participate and some participants elected not to answer sensitive questions. Third, outcome was assessed with a few items of unknown sensitivity. Fourth, those reared as boys were subjected to more surgery than those reared as girls.

### Recommendations Regarding Sex Assignment

Sex assignment for an intersex child is one of the most difficult decisions made by parents and health professionals, though it is natural to seek simple solutions. But just as it is no longer tenable to assume that gender identity is always consistent with the sex of rearing, evidence indicates that it is equally unwise to consider gender identity to result directly from fetal androgen exposure (inferred from genital appearance or another indicator). Although other aspects of behavior may relate to degree of fetal androgen exposure, gender identity does not. For example, among females with CAH, degree of prenatal androgen exposure (inferred from genetic mutation, salt-wasting status, and degree of genital virilization) is moderately associated with interest in boy-typical activities and sexual orientation,<sup>23,28-30</sup> but not gender identity.<sup>21-23</sup> Therefore, it is crucial to separate aspects of outcome (Table 3).

There is sufficient evidence to suggest that 46,XX CAH patients be reared as girls, given the documented good outcomes associated with such rearing. Nevertheless, there are no systematic studies of those reared as boys. It is reasonable to suggest that 46,XY micropenis patients be reared as boys, given the small studies of good outcomes in such cases and the need for surgery with rearing as girls, but it would be helpful to have more evidence comparing quality of life and sexual function in those reared as boys vs. girls. In all other cases, decisions will need to be made with the limited information available from case reports. All children should be assigned as boys or girls. Rearing children as intersex is not advocated by health professionals or activist organizations (including ISNA). Parents and health professionals should realize that an intersex individual may elect to change gender later in life. The accuracy of the sex assignment can only be judged by the patient. It is essential to recognize that gender identity is not synonymous with gender-role behavior or sexual orientation, so that childhood tomboy behavior in girls or homosexuality should not be taken as indications of incorrect sex assignment.

### EVIDENCE REGARDING SURGERY

Decisions regarding genitoplasty should be considered in light of the evidence regarding the stated need for surgery. Current practice is predicated on several assumptions: (1) sex-typical genital appearance is necessary for gender identity development consistent with rearing sex and for healthy psychological adjustment; (2) adjustment is hindered by unusual-appearing genitalia, through disruption in parent-child bonding, reactions from caretakers and peers, and difficulty in forming sexual relationships; (3) corrected genitalia are necessary for sexual activity, particularly intercourse. But some intersex patients as adults have

Table 2

### Summary of Traditional Care and Current Challenges in the Treatment of Children with Intersex Conditions

| <b>Sex Assignment/Gender Identity</b><br>Determinant of gender identity<br>Stability of gender identity<br>Role of genitalia<br>Decision-maker | <b>Traditional Practice</b><br>sex of rearing<br>fixed by age 2<br>crucial to identity & adjustment<br>physician                     | <b>Challenge</b><br>prenatal androgen<br>develops throughout life<br>reflect brain masculinization<br>family |
|--|--|--|
| <b>Genital Surgery</b><br>Rationale<br>Consequences<br><br>Decision-maker  | anatomy to match rearing sex<br>facilitates gender identity<br>facilitates adjustment<br>facilitates sexual intercourse<br>physician | surgery is for comfort of others<br>inhibits gender change<br>impairs sexual function<br><br>patient         |

Table 3

### Aspects of Outcome in Children with Intersex Conditions

|   |  |
|---|--|
| <b>Gender Identity</b>                              | Sense of self as male or female  |
| <b>Gender-role Behavior</b>                         | Aspects of behavior that differ between males and females; is multidimensional                               |
| <b>Sexual Orientation</b>                           | Sex of target of sexual arousal  |
| <b>Sexual Functioning</b>                           | Sexual sensitivity<br>Potential for orgasm<br>Capacity for intercourse, if desired                           |
| <b>Psychological adjustment ("quality of life")</b> | Happiness<br>Absence of distress<br>Satisfaction with specific aspects of life e.g., psychosexual adjustment |

complained that surgery does not prevent problems and may actually exacerbate them, because of adverse cosmetic and functional outcomes from surgery. These critics further contend that problems arise from the undue focus on the genitalia and not their appearance per se.

The surgical outcomes most often studied have been genital appearance and adequacy of genitalia for peno-vaginal intercourse. But the assumptions behind surgery and the concerns of patients make it clear that other outcomes need to be considered, particularly those related to the quality of sexual experience, including sensitivity and satisfaction, and general quality of life (Table 3).

#### Physical Outcomes of Surgery

There are no systematic outcome data regarding genital appearance and sexual function, especially for current surgical procedures. There are reports of suboptimal cosmetic outcome and self-reported sexual function, but they are based on limited assessments of selected patients with surgery of varying quality.<sup>26,27,31</sup> Therefore, it is difficult to know how surgery affects sexual function, and the factors that account for variations across individuals. Measures of clitoral responsivity suggest normal nerve conduction after surgery,<sup>32</sup> but it is unclear whether this translates into normal sensitivity. It is also important to remember that intercourse is only one part of sexual activity, and surgery to facilitate intercourse might compromise orgasmic response.

There is optimism that current techniques used by skilled surgeons produce better cosmetic and functional outcomes now than in the past,<sup>33</sup> but confirming evidence is essential. Outcome studies require detailed assessments and comparisons with subjects without intersex conditions, given the complexity of sexual response, the variations in arousal and orgasm among typical individuals without genital surgery,<sup>34</sup> and the limitations of self-report in assessing sexual response.<sup>35</sup>

### Psychological Impact of Genital Appearance

Both physicians and intersex advocates are concerned about psychological problems associated with intersexuality. Physicians suggest that children who look different will have difficulty forming a coherent self-concept, including gender identity, and receive negative reactions from others, with adverse effects on adjustment and life satisfaction. Some intersex advocates argue that problems result from stigma and shame induced by messages from physicians and parents that atypical genitalia are unacceptable.

Neither set of concerns have been empirically validated – or refuted. There are no data showing the relative importance or unimportance of normal-appearing genitalia for psychological outcome. The existence of gender dysphoria in individuals with and without intersex conditions indicates that normal-appearing genitalia are not sufficient for gender identity consistent with rearing sex, but there is no systematic study of the role (if any) that genital appearance plays in the development of gender identity. It is widely believed that boys with a small penis are teased, causing poor peer relationships and adjustment problems. Although this has not been systematically studied, males with micropenis appear to do well.<sup>24,25</sup> Relevant data from boys with hypospadias who had received genital surgery show psychological adjustment similar to that of control boys, with little relation between adjustment and genital appearance, but depression is associated with more surgery and hospitalizations.<sup>36</sup>

Evidence from individuals with other physical conditions reinforces the complex contributors to outcome. Problems in individuals with intersex conditions might not arise from specific aspects of the condition or treatment itself, but from the stresses they impose on the patient and the family.<sup>37</sup> Children's stress may arise from their own experiences, such as surgery, repeated physical exams and hospitalizations, responses to their unusual genital appearance, or from changes in parent-child interactions brought about by parents' stress. Parent stress may be independent of the child's physical illness or may result from it, for example, from concerns about the child's genital appearance, responsibilities of caring for a sick child, or financial burdens brought about by the child's illness. Additional risk may arise from children's problems with peer relationships,<sup>38</sup> but even here the cause is not simple. Peer problems are affected by more than physical appearance, such as frequent school absences and sex-atypical behavior.<sup>37,39</sup> Furthermore, the association between peer relationships and adjustment is bidirectional: poor peer relations place a child at psychological risk, but poorly adjusted children have difficulty making friends to start.

### Psychological Outcome in Intersexuality

Thus, there are many paths by which mental health might be affected in individuals with intersex conditions, but there is no evidence regarding any of them. Further, there is surprisingly little evidence about the ultimate mental health outcomes hypothesized to be affected by these paths, primarily because such studies are difficult. Scientific studies may undersample individuals with problems, but reports from intersex activists may overrepresent those with problems.<sup>40</sup>

The most systematic evidence regarding mental health in intersex individuals comes from females with CAH. Several studies show that their mental health is not different than that of controls, although they may have specific problems with body image and psychosexual function.<sup>41-46</sup> There are not enough data to know whether outcome is related to genital appearance or surgery.

These results on good adjustment might be surprising in light of assumptions described above. However, they are consistent with evidence that chronic illness, trauma, and other adverse life events have only transient effects on adjustment in the majority of people. Among individuals with a variety of physical disabilities (including quadriplegia), there is often an immediate period of depression, but after a short period (weeks to months), most report positive well-being.<sup>47,48</sup>

This mismatch between expectation and evidence is an example of the tendency to attribute outcome to the cause that is most salient, in this case, the appearance of the genitalia or the intersex condition itself. But, outcome is influenced by many factors, including temperament and life circumstances. People are not accurate at predicting factors that influence life satisfaction in others because they only focus on a small set of contributors.<sup>49</sup> This means that attributions about problems among intersex individuals must be validated empirically.

### Recommendations Regarding Surgery

The lack of systematic outcome data makes decisions about genital surgery very difficult. There are insufficient data regarding the functional consequences of genital surgery, but there are also insufficient data regarding the effects on a child of living with atypical genitalia. It is likely that the effects of both genital surgery and genital appearance are not the same for all individuals. Perceptions of and responses to the situation may be more important than its objective nature, and psychological support may help families develop coping strategies to foster mental health. It is important to remember that decisions should be made in the best interests of the child and not the parents.



## CONCLUSIONS

The discussions surrounding the treatment of children with intersex conditions have crystallized the assumptions and evidence underlying treatment. Changes to treatment must be informed by evidence or, consequently, dilemmas will arise again. Despite gaps in the evidence regarding outcome, there is some information available to guide treatment.

First, sex assignment cannot be based on the assumption that gender identity is determined by either sex of rearing or degree of fetal androgen exposure. Most individuals with 46,XX CAH do well when reared as girls, but there are no systematic studies of those reared as boys. Most individuals with 46,XY micropenis appear to do well when reared as boys, but this approach should be viewed cautiously until there is more evidence about psychological and sexual outcome with male vs. female rearing. There is insufficient evidence regarding other causes of intersexuality and cloacal exstrophy, but all children should be assigned as girls or boys, with the recognition that some may change gender later in life.

Second, decisions about surgery would benefit from systematic evidence regarding functional outcome of current procedures and consequences of atypical genitalia. Sexual function involves more than cosmetic

appearance and the ability to have intercourse. Given the dearth of evidence, assumptions and biases should be clearly articulated to families.

Third, there is a pressing need for additional systematic evidence that addresses the complex determinants of psychological outcome. It is not sufficient to examine outcome only in relation to characteristics of the intersex condition and its treatment. There must be recognition and consideration of the child's temperament, family situation, culture in which the child lives, and benefits of psychoeducational interventions to reduce stress and facilitate coping.

Outcome itself must be defined from the perspective of the patient, and include quality of life. The components of outcome are not interchangeable (Table 3).

Fourth, translation of findings to treatment requires that studies meet important methodological criteria regarding sampling, assessment, and inferences consistent with the limitations of the methodology (Table 4). It is important to avoid being swayed by studies that support preconceptions or provide simple solutions.

Recent debates have improved treatment of children with intersex conditions by forcing an articulation of assumptions and examination of evidence. Resolution of current controversies requires a commitment to

Table 4

### Considerations in Evaluating Outcome Studies of Children with Intersex Conditions

#### Sampling

What was the population sampled?

What proportion of potential participants were studied?

How do the participants compare to the nonparticipants?

How would results change if nonparticipants have different outcome?

What was the comparison group?

Were the samples of intersex and comparison individuals large enough to see effects of clinical significance, including group differences and predictors of outcome?

#### Outcome Assessment

Were different aspects of outcome carefully differentiated?

For example, was gender identity measured independently of gender role?

Was each outcome assessed in detail with reliable and valid measures?

Were patients compared to controls to be sure that outcome is specific to an intersex condition?

Were hypothesized predictors of outcome assessed in detail with reliable and valid measures?

#### Inferences

Were appropriate statistical comparisons made so that inferences can be made to the population?

To what populations can results be generalized?

Can outcome be empirically attributed to intersex condition itself?

Can outcome be empirically attributed to specific factors related or unrelated to intersex condition?

Are inferences appropriately qualified in light of (inevitable) methodological limitations?



evidence-based care and a recognition that outcome in intersexuality cannot be simply predicted from medical factors alone.

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## Commentary: Intersex Issues - A Series of Continuing Conundrums

Dr. Blizzard has abstracted and commented upon two extraordinarily important manuscripts by Migeon and colleagues. These investigators have provided the first analysis of the long-term outcome of 75 adults with male pseudohermaphroditism or micropenis (46XY or 45X/46XY) managed as children at Johns Hopkins Hospital. These children had been assigned to either the male or female gender. All of 18 patients with feminine external genitalia (androgen insensitivity syndrome or complete gonadal dysgenesis) were raised as females; 5/18 subjects with micropenis (stretched length <1.9 cm) without hypospadias were reared as females. In 39 subjects with ambiguous genitalia, 18 of whom were raised as female and in whom in depth information concerning their "sexuality" was sought, the assigned sex was at least "satisfactory" in the majority. Indeed, those reared as male had greater incidence of atypical external genitalia and greater dissatisfaction with perceived "body image". In general, however, the

outlook for normal adult heterosexual adjustment reared as either male or female was quite good in this group.

Until more complete data are available, these observations can serve as the basis upon which to counsel the parents of a neonate with male pseudohermaphroditism in regard to their choice in the gender assignment of their offspring. Dr. Blizzard correctly states that the "paternalistic" approach to medical practice is no longer tenable.

*In my opinion, in the context of this psychosocial emergency, it remains extremely important that the experienced physician assist, perhaps even guide, the parents through the decision making process. In the absence of androgen insensitivity, complete gonadal dysgenesis, deficiency of P450<sub>side chain cleavage</sub> or 17-hydroxylase/17-20 lyase, and related disorders, it seems most appropriate to rear the incompletely virilized male in the masculine gender if there is at all sufficient penile corpus to do so or to permit its surgical amplification.*

Dr. Blizzard critically analyzes the current thinking concerning the problem of when to perform reconstructive genital surgery in the patient with male pseudohermaphroditism assigned to the female gender.

*In my opinion, he correctly rejects the extremist position that no reconstruction be undertaken until the patient herself can consent. Clearly, this approach will lead to great duress in the lives of the patient and her parents. (One can barely imagine the stress that a parent would be under in raising a child whose gender may change or that of the child who will surely learn at a surprisingly early age that her genitalia differ from those of other girls.) While each child must be considered individually, cliteroplasty during infancy and vaginoplasty at adolescence seem reasonable in my opinion once feminine gender has been assigned until the long-term efficacy of earlier vaginal reconstructive techniques have been evaluated.*

Dr. Blizzard discusses the issue of intra-cultural differences in attitude toward the problem of intersex and the challenging question of whether all children with 46XX female pseudohermaphroditism should be reared as females.

*His thoughtful and insightful comments are seconded by this writer, although my inclination is to rear all females with virilizing congenital adrenal hyperplasia as girls. Individualization of care and informed parental choice*

*are the keystones upon which management of the neonate with atypical external genitalia must be based.*

*Readers who wish to be brought up-to-date concerning some of the conundrums of intersex issues and what the current concepts are concerning intersex issues will benefit from Dr. Blizzard's commentary.*

Blizzard RM. *Pediatrics* 2002;110(3):616-621.

Allen W. Root, MD

**Dr. Blizzard's Comment:** *Comments about one's commentary are not necessarily legitimate. However, I comment relating the above abstract and editorial comment by Dr. Root to the lead article in this issue by Dr. Sheri Berenbaum. Her studies and writings are always logical, intelligent, and scientifically based. In her article, Dr. Berenbaum demonstrates the applicability of my adjectives used to describe her approaches to solving the conundrums of intersex. I highly recommend each reader contemplate her description of the complexities in this field. Hopefully others will approach the conundrums of intersex in the same contemplative way as does she.*

Robert M. Blizzard, MD

#### Letter to the Editors:

In the December 2002 edition of *Growth, Genetics & Hormones* (Vol. 18, No. 4), two articles (Imaizumi K, et al. *Am J Med Genet* 2002;107:58-60; Kurotaki N, et al. *Nat Gen* 2002;30:365-366) were abstracted under the title *A Gene as a Major Cause of Sotos Syndrome Has Been Identified*. The authors are reported to state that the identification of a deletion or mutation of this mutated gene on chromosome 5 will sometimes help in the diagnosis of Sotos syndrome, etc. Both Dr. Judy Hall and Dr. William Horton gave cogent editorial comments.

However more recent evidence indicates that additional knowledge gained by Kurotaki and others should be considered by clinicians and investigators attempting to use identification of a deletion or mutation of this mutated gene (NSD1) to help in the diagnosis of Sotos syndrome. Specifically, at the ASHG meeting in October 2002, Kurotaki et al from Japan reported finding point mutations and deletions of the NSD1 gene in a large series of patients and Clech et al from Paris reported their findings in 39 patients. Only 14 were felt to have typical Sotos syndrome; four had a

NSD1 deletion of paternal origin. It had previously been suggested that based on similarity of the phenotypes, Sotos and Weaver syndromes might be allelic disorders. Rahman et al from the UK reported that >40% of patients with typical Sotos syndrome had intragenic mutations in NSD1 and 3 of 7 patients with Weaver syndrome had intragenic NSD1 mutations. In each of these series, patients with a combination of overgrowth and mental retardation, but without typical features of either Sotos or Weaver syndrome, were not found to have deletions or intragenic mutations of NSD1.

These reports collectively demonstrate that the majority of patients with typical Sotos and Weaver syndrome have intragenic mutations or deletions of NSD1, and thus, represent allelic disorders. However, the combination of overgrowth and mental retardation represents a heterogeneous phenotype in which only a portion is accounted for by abnormalities of NSD1.

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## Editorial Comment:

Sotos syndrome and Weaver syndrome are both overgrowth syndromes beginning usually prenatally. Such overgrowth continues during childhood. These two syndromes are similar in many respects; in respect to overgrowth, mental retardation, large hands and feet, advanced bone age, and tall stature but, usually, adult height within the normal advanced percentiles. However, they do differ in certain subtle respects. The patient with Sotos syndrome (cerebral gigantism) has a head that is dolichocephalic. The occiput tends to be flat in the patients with Weaver syndrome. The face tends to be smaller. There are hypoplastic facial bones and

macrognathia in Weaver syndrome, but pointed chin and normal mandibular development prompts one to think more of Sotos syndrome. The joints are limited in motion often in Weaver syndrome with limited elbow, ankle, wrist, hip, and knee extension. The long bones are widened or splayed in Weaver syndrome and camptodactyly is frequent. Further details concerning these two syndromes can be pulled from the pediatric database, although the update listed is 1994 (<http://www.icondata.com/health/pedbase/files/sotosynd.htm> - or - [weaversy.htm](http://weaversy.htm)). Comparable data can also be found on the web at <http://www.nlm.nih.gov>. At this web site you will have a choice to enter "Weaver".

Robert M. Blizard, MD

## Abstracts from the Literature

### Circulating Levels of IGF-1 Directly Regulate Bone Growth and Density

Previous studies by LeRoith and co-workers and Ueki et al have demonstrated that selective loss of liver-derived insulin-like growth factor-1 (IGF-1) or of acid labile subunit (ALS) does not substantially impair murine growth and development despite marked decline in circulating levels of IGF-1.<sup>1,2</sup> This has led to the suggestion that only the IGF-1 produced locally by bone is necessary for linear growth.<sup>3</sup>

In order to explore this question further, LeRoith and his colleagues developed double "knock-out" animals which were deficient in both liver IGF-1 and ALS (LID-ALSKO), and compared these with animals deficient only in liver IGF-1 (LIDKO) or ALSKO. As anticipated, serum concentrations of IGF-1 were decreased markedly, -65% in ALSKO, -75% in LIDKO, and -90% in LID-ALSKO relative to control animals with normal hepatic IGF-1 and ALS production. However, the rate of IGF binding protein-3 (IGFBP-3) degradation was also increased in these animals; thus free IGF-1 values were increased modestly in LIDKO (+150%), minimally in ALSKO (+108%), and markedly in LID-ALSKO animals (+350%). Growth hormone and insulin concentrations were greatly increased in LID-ALSKO mice. The clearance of IGF-1 was markedly accelerated in ALSKO (32 minutes) and LID-ALSKO (18 minutes) as compared with control (69 minutes) and LIDKO (73 minutes) mice, reflective of lack of binding of IGF-1 to IGFBP-3/ALS.

Intrauterine growth of all animals was apparently normal. By 3 weeks and 4 weeks of post natal age (Figures), the length and weight of the LID-ALSKO mice were less than those of the intact animals. Linear growth of the LIDKO and ALSKO animals did not differ from controls. However, the rate of weight gain of ALSKO mice was impaired to the same extent as that of the LID-ALSKO group. Tibial length, and heights of germinal, proliferating, and hypertrophic zones of the proximal

tibial growth plate, were significantly diminished in the LID-ALSKO mice but not in the two single "knock-out" groups. On the other hand, femoral length, total and cortical bone density, periosteal circumference, and cortical and trabecular bone volume were diminished in all "knock-out" groups, but to a substantially greater degree in the LID-ALSKO animals. Administration of exogenous IGF-1 increased linear growth, femoral length, and size of the proximal tibial growth plate, as well as IGFBP-3 concentrations, in all groups. IGF-1 mRNA levels in bone were similar in all groups.

The investigators concluded that circulating IGF-1 was important for linear and appositional bone growth and bone mineralization and that its effects were mediated through actions on periosteal osteoblasts as well as upon chondrocytes within the epiphyseal growth plates.

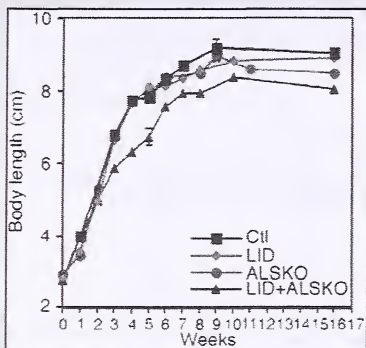
Yakar S, et al. *J Clin Invest* 2002;110:771-781.

**First Editor's Comment:** This important paper establishes the necessity of circulating IGF-I for normal growth and bone mineralization. It demonstrates that osseous synthesis of IGF-I alone is insufficient for normal linear growth of bone and mineral deposition. Thus, reexamination of the "somatomedin hypothesis" suggests that both liver derived and locally synthesized IGF-I are necessary for normal bone metabolism. Interestingly, "knock-out" of any of the IGFBPs has little effect upon the phenotype of the mutant mouse, but their over expression results in inhibition of growth.<sup>4</sup> One wonders what the phenotype of the mouse that lacks IGF-I, IGFBP-3, and ALS might be ... possibly lethal?

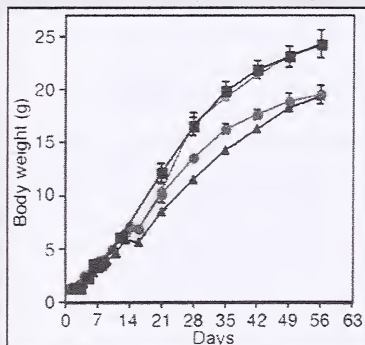
Allen W. Root, MD

## Figures

## Postnatal growth in LID+ALSKO mice



Body length was measured from nose to anus at weekly intervals ( $n = 20-30$  mice per group).



Body weight was measured at weekly intervals from birth to the age of 8 weeks ( $n = 30-60$  mice per group).

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4. Silha JV, Murphy LJ. *Endocrinology* 2002;143:3711-3714.

**Second Editor's Comment:** In *Growth, Genetics & Hormones* (Vol. 18, No. 3), an important lead article entitled *Somatomedin Hypothesis: Time for Reexamination* was written by Dr. Solomon Kaplan. He has been asked to write an editorial comment.

**Dr. Kaplan's Comment:** The paper by Yakar et al extends and amplifies the findings in a previous publication by the authors<sup>1</sup> on the role of circulating IGF-1 in promoting longitudinal growth in mice. They had already shown that despite inactivation of the IGF-1 gene in the liver, resulting in reduced concentrations of circulating IGF-1 by as much as 75%, the growth of the animals was not impaired. Their findings were consistent with the growing body of evidence against the validity of the somatomedin hypothesis, which holds that the effects of growth hormone on longitudinal growth are mediated through hepatic production of IGF-1.<sup>2</sup>

IGF-1 circulates in the serum largely as a 150-kDa complex comprised of the IGF-1 molecule, IGF binding proteins (mostly IGFBP-3), and the acid labile subunit (ALS). Others had previously shown that ALS knockout (ALSKO) mice experienced only mild growth retardation despite profound disruption of the circulating IGF system.

Yakar's current paper reported the effects of double gene disruption of the IGF system: inactivation of the hepatic gene for IGF-1 (LID) combined with ALSKO, on bone growth and density. In the mice carrying the double gene deletion, there was a reduction of circulating IGF-1 concentrations by as much as 85 to 90%; the animals also experienced significant growth impairment. There was a diminution in the amount of circulating IGFBP-3 protein and also in the free IGF-1 fraction. Loss of ALS led to more rapid disappearance of 125-I labeled IGF from the serum because absence of the ALS protein

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leads to proteolytic cleavage of IGFBP-3 and loss of its protective binding of IGF-1. The authors conclude that a minimum concentration of IGF-1 in the serum, higher than what they observed in the double gene-deletion mice, is necessary for normal bone and somatic growth.

Following administration of IGF-1 by injection, the animals with the double gene deletion experienced increased serum IGF and IGFBP-3 concentrations accompanied by restoration of normal bone growth and modeling, as well as increased somatic growth. These findings are consistent with their observation that the restoration of normal growth can be accounted for by increased serum IGF-1 concentrations above the minimal levels necessary for normal growth to occur.

This paper provides confirmatory evidence that hepatic derived IGF-1 and acid labile subunit are not necessary for normal growth provided minimal serum levels are maintained from non-hepatic sources including autocrine/paracrine production by target tissues.

Solomon A. Kaplan, MD

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## The BRCA2 Gene's Role in Fanconi Anemia and Various Cancers

Fanconi anemia (FA) is an autosomal recessive disorder in which affected subjects have great susceptibility to neoplasia early in life, including acute myeloid leukemia and squamous cell carcinoma. Bone marrow failure is also frequent, as well as mutations in at least 8 groups of FA patients (A, B, C, D<sub>1</sub>, D<sub>2</sub>, E, F and G) and germline mutations in six of these have been identified in 6 genes (A, C, D<sub>2</sub>, E, F and G). The FA cells manifest many broken and misshapen chromosomes reflecting that FA proteins participate in the repair of DNA damage, either stimulating or inhibiting normal repairs. Five of the 6 genes previously described combine in a multi-subunit nuclear complex which activates by ubiquitination of the protein product of a sixth gene (FANCD2) which is involved in the process of DNA repair. Howlett et al<sup>1</sup> identified a 7th gene by demonstrating that homozygous "loss of function" mutations occurring in the BRCA2 gene (causing breast cancer as does the BRCA1 gene) occurs in a subset of patients with FA.

Witt and Ashworth<sup>2</sup> stated in the introduction of their commentary; "Important discoveries are so neat and satisfying that, in retrospect, they seem obvious. Howlett et al disclosed that the inheritance of two defective copies of the BRCA2 breast cancer susceptibility gene can lead to FA. The BRCA2 protein is thought to be important in the repair of DNA damage. Cells lacking BRCA2 inaccurately repair damaged DNA leading to gene mutation and progression of tumors and are particularly sensitive to DNA cross-linking agents. Howlett et al demonstrated that one of the previously unidentified FA genes (FANCD1) is BRCA2." No BRCA1 mutations were found in the patients studied by Howlett et al. However, all the authors of all three papers speculatively agreed that the 6 previously cloned genes are linked in a common pathway with BRCA1 and BRCA2 genes.<sup>1-3</sup>

Venkitaraman<sup>3</sup> in his closing comments stated; "The network which connects BRCA and FA proteins in DNA

repair includes at least two other molecules - ATM (mutated in ataxia telangiectasia) and CHEK2 - whose inactivation is also associated with carcinogenesis in several tissues. Although the precise functional connections between the molecules in this network remain obscure, it is clear we are glimpsing an important tumour suppressor pathway whose disruption may underlie many different types of human cancer."

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**First Editor's Comment:** Heterozygous inactivating germline mutations in BRCA1 and BRCA2 have been linked to increased susceptibility to breast and ovarian cancer in women.<sup>1</sup> In the tumors that develop in these patients, there is loss of heterozygosity of BRCA1 or BRCA2. Both BRCA1 and BRCA2 are important for repair of DNA damaged by exposure to ionizing radiation and cross-linking, and do so by interrupting the cell cycle while promoting repair of the damaged DNA strands.<sup>1-3</sup> The carboxyl-terminal domain of BRCA2 likely binds to single strands of DNA at the site(s) of a double stranded DNA break and facilitates the binding of other repair factors such as RAD51, an important member of this family. This article is of interest because it demonstrates the difference in phenotypes that result from heterozygous as compared to homozygous germline mutations in BRCA2. How this mutation affects somatic growth and the reproductive endocrine system is unclear. However, Wajnrach et al<sup>4</sup> found aberrations of endocrine function in 44/54 primarily prepubertal patients with FA.<sup>4</sup> Abnormalities included short stature with mean height SDS -2.35 (due to growth hormone insufficiency in 44%), hypothyroidism (36%), hyperinsulinemia (72%), impaired glucose tolerance (25%), and diabetes mellitus (2%). Skeletal maturation

was approximately one year delayed behind chronologic age; predicted adult height in 22 subjects was -1.24 SDS.

## References

1. Wilson JH, Ellledge SJ. *Science* 2002;297:1822-1823.
2. Yang H, et al. *Science* 2002;297:1837-1848.
3. Witt E, Ashworth A. *Science* 2002;297:534.
4. Wajnrajch MP, et al. *Pediatrics* 2001;107:744-754.

Allen W. Root, MD

Robert M. Blizzard, MD

## Serum Zinc in Infants and Preschool Children in the Jeddah Area: Effect of Diet and Diarrhea in Relation to Growth

Dr. Bahijri has written a thoughtful analysis of the etiology and effect of zinc deficiency on wasting and stunting of 728 children in 5 age groups (4-6, 6-<12, 12-<24, 24-<36, and 36-72 months). Using the concept of weight for height, the subjects were classified according to their grade of wasting, and using the concept of height for age, the subjects were classified according to their grade of stunting. The dietary, auxological, and chemical evaluations were carefully done in accord with the most modern standards and techniques. The study was undertaken to determine the prevalence of zinc deficiency in the Jeddah (Saudi Arabia) area among preschool age children, to see whether such a deficiency is a cause of retarded growth, to determine whether a relationship exists between height for age and serum zinc concentrations, and if possible to determine the causes of zinc deficiency.

The authors presented serum zinc levels in the various age groups for subjects: (1) without stunting and wasting, (2) with various grades of wasting, (3) with various grades of stunting, and (4) with both stunting and wasting. Many subjects in each group had zinc levels <10.4  $\mu\text{mol/L}$  which is frequently cited in the literature as the cut off for normalcy. However, the lowest mean serum zinc levels were found in the patients in the group with stunting and wasting. Whereas those who had neither stunting nor wasting had the highest levels. The older stunted children (group 3) had lower zinc levels than those found in the younger children. All patients with wasting (group 2) had hypozincemia.

The authors concluded that diarrhea rather than low dietary intake mostly accounts for the low zinc levels in infants (4-12 months). As the subjects passed the 24 month mark, diet deficiency became the presumed major cause of hypozincemia and this cause became more dominant as the etiology in the oldest age group (36-72 months).

The importance of zinc in biology is well reviewed, including that zinc is known to influence cell division, growth and development, as well as sexual maturation. It is needed also as a membrane stabilizer, and is

**Second Editor's Comment:** The phenomena described in the papers given as references are phenomenal. The first 3 references read as a package will permit any reader not informed about such matters to advance into the upper elementary levels, both in respect to understanding the physiology and pathophysiology of Fanconi Anemia, breast cancer, and to the interactions of genes and gene products.

essential for the integrity of the immune system. More than 100 enzymes require zinc as a cofactor, and zinc seems to be involved in the proper storage and release of insulin, growth and repair of tissues, wound healing, ability to taste food, production of prostaglandins, mineralization of bone, blood clotting, function of vitamin A, and functions of the thyroid hormones.

Not commonly known, an important predisposing factor for zinc deficiency is the extensive use of cereal protein which limits the availability of zinc due to high phosphate and phytate content. The recommended dietary allowance of the Food and Nutrition Board and the National Academy of Sciences in the United States is 15 mg/day for adult males and 12 mg/day for adult females, with higher recommended levels during pregnancy and lactation. Requirements for infants and children are relatively high in relation to body size because of increased requirements for physical growth.

The best sources for zinc in the diet are meat and fish; the bioavailability of zinc from animal products is considered to be greater than that from plants. Diarrhea is associated with zinc deficiency and low serum zinc concentration. Suggestions have been made that growth retardation commonly seen in children in developing countries is related to zinc nutritional deficiency.

Unfortunately, it was not feasible to interpret the direct effect of zinc deficiency on wasting or stunting although a significant majority of subjects with wasting and/or stunting had severe deficiency. The author summarized: "The result of this work shows a high incidence of low serum zinc levels among Jeddah-area infants and young preschool children, which is associated with diarrhea and wasting in the first two years of life, and generally low dietary intake, wasting and/or stunting in older children. Zinc supplementation is recommended for certain categories of subjects to improve appetite and hence dietary intake, immunocompetence, and anthropometric measurements."

Bahijri SM. *Annals of Saudi Medicine* 2002;21:324-329.

**First Editor's Comment:** A complete reprint of this article will be sent to those who request it by e-mail to [rbllizzard@compuserve.com](mailto:rbllizzard@compuserve.com).

Unfortunately in nearly all studies of this type it is difficult to separate cause and effect. For example, does malnutrition or illness produce wasting and/or stunting accompanied by zinc deficiency or is the zinc deficiency etiologic in malnutrition and/or illness and/or stunting and/or wasting? In spite of this excellent study, the answer to this question remains an enigma. Moreover, zinc supplementation seems indicated to a much greater extent than currently in use.

Robert M. Blizzard, MD

**Second Editor's Comment:** Recently Brown et al<sup>1</sup> published a meta-analysis of randomized controlled trials of the effects of supplemental zinc on the growth and serum concentrations of prepubertal children. A total of 33 studies were compiled demonstrating that zinc supplementation produced a significant positive height response and an increase in serum zinc levels. Growth responses were greater in those children with low weight for age and low height for age. This paper was reviewed in *Growth, Genetics & Hormones in 2002* (Vol. 18, No. 4) and the importance of recognizing the value of zinc nutriture in "at risk" populations was emphasized.

However the note of caution noted below by Dr. Tarim should be kept in mind.

Fima Lifshitz, MD

# Reference

1. Brown KH, et al. *Am J Clin Nutr* 2002;75:1062-1071.

## Letter to the Editor:

I would like to add a precaution before suggesting zinc supplementation to anyone with nutritional growth retardation who lives in places where zinc deficiency may be prevalent. Iron deficiency which may co-exist with zinc deficiency may be aggravated during zinc therapy because these two minerals may block the intestinal absorption of each other.<sup>1</sup> Consequently, iron deficiency may also worsen growth retardation. Therefore, I suggest excluding iron deficiency, which is easier to diagnose than zinc deficiency, before initiating zinc supplementation.

Omer Tarim, MD

Director of Pediatric Endocrinology  
Uludag University Faculty of Medicine  
Bursa, Turkey

## Reference

1. Lifshitz F, et al. Nutritional Growth Retardation. In: Lifshitz F, ed. *Pediatric Endocrinology 3<sup>rd</sup> Edition*. New York: Marcel Dekker, 1996:103-120.

## Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

Because multiple laboratory tests are used in the diagnosis and management of this disease, the quality of the scientific evidence supporting the use of these assays varies. Therefore, an expert committee drafted evidence-based recommendations for the use of laboratory analysis in patients with DM. An external panel of experts (DB Sacks, DE Bruns, DE Goldstein, NK MacLaren, JM McDonald and M Parrott) reviewed a draft of the guidelines, which were modified in response to the reviewers' suggestions, and other steps were taken to gain a consensus of expert opinions. The guidelines, as published in *Clinical Chemistry*, consist of an Executive Summary of one page providing specific recommendations based on data published or expert consensus. Several analyses are of minimal clinical value at the present time and measurement of them is not recommended. The entire article is 42 pages. Those clinicians treating diabetics should at least scan the article and closely scrutinize the Executive Summary.

Highlights of the Executive Summary are now presented:

Glucose should be measured in an accredited laboratory to establish the diagnosis of DM and to screen high-risk individuals. Blood should be drawn after an overnight fast. Glucose should be measured in plasma. If plasma cannot be separated from cells within 60 minutes, a tube with glycolytic inhibitor should be used. On the basis of biological variation, glucose analysis should have analytical imprecision less than 3.3%, bias less than 2.5%, and total error less than 7.9%.

The OGTT is not recommended for the routine diagnosis of type 1 or 2 DM. The key limitation of the OGTT is its poor reproducibility. It is recommended for establishing the diagnosis of gestational DM.

Because of the imprecision and variability among glucose meters, they should not be used to diagnose DM and have limited value in screening. Noninvasive glucose analyses cannot be recommended at present as replacements for plasma glucose or measurements by an accredited laboratory. Glycated hemoglobin (GH<sub>1c</sub>) should be measured at least biannually in all patients with DM. US laboratories should use GH<sub>1c</sub> assays certified by the National GH Standardization Program



(NGSP) as traceable to the DCCT reference. GH<sub>5</sub> levels should be maintained at <7% and the treatment regimen should be reevaluated if GH<sub>5</sub> is >8% as measured by NGSP - certified methods.

Routine measurement of genetic markers is not recommended for the diagnosis or management of patients with DM. Likewise, autoimmune markers lack specificity and are not recommended for routine diagnosis or screening of DM.

An annual search for micro albuminuria should be performed on patients without clinical proteinuria. To be useful, semiquantitative or quantitative screening

tests must be shown to be positive in >95% of patients with micro albuminuria. Positive results must be confirmed by quantitative testing in an accredited laboratory.

All adults with DM should receive annual lipid profiles.

Sacks DB, et al. *Clinical Chemistry* 2002;48:3,436-472.

**Editor's Comment:** This is only the very essential infrastructure of the Executive Summary. The article is endowed with significant substance.

Robert M. Blizzard, MD

## Mutations of the *Great* Gene Cause Cryptorchidism

The investigators previously identified a mutant strain of mice (*crsp*) with high intraabdominal bilateral cryptorchidism due to a 550 kb deletion of the proximal arm of mouse chromosome 5. Within the deleted region, the investigators identified a G-protein coupled receptor gene (GPCR) termed "G-protein coupled receptor affecting testis descent" or *Great*. *Great* was expressed in testis, brain, and skeletal muscle. In the current paper, the authors developed a mouse "knock-out" model of this gene. The phenotypes of the wild type mice and those who were heterozygous (*Great*<sup>+/</sup>) were normal. However, animals who were homozygous for the mutation (*Great*<sup>-/-</sup>) were similar in phenotype to *crsp* mice. In (*Great*<sup>-/-</sup>) mice, there was failure of development of the gubernaculum (the ligament whose shortening is partially responsible for the inguinal-scrotal phase of testicular descent). The investigators then cloned human *GREAT* (chromosome 13q12-13), an 18 exon gene encoding a GPCR, and analyzed its structure in 61 men with bilateral (N=31) or unilateral cryptorchidism. In one subject with bilateral cryptorchidism, a heterozygous loss-of-function mutation was identified (exon 8, A C, Tyr222Pro was identified). The authors concluded that mutations in *GREAT* are responsible for cryptorchidism in some human males but the frequency of a *GREAT* as a cause of cryptorchidism mutation remains to be determined.

Gorlov IP, et al. *Hum Molec Genet* 2002;11:2309-2318.

**First Editor's Comment:** *GREAT* had been cloned by other workers and termed *LGR8 - Leucine-rich repeat-containing GPCR*. Relaxin had been identified as a ligand for *GREAT*. However, testicular descent is normal in the Relaxin "knock-out" male mouse. *InsI3 - insulin-like factor 3 - is a member of the relaxin family and is synthesized in the testes; its loss results in bilateral cryptorchidism due to maldevelopment of the gubernaculum. Thus, InsI3 may be the natural ligand*

*for GREAT. While homozygous loss of Great is needed for cryptorchidism in mice, apparently its heterozygous loss appears to be sufficient in humans to cause this malformation; the mechanism(s) of this species difference is/are not defined at present.*

*There are two phases of testicular descent - transabdominal and inguinal-scrotal. The first phase is conditioned by failure of development of a cranial suspensory ligament mediated by testosterone. The second phase is stimulated by development of the gubernaculum, demonstrated to be related to the interaction of InsI3 and GREAT. Mullerian duct inhibitory factor and its receptor also play a role in this phase of testicular descent. The manuscript also suggests that it would be inappropriate to tell another gentleman that he is "not so GREAT!"*

Allen W. Root, MD

## References

1. Overbeek PA, et al. *Genesis* 2001;30:26-35.
2. Nef S, Parada LF. *Nat Genet* 1999;22:295-299.
3. Teixeira J, et al. *Endocrine Rev* 2001;22:657-674.

**Second Editor's Comment:** This article is the best I have read concerning the development and descent of the testes. Work in mice and in humans is blended in describing the embryological development of both testes and ovaries. The 11 authors come from diverse and multiple fields - urology, genetics, pharmacology, embryology, molecular biology, etc., which largely accounts for the excellence of the article. Those interested in gonadal development, normal and/or abnormal, will be gratified in reading the article in its entirety.

Robert M. Blizzard, MD



## Kyphosis in Turner Syndrome

Elder and colleagues performed lateral thoracic spine and standing anterior-posterior scoliosis radiographs in 25 of 30 girls between the ages of 5 and 18 years with Turner Syndrome. Excessive kyphosis was defined as an A-P curvature greater than 40%, vertebral wedging as an A-P deformity greater than 5% at any vertebral body, and scoliosis as a lateral curve greater than 10%. Karyotype, age, height, weight, and body mass index percentile, and use and duration of growth hormone, oxandrolone (anavar), and/or estrogen were recorded and entered into a linear regression analysis to determine significant predictors of kyphosis or kyphosis and wedging. Of the 25 subjects studied, 15 (60%) had abnormal radiographic findings. Ten (40%) had excessive kyphosis, 10 (40%) had vertebral wedging, and 5 (20%) had scoliosis. All girls older than 14 years of age ( $N=8$ ) had excessive kyphosis and wedging.

The subjects were  $12.0 \pm 3.6$  years old. Sixty percent had a 45X karyotype, 80% had received GH therapy, and 36% had received estrogen therapy. Logistic regression analysis revealed that chronologic age alone was predictive of excessive kyphosis/wedging, ( $P=0.053$ ). Stepwise linear regression analysis also showed that chronologic age was predictive of the degree of kyphosis ( $P=0.032$ ). None of the other variables were predictive. The authors remarked upon the high prevalence of vertebral wedging and excessive kyphosis in their study population. They noted that this is markedly increased compared with the reported prevalence of 3% in the general population. The cause of the scoliosis is apparently multi-factorial, but may include mechanical factors, osteoporosis, adolescent growth spurt, and intrinsic bone defect. Girls with Turner syndrome are known to have a significant number of bony abnormalities, including hypoplasia of cervical vertebrae, and hemivertebrae, although these were not found in the study population. The authors also note

that their inability to determine the contribution of age and hormonal therapies to the development of kyphosis may be the result of the small number of subjects studied.

PediaLink.org (Vol. 109) 6/2002. PPE 93.

**Editor's Comment:** *With such a huge number of Turner subjects (40%) with reported excessive kyphosis, it is surprising that there are not more reports of its prevalence. Indeed this study suggests all girls with Turner syndrome should have routine radiographic screening and should be evaluated by an orthopedist. It is also surprising that more information is not available regarding the probable pathogenesis of these deformities. Since the vast majority of subjects in the study had received or were receiving GH, its contribution to the development of the kyphosis is impossible to determine. However, information from subjects in larger multi-centered databases of individuals who have and have not been treated with GH, would be important to access in order to determine its possible role in the genesis of this deformity. Some information regarding the prevalence of kyphosis in children treated with GH who either had or did not have GH deficiency also could be an important comparison group. Unfortunately this study raises many more questions than it answers, but will probably stimulate other centers to evaluate girls with Turner syndrome. Perhaps a multi-centered survey could help provide a better understanding of this problem. The Growth, Genetics and Hormones Editorial Board welcomes a letter to the editor from readers who have knowledge of data pertinent to the questions raised.*

William L. Clarke, MD

## Cancer Risk in Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann Syndrome (BWS) is a well-known syndrome of overgrowth. Macrosomia, neonatal hypoglycemia, midline abdominal defects, macroglossia, ear pits and the predisposition to embryonic cancers in infants and young children, including Wilms tumor, hepatoblastoma and neuroblastoma are the important clinical features of BWS. It is now possible to correlate the phenotypic features with specific genetic disturbances. Most recently, alterations in the imprinting and methylation of several genes in the 11p15 region have been implicated in its etiology. Different patients have different involvement phenotypically and genetically.

De Baun et al have correlated anomalies of DNA methylation of one of the relevant genes, *H19*, in patients with cancer, as compared to those without. Those with cancer are less likely to have abnormalities of the methylation of another gene in the area, *LIT1*. Conversely, abnormalities of methylation of *LIT1* are more likely to be associated with abnormal wall defects and macrosomia. Affected individuals with paternal uniparental disomy of 11p15 are more likely to have associated hemihypertrophy, cancer, and hypoglycemia than those without uniparental disomy.

These findings suggest that all individuals with BWS deserve a precise molecular evaluation in order to be

able to appropriately screen for expected complications. The cluster of genes related to BWS has been studied extensively because of its involvement in the epigenetic phenomenon of imprinting. Abnormal and loss of imprinting of the *IGF2* gene found in this region is present in a number of tumors. *H19* plays a role in the methylation of *IGF2* and so its abnormal methylation or expression may increase the risk of cancer by its relation to *IGF2*.

In the evaluation of BWS, one would expect that cancerous tissue might have different imprinting or methylation than other easier to study tissues. This is particularly frustrating when hemihypertrophy is present. It is interesting to note that any hypertrophy observed in patients with BWS is suggestive of mosaicism. To date, all of the reported patients with paternal UPD of 11p15 are in fact, mosaic. Thus, the two sides of the body probably have different manifestations of the Beckwith-Wiedemann gene cluster.

The hypoglycemia that can be seen in Beckwith-Wiedemann Syndrome also is associated with

uniparental paternal disomy. Since hypoglycemia can result in secondary mental retardation, both screening and watching for hypoglycemia in patients with BWS is extremely important during infancy.

DeBaun, et al. *Am J Hum Genet* 2002;70:604-611.

**Editor's Comment:** *Most of the conditions recognized to be involved in genomic imprinting are associated with abnormalities of growth. Thus, the possibility of genomic imprinting must be considered in any syndrome of abnormal growth. Further evaluation can obviously lead to unique insights about pathogenesis as are being developed in the BWS. This work is allowing recognition of the heterogeneity existing in BWS that may predispose to severe complications.*

Judith G. Hall, OC, MD

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# GROWTH

## Genetics & Hormones

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### LAWSON WILKINS - PIONEER IN PEDIATRIC ENDOCRINOLOGY AND GROWTH DISORDERS: REVISITED 2003

Robert M. Blizzard, MD  
Editor-in-Chief

#### EDITORIAL INTRODUCTION

In March 1987 in *Growth, Genetics & Hormones*, Vol. 3, No. 1, the lead article with the same title as above was published (the original article is available at the *Archive* section of [www.GGHjournal.com](http://www.GGHjournal.com)). Dr. Wilkins was the founder of pediatric endocrinology. His contributions to pediatrics and pediatric endocrinology were substantial. He was a consummate teacher, practitioner, and investigator, and his personal characteristics were of an exceptional human. He must be known by those who use his name frequently, including members of the Lawson Wilkins Pediatric Endocrine Society and those who utilize his articles in the pediatric literature as references for their own writing. It is for this reason that in this current issue of *Growth, Genetics & Hormones* the article published in *GGH* in 1987 is revisited. In respect to this updating, the two considerations incorporated include an updating of chronological time and the providing of references with highlights concerning Lawson Wilkins as a leader, teacher, pediatrician, and investigator.



Lawson Wilkins, MD

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Forty years have passed since 1963, when Dr. Lawson Wilkins died at the age of 69. His demeanor, his accomplishments, and the esteem in which he was held by his peers and his extended family of pediatric endocrine fellows whom he trained are not known to the third and fourth generations of pediatric endocrinologists who are members of the Lawson Wilkins Pediatric Endocrine Society. Since volumes could be written about each aspect of Dr. Wilkins' life, an abbreviated biography is inadequate. Nevertheless, a brief history of Dr. Wilkins' life presents the opportunity to update the image of a man who should be known by pediatric endocrinologists, pediatricians, and geneticists.

Lawson Wilkins was born in 1894 in Baltimore. His father, Dr. George Wilkins, was probably the most highly respected family practitioner in the city. Historical accounts indicate that George Wilkins was intellectually



curious, dedicated to his patients, and attentive to detail. His son exhibited the same characteristics. Mrs. Wilkins' death, when Lawson was five years of age, significantly strengthened the already close bond between father and son.

After receiving a baccalaureate degree from Johns Hopkins University in 1914, Lawson Wilkins began medical school there. In 1917, along with many other medical students, he volunteered to go to Europe and served as an orderly in a medical unit during World War I. After the war, he was accepted as an intern in internal medicine at Yale for a year. He then returned to Baltimore to serve a pediatric internship at Johns Hopkins Hospital where the influence of Drs. Blackfan, Park, Kramer, and the other giants of pediatric medicine of the period further whetted his keen intellectual appetite.

It was most likely his desire to follow in his father's footsteps as a practitioner that prompted him to enter pediatric practice in Baltimore in the early 1920s. Until the time he accepted a full-time academic position in 1946, Dr. Wilkins had practiced pediatrics for 25 years with intense intellectual curiosity and great compassion for his patients. This author has on several occasions in the past met adults in Baltimore who remembered Dr. Wilkins fondly as their pediatrician. These individuals had no idea that Dr. Wilkins had made major contributions to medicine as an endocrinologist and a geneticist.

In 1935, Dr. Edwards Park, who was instrumental in the development of various subspecialties in pediatrics, invited Lawson Wilkins to establish an endocrine clinic in the Harriet Lane Home of the Johns Hopkins Hospital. Dr. Wilkins was reluctant since endocrinology at that time was the trade of quacks and charlatans. He accepted the position, however, and with Drs. Fuller Albright, John Eager Howard, George Thorn, Robert Williams, and a few others, he transformed endocrinology into a respectable subspecialty.

Wilkins focused on the problems in pediatric endocrinology - particularly problems of growth and genetics - while his confreres tended to the accumulation of knowledge about endocrinology in adults. Although he was intensely interested in the metabolism and control of carbohydrate and fat metabolism, he assiduously avoided a clinical interest in diabetes. Possibly this was because Dr. Harriet Guild of the Harriet Lane staff had established a diabetes clinic and, characteristically, Dr. Wilkins would not intrude on the work of others unless invited. Interestingly, he never considered diabetes a disease of the endocrine system, although he believed hypoglycemia was.

Lawson Wilkins was more than a scientific giant. He was a man of great magnetism and personality. Few who knew him could forget his bass voice which he put to good use singing ballads and bawdy songs long into the night. He loved to sail his boat on the Chesapeake Bay and tell jokes, which he masterfully embellished. He also adored - and was adored by - Lucile Mahool, his first wife, and Teence Anderson, to whom he was married after Lucile died in 1959.

At a meeting in Baltimore of the Lawson Wilkins Pediatric Endocrine Society in the mid-1960s, Dr. John Eager Howard\* related the following about Dr. Wilkins: "When I first met Wilkins, which was at a time I had heard about his studies that Dr. Park exalted, I was even more impressed by the vitality of the man than by his scientific studies. In response to my knock on the door, the rafters fairly reverberated to the booming voice that urged us to come in. His whispers in a conference could cause consternation, for his 'That fellow is putting out pure hogwash' might have been heard all over the room. But I should hasten to say that his comments were rarely uncomplimentary, for an immense generosity toward others was one of his most endearing qualities." In accord with Dr. Howard's observations, this author found Dr. Wilkins to be a paradox in that he was gruff but gentle. And while he always dominated the situation, he never exhibited dominating behavior toward individuals.

Another mark of the quality of Dr. Wilkins' personality was the grace with which he relinquished his pediatric endocrine clinic and training program to Dr. Claude Migeon and this author in 1960. During the next three years, before he died in 1963, he was present much of the time, he remained intellectually curious, and he continued to contribute in all respects.

## SCIENTIFIC CONTRIBUTIONS

Lawson Wilkins greatly expanded our knowledge of endocrine physiology and pathophysiology. Some of us have been fortunate enough to have shared in his experiences in establishing pediatric endocrinology as a subspecialty. Drs. Albert Bongiovanni,\* Claude Migeon, and Walter Eberlein shared his interest in adrenal steroid metabolism and the pathophysiology produced by deficiencies of various enzymes for cortisol synthesis, including defects in 21 hydroxylation and 11 hydroxylation that produce congenital virilizing adrenal hyperplasia. In 1950, Drs. John Crigler, Robert Klein, Lytt Gardner,\* Claude Migeon, and Eugenia Rosemberg joined Dr. Wilkins in successfully treating the first patients with congenital virilizing adrenal hyperplasia with cortisone. As always, Dr. Wilkins applied the knowledge he gained from his physiologic studies to therapy.

(\*Deceased)

Drs. Melvin Grumbach and Judson Van Wyk worked with Dr. Wilkins in his studies of sexual differentiation. In this area, Dr. Wilkins applied what had been learned from the animal experiments of Alfred Jost to postulate and prove that the anatomy in gonadal agenesis and pseudohermaphroditism in human beings could be explained by the presence or absence of androgens and Mullerian inhibiting factor.

It was with Dr. Wilkins that Lytt Gardner\* developed his interest in genetics and cytogenetics. It was Dr. Wilkins and his students who were among the first to apply the cytological techniques of Dr. Murray Barr to identify the inactivated X chromosomes (Barr bodies) in the nuclei of patients with Klinefelter's syndrome and in female pseudohermaphrodites. These diagnostic aids facilitated the diagnosis and therapy of patients with abnormalities of sexual development.

With Dr. Wilkins, Dr. George Clayton demonstrated that enzyme defects in the synthesis of thyroid hormone metabolism produce pathologic changes in the thyroid that simulate thyroid carcinoma. Dr. Wilkins had previously demonstrated during his years in practice the effect of thyroid hormone on cholesterol and creatinine metabolism.

Dr. David Smith\* and this author benefitted from Dr. Wilkins' astute record keeping; he was a master in maintaining growth charts and other documents. With him, we published the effect of thyroxin treatment on the mental development of cretins.

These were classic physiologic studies in which the effects of a hormone were investigated clinically. He had demonstrated during this same period that the epiphyses in patients with thyroid deficiency were misshapen as they calcified (epiphyseal dysgenesis) and delayed in appearance, and that epiphyseal dysgenesis was a frequent finding in the untreated cretin. With treatment, the epiphyses that had not appeared because of thyroid hormone deficiency were often dysgenetic when they did appear, but the epiphyses that were expected to appear following the chronologic age that treatment was begun were always intact in their development.

## THE SECOND GENERATION AND BEYOND

Other pediatric endocrinologists from the United States who trained with Dr. Wilkins between 1946 and 1960 were Drs. Thomas Shepard, Gerald Holman, José Cara,\* David Mosier, William Cleveland, Ralph David, Orville Green, Malcolm Martin, Samuel Silverman, and Robert Stempel. Many students from abroad who are now professors also trained with Dr. Wilkins. These include Drs. Jean Bertrand, John Eckert, John Gerrard, Casaer

Bergada, Theodoros Papadatos,\* and Andrea Prader\* who followed in Lawson's image as a major founder of pediatric endocrinology in Europe, and Henning Anderson.\* These endocrinologists and professors have trained the third generation of pediatric endocrinologists who in turn have trained the fourth generation.

Dr. Wilkins wanted to be called "Lawson" by "his boys" as he called those who trained under him, but esteem for him was so great that he remained "Dr. Wilkins" to most for many years.

It is not by chance, however, that there was only one female fellow, Dr. Eugenia Rosenberg, prior to 1960. It was simply Dr. Wilkins' policy not to accept women as fellows. He respected the intellect of female physicians, but he was reluctant to let them examine the male teenagers who came to him for consultation. With the acceptance of Drs. JoAnne Brasel, Virginia Weldon, and Irene Solomon as pediatric endocrine fellows at Johns Hopkins in the early 1960s (when he was professor emeritus but still active), he relented and realized that he had been unduly restrictive.

We in pediatric endocrinology, pediatrics, and genetics are indeed blessed to have had such a man to lead us. The history of Lawson Wilkins is well worth passing along to the third and fourth generations of pediatric endocrinologists, and it is to be hoped that they will pass it along to the fellows who train with them.

(\*Deceased)

## REFERENCES AND THEIR HIGHLIGHTS

1. Wilkins L. Presidential Address to American Pediatric Society. *Am J Dis Child* 1962;104:449-456.

Dr. Wilkins wished to chastise pharmaceutical firms for their focus on the commerce of manufacturing and marketing drugs and to warn physicians to avoid the pitfalls of over prescribing medications and/or prescribing the newest medicine in the pipeline when its efficacy and the potential long-term toxicity are obscure. This masterful presentation was both educating and chastising. The following capsulizes Wilkins' closure: (1) Remember the Oath of Hippocrates, (2) Give no drug if it is not needed. Placebos rarely have a place in pediatrics, (3) Remember that practically every effective drug has potentials for toxic side-effects, (4) Neither discuss nor prescribe drugs by brand name, (5) Never use a drug or mixture without full knowledge of its chemical nature and pharmacological action, (6) Do not attempt to learn your new therapeutics from the handy brochures or even the PDR, (7) Do not ignore the 400+ new drugs coming on the market each year.

particularly if they are variants of drugs with which you already have had experience, (8) Wait, wait, wait - and then wait. Let the other fellow poison his patients.

2. Bongiovanni AM. Presentation of the John Howland Medal and Award of the American Pediatric Society to Dr. Lawson Wilkins. *J Pediatr* 1963;63:803-807.

Dr. Bongiovanni pays tribute to Lawson Wilkins for all of his accomplishments with the help of Wilkins only sibling and records: "He had a child like curiosity and spirit of inquiry that kept him young. He was never struck with the prejudices of a prior era. His advantages were scholarly acquaintance with earlier discoveries, an intimate knowledge of clinical aspects, and a firm hold on the basic sciences. His multiple interests are reflected in the diversity of titles to his innumerable publications, which include studies on serum potassium, ulcers of the tongue, rickets, immunization against dysentery, meningitis, pyuria, epilepsy and many diverse aspects of endocrinology." The presentation in this reference was a remarkably successful rendering of insight about the personality and personal characteristics of Lawson Wilkins.

3. Wilkins L. Acceptance of the Howland Award. *J Pediatr* 1963;63:809-811.

Dr. Wilkins paid extensive gratitude to his mentors and colleagues, including fellows, which reflected his true sincerity for his colleagues' contributions and collaborations, and to educate his listeners. As he stated, "I wish to take the privileged opportunity to emphasize the importance of the clinician and clinical investigator in contributing to basic and fundamental knowledge." His views about clinical investigation in abbreviated wording was as follows: It is the clinician who must seek out and bring to attention the human experiments of nature . . . no one can reproduce in the laboratory most of the inborn enzymatic defects

. . . I always permitted my assistants to delve into any type of problem which interested them . . . The scientist must have an insatiable curiosity to seek knowledge along any lines

. . . The clinical investigator must have curiosity and, if he has such curiosity, nearly every patient he sees will call forth many questions of real importance which have never been answered. The clinical investigator will be impelled to attempt to answer these questions by studies upon the patient.

4. Wilkins L. The Evolution of Endocrine Diagnosis and Treatment: The Addison Lecture. *Guys Hospital Gazette* 1954;March 19th, pages 1-9.

Dr. Wilkins gave a masterful presentation of the history of clinical endocrinology beginning with Graves' classical description of thyrotoxicosis in 1834 and a current (1954) discussion of the interrelationships of the endocrine glands and their hormones including diagnostic methodology available, differentiation of CAH in males from other types of sexual precocity, diagnosis of sexual infantilism, etc. The result was a very erudite lecture revealing how successful Dr. Wilkins was in sorting out the diagnoses and treatment of various pediatric endocrinopathies. The content of this lecture was incorporated into the 2nd Edition of his textbook, *The Diagnosis and Treatment of Endocrine Disorders in Adolescence and Childhood* (1957).

5. Blizzard RM. Pediatric Profiles: Lawson Wilkins (1894-1963). *J Pediatr* 1998;133:577-580.

Dr. Blizzard was invited to write such a profile as the *Journal of Pediatrics* was composing a series on the profiles of those who had pioneered in the specialty of pediatrics. His initial goal was to introduce an unusual story to the readers of his first encounter with Lawson Wilkins. This unusual encounter characterized Wilkins' personality - honesty, directness, a no nonsense approach, leadership, preciseness, and the expectation that one hearing a private conversation would keep the confidence of the discussants. The paper also describes in subsections The Wilkins personality, Wilkins as a physician, Wilkins as an investigator, and Wilkins as a teacher. The article ends with brief descriptions of his last years and conclusions.

6. Bongiovanni AM, et al. To Honor Lawson Wilkins, MD in His 65th Year. *J Pediatr* 1960;57:317-325.

Dr. Bongiovanni provides a personal accounting given by Dr. Edwards A. Park (pages 317-322) of his professional relationships with Lawson Wilkins and accountings of personal relationships with Lawson Wilkins by some of his colleagues of the early historic days, including Douglas Hubble of Scotland. The accountings of Hubble and Park are particularly insightful and should be read by those wishing to more completely understand Dr. Wilkins as a clinical investigator and as a unique personality.

7. Money J. Foreword to the 3rd Edition of *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. By Lawson Wilkins with the editorial assistance of Robert M. Blizzard and Claude J. Migeon; 1965;pages vii-xi.

Dr. Money wrote this foreword after Lawson Wilkins' death with the primary objective of recording Dr. Wilkins' professional and personal characteristics by one who had worked closely with him for more than a decade.



Dr. Money delivered a very thorough and appropriately lengthy personal and professional history of Dr. Wilkins. Dr. Money's closing paragraph is particularly pertinent as it is conceptually flattering and truthfully accurate: "Lawson Wilkins achieved fame, but as a by-product of accomplishment. His life's goal had been to achieve, not to become famous."

8. Fisher DA. A Short History of Pediatric Endocrinology in North America. *J Pediatr* 2003 (In preparation).

The purpose of this article is to record for posterity a historical perspective of the founding and development of pediatric endocrinology as a subspecialty, of the Lawson Wilkins Pediatric Endocrine Society, of pediatric

endocrine training programs, of pediatric diabetes as a discipline, and of advances in understanding, diagnosing, and treating pediatric endocrinopathies since 1950. A very excellent and complete presentation of the topic has been written by Dr. Fisher. As part of this, Lawson Wilkins' major roles as pediatrician, founder of the subspecialty, clinical investigator, and academician are evident.

9. Migeon CJ. *The Origins and Establishment of the LWPES*. <http://www.lpwes.org/history.html>. The concept and history created by Lawson Wilkins invitation in 1963 of a scientific gathering to the formal creation in 1972 of a Society is interestingly detailed.

#### Abstracts from the Literature

### Body Mass Index and Segmental Proportion in Children with Different Subtypes of Psychosocial Short Stature

Psychosocial short stature (PSS) has been classified by the authors into 3 categories: (1) Type IIA are hyperphagic children, in whom there is reversible growth hormone (GH) insufficiency with rapid catch-up growth with a change in their living environment but with minimal response to exogenous GH; (2) Type IIB is a heterogeneous sub group of non-hyperphagic children who have normal GH secretory dynamics and minimal or absent increase in growth rate with change in their environment and variable response to GH; and (3) Type III are children with anorexic eating habits, with an onset as early as infancy, with failure to thrive, depression, normal GH secretory dynamics, and significant growth response to exogenous growth hormone. Gohlke et al

report anthropometric evaluations of 46 children with PSS, before and after change in their environment (Table 1).

Significant improvement in height velocity SDS after intervention was observed in all groups. ANOVA failed to show any significant differences in growth velocity between groups. There was no significant change with treatment in body proportion in type IIA (hyperphagic) or in type IIB (heterogenous) children. In type III (anorexic) children, the body proportions decreased significantly after intervention indicating relatively shorter upper segments after treatment. In those who received GH treatment (n = 21), there was no significant change in body proportion after GH therapy. Body Mass Index

Table 1

#### Clinical Data of 46 Children with PSS

| Classification                                   | Type IIA (n = 20) | Type IIB (n = 16) | Type III (n = 10) |
|--|-------------------|-------------------|-------------------|
| Mean age at presentation (years)                 | 8.6               | 7.6               | 10.2              |
| Age range (years)                                | 4.9-15            | 3.8-14.9          | 5.4-14.9          |
| Sex  | 9F. 11M           | 4F. 12M           | 6F. 4M            |
| IUGR   | 2F. 2M            | 0F. 5M            | 2F. 2M            |
| Mean bone age delay at presentation (years) (SD) | 1.69 (1.0)        | 1.69 (1.3)        | 2.0 (1.5)         |
| Prepubertal at presentation                      | 18                | 15                | 9                 |
| <b>Type of intervention</b>                      |                   |                   |                   |
| Social services only                             | 17                | 5                 | 3                 |
| Social services and GH therapy                   | 3                 | 11                | 7                 |

Adapted from: Gohlke BC, et al. *Eur J Pediatr* (220) 161: 250-254.



(BMI) did not increase in any of the groups after intervention and there were no significant changes in bone age. Multiple regression analysis showed that the type of PSS was a predictor for height velocity after intervention. The greatest effect in removal from adverse home events were in the type IIA (hyperphagic) subjects. The authors state that their findings should be helpful to clinicians managing children with PSS because of the emphasis on appetite disturbance and the variable treatment responses.

Gohlke BC, et al. *Eur J Pediatr* 2002;161:250-254.

**First Editor's Comment:** PSS, first described in 1947 by Talbot et al,<sup>1</sup> is often difficult to diagnose. Variable GH secretory dynamics, and responses to exogenous GH therapy make it important to attempt to better understand the etiologies involved and their potential response to psychosocial changes. The current manuscript report data on a large number of subjects with PSS and suggested that BMI is not useful in predicting response to treatment, but that categorization based on appetite may be of use in predicting growth changes. It is unfortunate that their data were not analyzed separately for those with intrauterine growth retardation (IUGR) and for those with and without GH insufficiency. However, the heterogeneous composition and variable treatment of these children strengthen the conclusions based on categorization of subjects by their eating behavior.

## Reference

1. Talbot NB, et al. *N Engl J Med* 1947;236:783-789.

William L. Clarke, MD

**Second Editor's Comment:** PSS should be considered by pediatric endocrinologists or pediatricians in the differential diagnosis of short stature when a short child is seen in the clinic. If the possibility of this diagnosis is not considered and explored in the history, the diagnosis will be missed. PSS occurs much more frequently than is realized. Many parents of children with PSS (particularly Type II A of the English classification) are not concerned about their child's stature because the parents are psychologically rejecting the child.

PSS is a spectrum of entities as Gohlke, Frazer, and Stanhope state. The classification is muddy for this reason. In the patients reported by Gohlke et al there was no child less than 3.8 years of age. In the classification listed in Lifshitz's *Pediatric Endocrine Text*<sup>1</sup> (3<sup>rd</sup> Edition, 1996), infants with PSS comprise a broad clinical spectrum. This should be kept in mind so that the diagnosis of PSS is made and treatment properly

instituted, which in my opinion is not GH, but removal of the child from the adverse environment, particularly for type II.

## Types of PSS as Described in the 3<sup>rd</sup> Edition of *Pediatric Endocrinology*<sup>1</sup>

At least three subtypes of psychosocial short stature have been recognized (Table 2). The first (type I) occurs in infants and children 2 years of age or younger. These infants usually have failure to thrive (nutritional deficiency), as well as short stature, and have been very adequately described by Krieger, Whitten, and colleagues.<sup>2-6</sup> There is no evidence that these children have a hormonal disturbance, such as growth hormone deficiency, and they usually recover when sufficient calories are ingested. Their parents do not usually blatantly reject the child. The mothers characteristically have multiple children or responsibilities. They are usually disorganized, and the children do not receive the food or the attention they need, but the attention they receive is usually adequate for infants to again grow, if they are given adequate nourishment. Nevertheless, growth in some may be inadequate without further psychosocial interventions, as reported by Bithoney et al.<sup>7-9</sup>

Type II PSS has been called transient hypopituitarism, reversible hyposomatotropism, emotional deprivation, maternal deprivation, psychosomatic dwarfism, abuse dwarfism, and the "garbage can" syndrome. The term PSS is preferable to definitions that include the presence or absence of GH, the presence or absence of overt psychologic abuse, or emotional deprivation. This type occurs characteristically in children 3 years of age and older.

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Table 2  
Characteristics of Various PSS Syndromes<sup>1</sup>

| Type | Age of Onset     | Failure to Thrive      | Bizarre Behavior | Depression | GH Secretion              | Parental Rejection     | GH Responsiveness        |
|------|------------------|------------------------|------------------|------------|---------------------------|------------------------|--------------------------|
| I    | Infancy          | Usually                | No               | Often      | Normal                    | No (see text)          | ?                        |
| II   | ≥3 years         | Some & some overweight | Usual            | Very often | Decreased or absent often | Usual                  | Minimal at doses used    |
| III  | Infancy or later | Not usual              | Not usual        | Yes        | Normal                    | Concern, not rejection | Significant at dose used |

Adapted from: Blizzard RM, Bulatovic A. In: Lifshitz F, ed. *Pediatric Endocrinology 3<sup>rd</sup> Edition*. New York: Marcel Dekker, 1996:83-93.

There is a greater psychologic component, and GH response may be inadequate after stimulation with pharmacologic agents, such as arginine or insulin. Other abnormalities indicating adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone, and gonadotropin deficiency may be noted; however, GH deficiency is the most common endocrine aberrancy. The parents in this group usually reject their children and abuse them psychologically. The fathers and/or mothers are frequently chronic alcoholics. Occasionally type I patients are observed to advance into type II, which is not surprising.

Type III of PSS was described by Boulton et al,<sup>10</sup> who studied seven children aged 3.6 – 11.6 years who did not have the bizarre signs and symptoms of type II patients. They were significantly depressed and/or had a disorder of attachment often dating from infancy. In contrast to previously reported patients they secreted GH when tested and had a significant increase in growth when given growth hormone treatment. A lesser response was obtained with a placebo. The authors emphasized that type III PSS patients did not show lack of discrimination in relationships, nor did they display the self-destructive behavior, pain agnosia, or bizarre eating and sleeping disorders seen in many type II patients. In addition, the parents were not indifferent and rejecting, as are those with PSS type II. The parents also had insight into the problem, which was not characteristic of the parents of other patients with PSS and several felt guilty and/or had depression.

The classifications discussed here by the English group and that presented in Lifshitz's *Endocrine Text* are compatible. Type I, as described above, should remain as type I and be applied to infants and very young children. Type II pertains to children with severe PSS of the hyperphagia type. In my opinion, type II should be limited to this group. Type III is where further subclassifications should be placed. For example, type IIIA could (should) be the group described by Boulton<sup>10</sup> and type IIIB of the type referred to by Gohlke et al. With this classification type IIIA & B can be subdivided or a type IV added as further subgroups are recognized. I wonder if Drs. Gohlke et al or others agree with my thinking? A letter to the Editors of GGH will be most welcome.

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Robert M. Blizzard, MD

## Leanness, Extended Lifespan & IGF-1 Receptor Mutations in Mice: Fascinating Observations

In flies and worms, loss-of-function mutations in insulin and insulin-related cell signaling pathways have led to increase in life span of the species studied. In order to evaluate these pathways in a mammalian species, the

present investigators developed mice with hemizygous loss of one insulin-like growth factor-1 receptor (IGF-1R) allele and studied their longevity. The hemizygous IGF-1<sup>+/−</sup> mice were generated by deletion of exon 3 of

the gene encoding IGF-1R; these mice had 50% of the IGF-1R levels that intact animals had. Homozygous inactivation of the gene encoding the IGF-1R (IGF-1R<sup>-/-</sup>) was lethal. During nursing, IGF-1R<sup>-/-</sup> and intact (IGF-1R<sup>+/+</sup>) mice grew identically; after weaning there was a slight decrease in growth (-6% to -8%) in hemizygous mice relative to intact animals through 11 weeks of age. IGF-1R<sup>-/-</sup> female mice lived 33% longer and males 16% longer than did IGF-1R<sup>+/-</sup> mice, and female hemizygous mice outlived their male counterparts. (Figure) As anticipated, serum IGF-1 concentrations were higher in IGF-1R<sup>-/-</sup> mice than with control animals, while insulin levels were normal. Glucose tolerance was impaired in IGF-1R<sup>-/-</sup> male but not female mice. Energy balance in mutant and control animals was similar in food intake, body temperature, physical activity, metabolic rate and fertility. The ability to withstand an oxidative stress was greater in mutant than control animals both *in vivo* and *in vitro*. In cultured fibroblasts, the amounts of several signal transduction molecules downstream of the IGF-1R were decreased relative to the activity of control fibroblasts. In particular, levels of phosphorylated p66 shc, an activator of mitogen activated protein (MAP) kinase, were reduced by one-half, suggesting that perhaps a decrease in the rate of cell division might be an important factor in increasing longevity. The investigators conclude that in mice the partial inhibition of IGF-1 signaling leads to increase in life span.

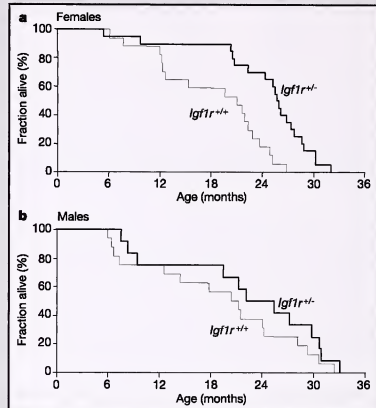
Blüher et al demonstrated that in mice in which there has been localized "knock out" of fat specific insulin-receptors (FIRKO) (in contrast to generalized loss of IRS which leads to insulin resistance, diabetes mellitus, and obesity), there was extension of life span despite normal caloric intake and without clinical or biochemical abnormalities. FIRKO mice were approximately 20% lighter and their body fat content approximately 60% lower than control animals, despite eating similar quantities of food. Control animals lived an average of 753 days, while FIRKO mice lived 887 days (+134 days, +18%); median life span in FIRKO was increased by +3.5 months and maximum life span by +5 months. Fertility of the FIRKO mice was not reported. The investigators concluded that low body fat content (leanness) rather than decreased food intake was the primary factor contributing to increase in life span of the FIRKO mice.

Holzenberger M, et al. *Nature* 2003;421:182-186.

Blüher M, et al. *Science* 2003;299:572-574.

**Editor's Comment:** There is increasing evidence that insulin, growth hormone (GH), and IGF-1 are intimately involved with the duration of life. Experimentally, partial caloric deprivation increases life span while decreasing serum concentrations of IGF-1. Mice with GH deficiency

Figure  
Lifespan extension in *Igf1*<sup>-/-</sup> mice  
with respect to *Igf1*<sup>+/+</sup> (WT) mice



a - *Igf1*<sup>-/-</sup> females (thick line) live a mean of 33% longer than their wild-type littermates (756 ± 46 compared with 568 ± 49 days; *P* < 0.01, *t*-test). Kaplan-Meier analysis of survival revealed a later decline in *Igf1*<sup>-/-</sup> mice compared with wild type (*P* < 0.001, Cox's test). b - *Igf1*<sup>-/-</sup> males live 15.9% longer than wild-type littermates (679 ± 80 compared with 585 ± 69 days; NS).

Reprint with permission from: Holzenberger M, et al. *Nature* 2003;421:182-186.

(GHD) such as Ames (*Prop<sup>dw/dw</sup>*) and Snell (*Pit1<sup>dw/dw</sup>*) mice are extremely long-lived albeit dwarfed and infertile, as are mice in which the GH receptor has been "knocked-out."

The manuscripts present several interesting observations in addition to those on longevity. Thus, partial inactivation of the IGF-1R gene led to slightly subnormal growth in mice, suggesting that variants of this gene might play a role in the diversity of height in man. Also of interest were the gender specific effects of partial loss of IGF-1R which was more pronounced in females than males which indicated that sex-specific factors may modulate the effects of IGF-1R.

While it is not possible to transpose these data to man, they make one wonder whether we may be adversely affecting life span by treating our GHD adult patients with rhGH. Perhaps it might be less risky to treat the cardiovascular and skeletal abnormalities of the adult with GHD with agents other than rhGH.

Allen W. Root, MD



## Hypothalamic Insulin Signaling is Required for Inhibition of Glucose Production

Insulin has many energy modulating actions that take place in the hypothalamus, such as inhibition of feeding. The investigators studied the effects of infusing insulin, an insulin mimetic, and inhibitors of insulin action. Infusion was done in the intra-third cerebral ventricle (ICV). Hepatic glucose production and peripheral glucose consumption were determined. Steady state of serum insulin concentrations were achieved by using systemic pancreatic-insulin clamps.

ICV infusion of insulin/insulin mimetic at basal insulin concentrations led to a 7-fold increase in glucose infusion rate to maintain euglycemia. Thus, ICV glucose enhanced peripheral insulin action. Employing radiolabeled glucose and kinetic glucose studies, the investigators demonstrated that ICV insulin decreased the rate of hepatic glucose production by 40+% while not altering peripheral glucose consumption. Inhibition of insulin action in the hypothalamus by co-infusion of insulin antibodies or an antisense disrupter of insulin receptor synthesis antagonized the effect of insulin on glucose production. Further studies demonstrated that the intracellular mechanism(s) through which hypothalamic insulin exerted its effect on glucose production involved the phosphoinositide-3-kinase signal transduction pathway and ATP sensitive potassium channels. However, the manner in which

hypothalamic insulin impaired hepatic glucose production was not identified by these studies. The authors suggest that hypothalamic insulin (as well as other factors such as leptin and melanocortins) may monitor and modulate exogenous energy intake relative to endogenous energy consumption. Failure of hypothalamic insulin function may lead to peripheral insulin resistance and may be a factor in the pathogenesis of the dysmetabolic syndrome and type 2 diabetes mellitus.

Obici S, et al. *Nature Med* 2002;8:1376-1382.

**Editor's Comment:** *The physiological importance of insulin action within the central nervous system is well described in the content of this manuscript. The demonstrations reported open yet another site at which a metabolic error may lead to clinical illness. It is crucial to determine the specific mechanisms by which the hypothalamic action of insulin is recognized at the hepatic level and to develop a method(s) by which one may assess hypothalamic insulin function in the intact human.*

Allen W. Root, MD

## Hyperzincaemia and Hypercalprotectinaemia: A New Disorder of Zinc Metabolism

The authors describe five patients (including a mother and her son) who had a multidimensional illness comprised of recurrent infections, rash, arthritis/vasculitis, hepatosplenomegaly, and growth retardation in infancy and childhood. Although these findings were consistent with zinc deficiency, the patients had marked hyperzincaemia due to its binding to greatly elevated amounts of a zinc-binding protein called calprotectin. Calprotectin is a calcium and zinc binding protein complex of two S100 plasma proteins termed S100A8 and S100A9 (also termed proteins MRP8 and MRP14, respectively). It is present in the cytosol of phagocytes and is released into plasma as phagocytic neutrophils are destroyed. In these patients, plasma zinc concentrations were 5-10 times higher than the upper normal range (18 µmol/L), while calprotectin concentrations were 1000 fold greater than the upper normal value (850 µg/L), suggesting that free plasma zinc concentrations were likely to be low. Individual patients were anemic, thrombocytopenic, and had low numbers of monocytes and B lymphocytes.

Chromatographic analysis of S100A8 and S100A9 proteins was normal, suggesting no major mutations or post-translational modifications of calprotectin. Since there was no evidence of increased neutrophil turnover rate, the investigators hypothesized: (1) that the increased plasma concentrations of calprotectin reflected its decreased rate of degradation; (2) that the patients were zinc deficient because of the high affinity of calprotectin for zinc; and (3) that calprotectin itself may have been cytotoxic to neutrophils and other tissues.

Sampson B, et al. *Lancet* 2002;360:1742-1745.

**First Editor's Comment:** *The new syndrome comprises patients with an apparent "functional zinc deficiency" despite high plasma concentrations of this element. Although "free zinc" concentrations were not measured, they were thought to be low. In addition, the authors did not report the effects of a trial of therapy with supplemental zinc in these subjects. Thus, the*



Table

## Clinical and laboratory data of patients

|  | Patient 1       | Patient 2       | Patient 3       | Patient 4          | Patient 5                    |
|--|-----------------|-----------------|-----------------|--------------------|------------------------------|
| Age (years)  | 18              | 9               | 14              | 35                 | 21                           |
| Sex  | M               | F               | M               | F                  | M                            |
| Growth failure                                     | <3rd percentile | <3rd percentile | <3rd percentile | Normal             | Normal                       |
| Hepatosplenomegaly                                 | Yes             | Yes             | Yes             | Yes                | Yes                          |
| Dermatological symptoms                            | Vasculitis      | None            | None            | Vasculitis, eczema | Vasculitis, furuncles ulcers |
| Rheumatic symptoms                                 | Arthritis       | Arthritis       | Arthritis       | Arthritis, uveitis | Arthritis                    |
| Plasma C-reactive protein (mg/L)†                  | 41-143          | 100-200         | 22              | 17                 | 45-146                       |
| Haemoglobin (g/L)                                  | 80              | 90              | 109             | 125                | 80                           |
| Total white-cell counts (10 <sup>9</sup> cells/mL) | 2.0             | 3.7-5.0         | 1.5             | 5.0                | 3.8                          |
| Monocytes  | 0               |                 | 1.9%            | 1.9%               | 4.3%                         |
| Plasma zinc (mol/L)‡                               | 180-200         | 82-96           | 160-200         | 175                | 77                           |
| Plasma calprotectin (g/L)§                         | 6.5             | 1.4, 2.55       | 9               | 6.1                | 1.5                          |

†Reference <10 mg/L. ‡Reference 10-18 mol/L. §Reference <1 mg/L

Adapted from Sampson B, et al. *Lancet* 2002;360:1742-1745.

*hypothesis regarding "functional zinc deficiency" remains unproven. Since calprotectin is a calcium binding protein, it would have been of interest to report total and ionized calcium values in these patients.*

*Zinc deficiency may be congenital or acquired. Acrodermatitis enteropathica (OMIM 201100) is an autosomal recessively transmitted disease characterized by bullous lesions of the skin, alopecia, diarrhea, and growth failure with hypozincemia. Administration of supplemental zinc ameliorates these abnormalities. Approximately 50% of patients with acrodermatitis enteropathica have a loss-of-function nonsense or missense mutation in SLC39A4 (Solute Carrier Family 39 [Zinc Transporter], Member 4) encoding a renal- and intestine-specific transmembrane zinc transporter protein (OMIM 607059, chromosome 8q24.3). Zinc deficiency may be acquired due to dietary*

*deficiency, decreased absorption due to co-ingestion of zinc-binding materials such as clay or phytates, malabsorption as in patients with chronic inflammatory bowel disease, or excessive excretion as in patients with sickle cell disease and hyperzincuria.*

Allen W. Root, MD

**Second Editor's Comment:** *I am puzzled by the possibility of copper deficiency in these patients. The clinical picture and the anemia and leucopenia are typical of it. A deficit of this mineral would likely result in a deficiency of Ca/Zn SOD (super oxidase desmutase) though I do not know of studies of its effects on calprotectin.*

Fima Lifshitz, MD

## Initial Treatment Dose of L-Thyroxine in Congenital Hypothyroidism

The American Academy of Pediatrics (AAP) recommends an initial L-thyroxine dose of 10 to 15 mcg/kg/d for the treatment of congenital hypothyroidism (CH).

Several studies have shown that early high dose therapy which quickly produces serum T-4 levels within the "normal" neonatal range may be associated with the

development of near normal IQ scores; whereas therapy with lower dosages are associated with a delay in achieving normal T-4 concentrations by as little as 1 week may result in lower IQ scores. Thus, pediatricians and pediatric endocrinologists need to be familiar with treatment regimens that achieve the T-4 goal with as little delay as possible, yet do not produce untoward side effects such as craniosynostosis.

Selva and colleagues present data obtained in 47 congenitally hypothyroid neonates (BW 3-4kg) using a prospective randomized study of 3 different L-thyroxine dosing regimens (Group 1 – 37.5mcg/d; Group 2-loading dose 62.5mcg/d x 3d, then 37.5mcg/d; Group 3 – 50mcg/d). Serum T-4, free T-4, T-3, free T-3, and TSH were measured at baseline, 3 days, and 1, 2, 4, 8, and 12 weeks after starting treatment. No changes in treatment dose were made for 2 weeks. At that time, dosages were altered using the following important algorithm to maintain serum T-4 concentrations between 10 – 15 mcg/dL; a) T-4 < 8.5mcg/dL, increase dose by 12.5mcg/d, b) T-4 between 8.5 and 9.9mcg/dL, increase dose by 6.25mcg/d, c) T-4 between 15.1 and 16.5mcg/dL, decrease dose by 6.25mcg/d, d) T-4 greater than 16.5mcg/dL, decrease dose by 12.5mcg/d.

Pre-treatment thyroid levels were similar in all three groups. Infants in Groups 2 and 3 achieved target T-4 levels by 3 days, while infants in Group 1 did so by 1 week of age. Subjects in Group 3 had T-4 levels above 16mcg/dL by 1 week, while the others were in the target range at both 1 and 2 weeks. TSH remained elevated in Groups 1 and 2 for the first 2 weeks. After 2 weeks, serum T-4 remained within the target range in all three groups, but doses were adjusted as outlined above. At 12 weeks, mean L-thyroxine dose was 36.7 mcg/d (approximately 6mcg/kg/d) in all groups, which was associated with ideal target levels of T-4, T-3, and TSH. Free T-4 levels rose above normal by 1 week and remained above normal at 12 weeks in all age groups. There were no significant differences in TSH concentrations at 12 weeks among the groups.

When patients were divided into severe and moderate CH categories based on serum T-4 above or below the median value, the differences in initial T-4 levels were abolished by 3 days for Group 3 infants and by 1 week for the others.

The authors state that their data shows that a loading dose of 62.5mcg/d x3 days followed by a dose of 37.5mcg/d raises serum T-4 levels quickly but does not normalize TSH levels. However, the sustained dose of L-thyroxine (50mcg/d – Group 3) normalized TSH levels within 2 weeks and abolished any difference in serum T-4 levels between severe and moderate CH infants by 3 days. Consequently, they recommend the use of a higher target range of 10 to 18mcg/dL for T-4 for the first

2 weeks of therapy to insure that the benefits of therapy are maximized.

Selva K, et al. *J Pediatr* 2002;141:786-792.

**Editor's Comment:** *It may seem surprising to read a paper dealing with the "correct" L-thyroxine dose for treating infants with CH, when most neonatal screening programs have been in place for approximately 20 years and have been highly successful in identifying these infants and seeing that they receive what has been considered "appropriate" treatment. However, the medical community, despite well-delineated guidelines from the AAP, has yet to define "appropriate" treatment. The article by Selva et al helps clarify three different treatment regimens. They are to be commended for the prospective randomized protocol followed. It is interesting that they refrain from "recommending" a single or favorite regimen. Indeed all three regimens work well if the goal is to normalize serum T-4 within 1 week. Quicker attainment of the target range requires a loading dose for three days. The accompanying algorithm for adjusting L-thyroxine doses is helpful and all of these data and recommendations need to be disseminated to those caring for neonates.*

William L. Clarke, MD

**Second Editor's Comment:** *This detailed analytical study is accompanied by a detailed analytical report pointing out that several groups have demonstrated as much as a 20 point IQ deficit in severely affected CH infants who did not have rapid and complete conversion of serum hormonal levels of T-4, T-3, free T-4 and T-3, and TSH to normal. The article convinced me that a treatment protocol as used for group 3 is currently the best available.*

*In an accompanying editorial by Dr. Nancy Hopwood of the University of Michigan, emphasis is given to the importance of using only tablets of T-4 because liquid preparations may be unreliable. She also points out that persistent TSH elevation can result from faulty absorption of T-4 in patients with milk allergy, malabsorption of various causes, with soy formulas, iron therapy, and with acidic juices in children of all ages. The article by Selva et al and the editorial by Dr. Hopwood fit together splendidly.*

## Reference

1. Hopwood NJ. *J Pediatr* 2002;141:752-4.

Robert M. Blizzard, MD

## Survival Profile for Down Syndrome

Down syndrome is the most common form of inherited intellectual disability. In addition, it is associated with growth deficiency, hypotonia, characteristic craniofacial appearance and developmental anomalies involving the heart and other organ systems. Survival of these patients has changed dramatically over the last several decades primarily because of surgical intervention for cardiac defects. For example, life expectancy increased from 12 years in England in 1949 to recent estimates of over 50 years in western countries. These estimates are based on cross-sectional data because there is little longitudinal information available. Moreover, it is known that adults with Down syndrome are predisposed to a number of disorders including obesity, hypothyroidism, epilepsy, dementia, and Alzheimer's disease; however the impact of these disorders on survival is unknown.

To define the survival profile for those with Down syndrome, Glasson and colleagues assessed survival in 1,332 patients (45% female) born between 1902 and 2000, mostly in Australia. Most patients had had standardized intelligence testing. Death had occurred in 20%. Kaplan-Meier survival probabilities were calculated separately for sex, level of intellectual disability and decade of birth.

The analysis showed that the overall life expectancy for patients with Down syndrome approaches that of the general population in Australia. Seventy-five percent of cases had survived to 50.0 years, 50% to 58.6 years

and 25% to 62.9 years of age. The mean life expectancy for males was greater than females by 3.3 years with the median survival probabilities of 61.1 for males and 57.8 for females. The difference was attributed to a higher incidence of heart defects in females. When examined by decade born, each successive birth group showed increased survival consistent with progressive improvement in medical care. No association was found between level of intellectual disability and survival, which was surprising to the authors because an association had apparently been found in an earlier study.

Approximately 25% of all Down syndrome deaths occurred between the ages of 58 and 63 years. No clear explanation for this was found nor is there any certainty that the trend will continue in patients born more recently. The authors raise the possibility that it could reflect mortality associated with the above mentioned chronic diseases to which adults with Down syndrome are predisposed.

Glasson EJ et al. *Clin Genet* 2002;62:390-393.

**Editor's comment:** *The information contained in this paper should be very useful to physicians, genetic counselors and others who deal with families concerned about long term survival in Down syndrome.*

William Horton, MD

## Mutagenesis Does Not Explain Paternal Age Effect in Achondroplasia

Achondroplasia is the prototype of chondrodysplasia in humans. Its major features include short limb dwarfism and a large head with mid-facial hypoplasia. Achondroplasia arises most often as a sporadic event to normal parents and there is a pronounced paternal age effect. It results from activating mutations of Fibroblast Growth Factor Receptor 3 (*FGFR3*), which encodes the transmembrane receptor. *FGFR3* mutations have several unique features including that they arise *de novo* almost exclusively during spermatogenesis and that almost all involve the same G-to-A transition at base pair 1138 (G1138A) of the gene resulting in a glycine to arginine substitution in the transmembrane domain of the receptor. Taken together, these observations have led to the commonly accepted views that *FGFR3* is exceptionally mutagenic and that the paternal age effect reflects replication errors that occur during spermatogenesis. Spermatogenesis continues throughout life and presents many more opportunities for erroneous copying of DNA than does oogenesis in which replication ceases before birth.

Although this explanation makes good sense, there is now evidence that *it is incorrect*.

To test if increased mutagenesis accounted for the paternal age effect in achondroplasia, Tiemann-Boege et al determined the frequency of the common G1138A *FGFR3* mutation in sperm from 118 healthy men ranging in age from 18 to 80 years. They expected to detect a progressive increase in sperm mutation frequency comparable to the increase in number of achondroplasia births to older fathers. However, to their surprise, using a carefully controlled polymerase chain reaction assay, they found only a small increase in the G1138A mutation which by itself could not account for the paternal age effect.

More specifically, they observed that the mutation rate for G1138A averaged about 1 per 11,000 haploid genomes over all ages. Broken down by age, the mutation frequency changed little between the ages of 18 - 40 and 55 - 80 years. It increased about 2-fold between the two age groups, but this was nowhere near



the increased frequency of achondroplasia births in older fathers.

The authors addressed in considerable depth various possible explanations for their findings. Several involve experimental biases or artifacts. For example, fathers of children with sporadic achondroplasia may constitute a subgroup of men with distinct mutation properties that differ from the sperm donor population. There may be unappreciated ascertainment biases with regard to the makeup of donor population or in previous studies. Despite extensive controls, there could have been underreporting of mutations in the PCR assay. These studies may have led to overestimating the magnitude of the paternal age effect.

Two of the possibilities deserve special attention. The first is that there may be an age-dependent increase in germ-line permutations at the G1138A site that are neither converted to a full mutation or repaired before fertilization. One candidate lesion would be an unrepaired G/T mismatch resulting from deamination of 5-methyl cytosine. The cytosine at position 1138 is known to be highly methylated in sperm and therefore a candidate for such a premutation, which might go undetected under conditions of PCR.

Another possibility is that the G1138A mutation gives a selective advantage to sperm that carry it. The authors acknowledge the highly speculative nature of this possibility, but point out that FGFR3 is expressed and presumably active in mature sperm cells. They also caution that invoking this possibility must include an explanation of how a potential selective advantage would increase with age.

Tiemann-Boege et al. *PNAS* 99 2002;14952-57.

Hurst LD, Ellegren H. *Nature* 2002;420:365-66.

**Editor's comment:** Many observations over the last several years have led to the dogma that FGFR3, especially the site where achondroplasia mutations arise, is extraordinarily mutable during spermatogenesis and that this mutability increases dramatically with age. The idea that DNA is prone to replication or mitotic errors, that there are many more opportunities for such errors to occur during spermatogenesis compared to oogenesis, and these can somehow accumulate with age has been conceptually appealing and is easy to explain during counseling. However, the results reported here cast serious doubt on its validity. Assuming they hold up, which seems highly likely given the considerable lengths to which the authors went to control their experiments and validate their results, the dogma will need to change.

The notion of genetic premutation in achondroplasia is not new. It was proposed by John Opitz and others long before mutations of FGFR3 were discovered. It never gained much momentum, probably because it lacked experimental data with regard to a specific locus or mutation; however, the paper by Tiemann-Boege et al may add new life to this concept.

The possibility that sperm which harbor activating mutations of FGFR3 have a selective advantage for motility, fertilization or the like, is intriguing. Of note is that activating FGFR3 mutations found in the achondroplasia family of disorders have been detected in several types of cancer, including multiple myeloma and bladder, breast and colon carcinoma. The mechanisms through which the mutations contribute to neoplasia are not well understood. However, they may well give the cancer cells a competitive advantage over the normal cells.

William Horton, MD

## Is Insulin-Like Growth Factor-1 Monitoring Useful in Assessing the Response to Growth Hormone of Growth Hormone-Deficient Children?

In order to assess the relationship between insulin-like growth factor-1 (IGF-1) and the growth hormone (GH) dose utilized to treat GH-deficient children, the IGF-1 response was compared with the changes noticed in height-standard deviation scores (H-SDS) and height velocity during treatment.

The study was carried out in 24 prepubertal GH-deficient patients with a mean age of  $10.5 \pm 1.8$  years and a mean bone age of  $8.4 \pm 2.1$  years. H-SDS for chronological age and bone age before therapy were  $-2.6 \pm 0.8$  and  $-1.2 \pm 0.8$ , whereas height velocity was  $-1.1 \pm 1.5$  cm. Serum IGF-1 and insulin-like-growth factor binding protein-3 (IGFBP-3) levels were measured before, after 6 months and 12 months of GH treatment,

and correlated with the GH dose. IGF-1 increased significantly during the first six months of therapy, but did not increase any further at twelve months, despite the use of higher GH dosages ( $0.14$  vs.  $0.1$  IU/kg/day), whereas IGFBP-3 increased at both 6 and 12 months. There was no correlation between GH dose and IGF-1 and IGFBP-3 levels. Height velocity as well as height for chronological age and bone age were significantly greater after one year of treatment with GH. The authors concluded that the increment in IGF-1 during therapy did not correlate with the interval height increase and was found to be less useful than height increments in adjusting the GH dose needed to treat prepubertal GH-deficient children.



Lanes R, Jakubowicz S. *J Pediatr* 2002;141:606-610.

**Editor's Comment:** The monitoring approach that individualizes therapy and includes both biochemical and auxological determinations to titrate the GH dose utilized to treat GH deficiency is considered standard practice in treatment with GH. A common practice is to monitor height increments and serum IGF-1 and IGFBP-3 concentrations to guide with the treatment of GH-deficient patients. However, in this study IGF-1 and IGFBP-3 levels were not found useful in assessing the response to GH treatment. There are wide variations in IGF-1 levels during the day, as well as different stages throughout time, and even in the same individual. Of great importance is the nutritional status and intake of the patients in relation to the IGF levels. Any one or several of these factors might have played a role in the

lack of a clinically relevant, as well as statistically significant, difference in IGF levels found in this small group of patients studied. The reader is advised to read the editorial on this paper published in the same journal by Dr. Barry Bercu entitled "Titration of growth hormone dose using insulin-like growth factor-1 measurements: Is it feasible in children?" This study once again demonstrates that careful measurements of height and the monitoring of growth progression is the most important marker in the assessment of short children with or without GH deficiency, as well as during treatment with GH.

## Reference

1. Bercu B. *J Pediatr* 2002;141:601-5.

Fima Lifshitz, MD

## Leptin Measurement in Urine and its Relationship to Other Growth Peptides in Serum and Urine

Leptin is a 167 amino acid product of adipocytes that has multiple physiologic effects including appetite suppression, alteration in energy balance, acceleration of pubertal onset, and both stimulatory and inhibitory effects on bone mineralization. Its role in human physiology other than for appetite suppressive effects and possible hypogonadotropism, is uncertain. The authors have adapted a two-site immunoradiometric assay (IRMA) for measurement of leptin in serum to its determination in urine. In this assay, two mL of urine (unmodified by acidification or dialysis) are incubated initially in a plastic tube coated with antibody (#1) to leptin, followed by incubation with a second, radiolabeled antibody (#2) to leptin with specificity to a different epitope. Free labeled antibody (#2) is removed and radiolabeled bound antibody (#2) quantitated. Leptin in urine (lep/u) is calculated by comparison to standards of leptin similarly prepared. Lep/u was quantitated in timed overnight urine collections in 188 (100 females) children and adolescents 5-19 years of age. Serum and/or urinary levels of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), and IGF binding proteins (IGFBP3 and IGFBP-1) were also determined. The IRMA for lep/u was validated by dilution and recovery experiments. In the cross-sectional survey, total lep/u was similar in prepubertal boys and girls (0.2 ng/night). Lep/u values increased to a peak in boys at Tanner genital stage III (0.8 ng) and then declined; in girls, lep/u continued to increase through breast stage V (1.1 ng) and values were significantly higher in adult females than in males. The maturational patterns of lep/u were similar to those described for serum leptin (lep/s) changes. Log transformed values of lep/u and

random lep/s were highly correlated. Lep/u levels were variable related to age, stage of sexual maturation, BMI, IGF-I, and IGF-II. In two adults in whom overnight urines were collected consecutively for more than 30 nights, nocturnal lep/u values varied night-to-night by 42-75%. In a substantial number of specimens (20+%) obtained from both the children and adults, lep/u was not measurable. The authors conclude that measurement of timed overnight lep/u is a feasible method for longitudinal assessment of leptin production in children, adolescents, and adults.

Zaman N, et al. *Clin Endocrinol* 2002;58:78-85.

**Editor's Comment:** The majority of secreted leptin is catabolized in the kidney to smaller peptides. The investigators relied, in part, upon the specificity of two antibodies directed to different epitopes of leptin to validate the IRMA for lep/u. However, it would have been of interest to examine the physicochemical properties of urinary leptin by size exclusion chromatography and/or mass spectroscopy to determine more accurately the nature of the peptide measured by the IRMA. It would also have been of interest to have measured urinary/serum levels of gonadotropins and sex hormones and to assess their relationships to lep/u and stages of sexual development (perhaps a manuscript already in preparation). Nevertheless, the data are of interest and the described method may be helpful in furthering our understanding of the relationship between growth, sexual maturation, and leptin.

Allen Root, MD

**Letter to the Editor: Misconceptions - Epiphyseal Fusion Causes Cessation of Growth**

Dr. A. Michael Parfitt brought to the attention of the Editorial Board his article published in a journal not often reviewed by *Growth, Genetics & Hormones*. I have summarized some of the highlights of this very interesting article and recommend that the readership review the full paper, as it is of great interest.

Parfitt AM. Misconceptions: Epiphyseal Fusion Causes Cessation of Growth. *Bone* 30:2002;337-339.

This paper brings to light the fact that when the bone reaches its appointed genetically determined length, the following takes place: the longitudinal growth ceases, the epiphysis fuses with the metaphysis, and the growth plate disappears. Pediatric endocrinologists have always believed that growth stops because the epiphysis fuses, and that short adult stature could result from early fusion of the epiphyseal growth plate. The reverse is also true - a sustained linear growth through puberty could be a consequence of failure of epiphyseal fusion. However, Dr. Parfitt suggests that the epiphysis fuses because growth stops. In other words, fusion is the marker of growth cessation, not a determinant of it.

Epiphyseal fusion is an active process that might not necessarily be preceded by, nor automatically follow, the cessation of growth. Endochondral ossification represents the culmination of a sequence of changes in the cartilage cells and their associated matrix. These events must always occur in the same order, requiring a minimum period of time. It has been shown that the growth plate narrows, not because cartilage replacement occurs earlier, but because cartilage addition occurs more slowly as the rate of chondroblast proliferation declines. The growth plate

does not begin to disappear until proliferation has stopped altogether. Collectively, the data demonstrate that epiphyseal fusion does not precede, but rather follows the cessation of growth. Nevertheless, fusion is not simply the result of continued cartilage replacement with no further cartilage addition; this is an active process with its own hormonal controls, cellular mechanisms and structural features. For example, if there is estrogen deficiency, the epiphyses may remain unfused long after growth has stopped, with resumption of the normal timetable of fusion after replacement of the missing hormone. However the complexity of estrogen action at the growth plate has contributed to the current confusion. Estrogen has separate and independent effects on chondroblast proliferation and on active epiphyseal fusion. It has a biphasic effect on proliferation, which is stimulated by low levels and inhibited by high levels. The latter predominate in late adolescence in both sexes, leading initially to growth cessation and subsequently to active fusion. Dr. Parfitt concludes that recognizing the correct temporal relationship between growth cessation and fusion is an essential first step to understanding the complexities of growth plate function, but evidently a great deal more work is needed to clarify all the sequences.

**Editor's Comment:** *The effects of the high levels of estrogens found in sexual precocity may account for the early fusion of the epiphyses and the reduced height of the patients. The biphasic effect of estrogen on chondroblast proliferation as stated by Dr. Parfitt would account for these findings.*

Fima Lifshitz, MD

**Gastrointestinal Complications of Russell-Silver Syndrome**

A survey was conducted among members of the support group MAGIC, which includes individuals with Russell-Silver Syndrome (RSS) and their families. Completed surveys were returned from 135 individuals. Of those, 65 were determined to have clear-cut RSS on the basis of the criteria of: small for gestational age (IUGR), small for age during childhood, and having preservation of head circumference. Asymmetry is often seen in RSS as well. To be included in the study, it was necessary for the subjects to have at least three of four findings. If they had only three distinctive minor clinical features, other features were sought, including hypospadias, clinodactyly, triangular face and hypoglycemia to confirm the affected individual as a "clear cut" case.

In carefully reviewing these "typical" RSS cases, a surprisingly high frequency of gastrointestinal (GI) symptoms were found. Among the many areas of complications surveyed, GI problems stood out. Out of 65 subjects with typical RSS, 77% (50 subjects) had gastrointestinal symptoms. The major symptoms included gastroesophageal reflux disease (34%), food aversion (32%), and esophagitis (25%). The latter two are often a result of gastroesophageal reflux.

These observations suggest that the GI problems are often significant components of typical, "clear cut" RSS. The high incidence of reflux and esophagitis resulted in Nissen funduplications in many affected individuals (18%). The group with GI complications also showed a high frequency of hypoglycemia (36%) as

compared to the overall group (25%). Blue sclera and kidney abnormalities were also more common among those with GI complaints.

These findings have important implications for management. In IUGR children with failure to thrive and presenting with severe GI symptoms the diagnosis of RSS should be considered.

Anderson J, et al. *Am J Med Genet* 2002;113:15-19.

**First Editor's Comment:** Among children with RSS, about 10% have uniparental maternal disomy for chromosome 7. It is not yet clear whether they also have this very high frequency of GI symptoms. This type of

*phenotype/genotype associations needs to continue to be explored since they are so important for natural history and management.*

Judith G. Hall, OC, MD

**Second Editor's Comment:** The association of failure to thrive, gastroesophageal reflux disease, and hypoglycemia is important. Inadequate nutrient intake increases the risks of hypoglycemia. This complication must be considered and hopefully prevented in these patients.

Fima Lifshitz, MD

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## Growth Hormone Deficiency in Salt-Losing Congenital Adrenal Hyperplasia

This short report describes the identification of 4 children with 21-hydroxylase deficiency with defects in the CYP21 gene who presented with growth hormone deficiency between ages 2.1 and 12.9 years of age. These children were receiving steroid replacement at traditional doses of hydrocortisone (12 – 15 mg/m<sup>2</sup>/d) and fludrocortisone (100 – 150 mcg/m<sup>2</sup>/d) and were compliant with their treatment. Neuroimaging in two of the children revealed small, but present pituitary glands. All four grew well with growth hormone (GH) therapy. The authors speculate that these children may have sustained pituitary damage during salt-losing crises with associated hypotension and suggest that GH deficiency be considered in children with 21-hydroxylase deficiency who are growing poorly on traditional glucocorticoid and mineralocorticoid replacement doses.

Tirendi A, et al. *Eur J Pediatr* 2002;161:556-558.

**Editor's Comment:** Unfortunately these authors do not present the denominator. How many children, out of a population of what size with 21-hydroxylase deficiency and poor growth, is the question to be answered. How many children with adrenal crises have poor growth? Despite these obvious and important questions, the take home message remains clear. Twenty-one-hydroxylase deficiency need not occur as an isolated disorder. Children with 21-hydroxylase deficiency, as pointed out in the manuscript, are not necessarily short. It is important to carefully consider all possible causes when evaluating growth failure in any child.

William L. Clarke, MD

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# GROWTH

## Genetics & Hormones

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### METABOLIC SCREENING IN THE NEWBORN

**David Millington, PhD; Dwight Koeberl, MD, PhD**  
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#### INTRODUCTION

The concept of metabolic screening for the recognition, diagnosis and treatment of inborn errors of metabolism has evolved as new methodology for detection and improved treatment have become available.<sup>1</sup> The diagnosis of metabolic disorders is challenging because of (1) the episodic nature of metabolic illness, (2) the wide range of clinical symptoms that are also associated with more common conditions, (3) the low incidence of these disorders, (4) the consequent lack of experience among the pediatric sub-specialties, and (5) the need for specialty testing. Although the incidence of each disorder is in the range of  $1:10^4$  to  $1:10^7$ , there are thousands of known patients with metabolic disorders. It is probable that collectively, the total incidence exceeds 1:4000. Consequently they certainly account for significant morbidity and mortality in the newborn population.

Without doubt, the most opportune time to diagnose an inborn error of metabolism is at birth. Early recognition

and correct diagnosis enables appropriate treatment, without which tragic outcomes are all too common. Public awareness of metabolic diseases was all but unknown in the United States until 1964; at that time widespread neonatal testing was introduced for phenylketonuria (PKU), a disease resulting from lack of phenylalanine hydroxylase activity and affecting about 1:23,000 newborns. Since then, most states have expanded screening to a handful of additional diseases that fit the "PKU paradigm" – a treatable disease for which an inexpensive screening test is available and that has dire consequences if left untreated.<sup>2</sup> Currently, most states are screening for at least four disorders: PKU, congenital adrenal hyperplasia of the 21-hydroxylase type, galactosemia because of galactose-1-phosphate uridylyltransferase deficiency, and congenital hypothyroidism due to defects of thyroxine synthesis.

The case of PKU screening exemplifies the benefits of early diagnosis of a metabolic disease to patients, their families and society as a whole. The benefits of finding and treating these patients far outweigh the costs of screening the entire population.

Expanded newborn screening is a very recent development that utilizes tandem mass spectrometry (MS/MS) to screen for more than 20 inborn disorders of metabolism from a single blood spot.<sup>1-3</sup> This review explores the development and application of MS/MS as a clinical diagnostic testing method and its impact on newborn screening.<sup>2,4</sup>

#### ACYLCARNITINES AND DISORDERS OF FATTY ACID AND AMINO ACID CATABOLISM

The driving force for applying MS/MS in clinical diagnostics was the need to analyze a class of compounds called the acylcarnitines which can accumulate from the defective catabolism of fatty acids and certain amino acids, especially leucine, isoleucine and valine.<sup>1-3</sup> These normal metabolic pathways are located in the mitochondria, and are mediated by coenzyme A (CoA) leading to metabolic end-products, such as acetyl-CoA. When there is a metabolic block, abnormal acyl-CoA species accumulate inside the

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mitochondria, and can only escape by biochemical transformation using alternate pathways. One of the most important detoxification pathways is an exchange reaction to form a corresponding acylcarnitine – a biochemical end-product that can cross mitochondrial membranes and exit the cell (Figure 1).

A patient with a defect of fatty acid oxidation typically develops symptoms after several hours of fasting, as may occur during an intercurrent illness. Reserves of glucose are exhausted and the cell switches to the fatty acid and gluconeogenic amino acid oxidative pathways as the primary energy sources. In a defect of fatty acid oxidation, abnormal metabolites can accumulate very rapidly and result in overwhelming cellular dysfunction – causing the symptoms of metabolic decompensation. Depending on the pathway affected, these symptoms can include vomiting, lethargy, respiratory distress, apnea, coma, cardiac arrhythmias, often accompanied by acidosis, ketosis, hypoglycemia and hyperammonemia. It is during such episodes that patients are at high risk for permanent neurological damage. A delay in emergency treatment of a few hours can be fatal. If intravenous glucose is administered on time, the symptoms and the biochemical abnormalities are rapidly ameliorated. The most common defect of fatty acid oxidation is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. It may present with Reye-like symptoms, or sudden death, yet there can be affected asymptomatic siblings within the family. Severe outcomes are entirely preventable by appropriate treatment.

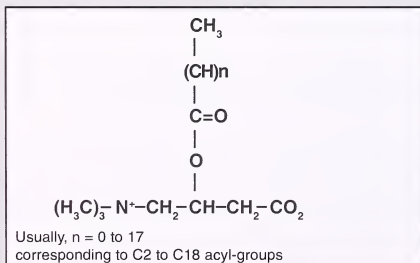
The acylcarnitines in blood reflect the primary accumulating mitochondrial acyl-CoA metabolites in

disorders of fatty acid and amino acid catabolism. Thus, an acylcarnitine “profile” will recognize almost all of the defects in these pathways. While older methods cannot detect acylcarnitines, these metabolites are readily amenable to MS/MS coupled with a “soft” ionization technique such as electrospray (ESI) or fast atom bombardment (FAB).<sup>1-3,5</sup>

## TANDEM MASS SPECTROMETRY AND THE ANALYSIS OF MIXTURES

The tandem mass spectrometer, MS/MS, usually consists of a pair of analytical quadrupole mass analyzers separated by a reaction chamber or collision cell. The triple quadrupole MS/MS is a modern system for analyzing complex mixtures. The mixture to be analyzed undergoes a “soft” ionization to create predominantly quasi-molecular ions, and is injected into the first quadrupole, which separates the molecular ions from each other. The ions then pass in order of mass/charge ( $m/z$ , ratio) into the reaction chamber or collision cell, where they are subjected to controlled fragmentation by collisions with an inert gas such as argon or helium. These fragments of the molecular ions then pass into the second analytical quadrupole where they are analyzed according to their  $m/z$  ratio. Electrospray ionisation is a ‘soft ionisation’ technique which enables the direct analysis of polar or high molecular weight biological substances like amino acids, acylcarnitines and proteins. These compounds can be detected and quantified directly from the solution without need to volatilize the sample. It offers excellent sensitivity (sub-picomole detection limits). Because separation of compounds in the mixture is by differences in mass spectral behavior instead of by column

Figure 1  
Acylcarnitine



Structure of acylcarnitine intermediates in fatty acid oxidation inside the mitochondria. For example, in MCAD deficiency the accumulated acylcarnitine has a side chain containing 8 carbons, such that  $n = 7$  as depicted here.

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chromatography, the entire process from sample injection and ionization to mixture analysis and data acquisition by computer takes only seconds.

The acylcarnitine "profile", generated from a small amount of blood either spotted into filter paper or after coagulation as plasma or serum, can identify more than 20 metabolic defects of fatty acid oxidation and organic acid metabolism, including MCAD deficiency (Table 1). A specimen can be sent to a diagnostic facility by overnight courier and the MS/MS analysis be completed by lunchtime on the day of arrival. MCAD gives a clear diagnostic acylcarnitine pattern as compared with normal controls (Figure 2). This is also true for most of the other disorders of fatty acid and amino acid catabolism. Thus, acylcarnitine analysis has become a valuable front-line diagnostic test for these disorders.

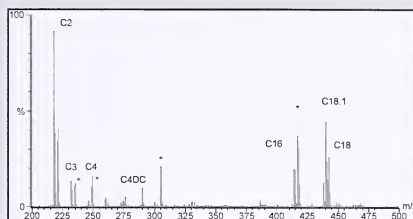
## TANDEM MASS SPECTROMETRY AND EXPANDED NEONATAL SCREENING

Five steps are critical to effective newborn screening: screening, follow-up, diagnosis, management, and evaluation.<sup>4</sup> The following sections discuss the experience with each of these steps in respect to MS/MS newborn screening.

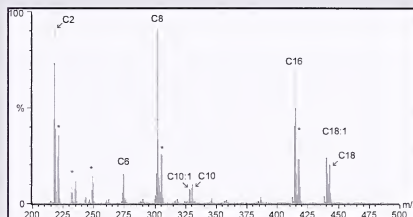
**Screening.** Table 1 summarizes 2 years of initial experience by the North Carolina State Laboratory of Public Health, when 237,774 babies were screened.

In accordance with other newborn screening programs, MCAD deficiency was detected with the highest

Figure 2  
Acylcarnitine Analysis



Normal



MCAD Deficiency

Acylcarnitine profile generated by MS/MS precursor ion scan of normal plasma (upper) compared with that of a patient with MCAD deficiency (lower). Peaks represent molecular species (C2 = acetylcarnitine, etc). Note the marked accumulation of medium-chain species in the disease profile. Peaks marked "\*" are added internal standards.

Table 1

### Disorders of metabolism detected by MS/MS newborn screening (4/20/99 until 4/15/01)<sup>6,7</sup>

#### Fatty acid oxidation

- MCAD (medium chain acyl-CoA dehydrogenase) deficiency (21)
- VLCAD (very long chain acyl-CoA dehydrogenase) deficiency (1)
- SCAD (short chain acyl-CoA dehydrogenase) deficiency (3)
- GA (glutaric acidemia) type II\*
- CPT II (carnitine palmitoyl transferase II) Deficiency\*
- LCHAD/TFP (long chain 3-hydroxyacyl-CoA dehydrogenase) deficiency\*

#### Organic acid metabolism

- 3-MCC (3-methyl crotonyl-CoA carboxylase) deficiency (7)
- Propionic acidemia (1)
- Methylmalonic acidemia (2)
- Glutaric acidemia, type I (1)
- $\beta$ -ketothiolase (SKAT or mitochondrial acetoacetyl-CoA thiolase) deficiency (1)
- Isobutyryl-CoA dehydrogenase deficiency (1)
- 2-methylbutyryl-CoA dehydrogenase deficiency (1)
- Isovaleryl-CoA dehydrogenase deficiency (3)
- Malonic Acidemia\*

#### Amino acid metabolism

- Phenylketonuria (14)
- Argininosuccinic acid lyase deficiency\*
- Citrullinemia (1)
- MSUD\* (Maple Syrup Urine Disease)

\*Cases of these disorders, reported by other screening programs, had not yet been detected in North Carolina. (n)= number of patients. Total number of neonates screened 237,774.

frequency. The incidence of MCAD deficiency was estimated at 1 in 13,600 live births in North Carolina. The overall incidence of disorders of metabolism detected by MS/MS newborn screening was 1 in 4,400 live births.

Beyond implications for the affected infant, newborn screening can have implications for maternal health. An association between the risk of serious complications of pregnancy, especially in the HELPP syndrome (hemolysis, elevated liver function tests and low platelets) with the occurrence of acute fatty liver of pregnancy in the mother and a fetus affected with LCHAD deficiency, was first established 10 years ago. Since then there has been a growing awareness that the presence of other fatty acid oxidation disorders, including MCAD deficiency, can also cause pregnancy complications.

**Follow-up.** Initial follow-up was directed according to cut-off values for each metabolite, typically set at 4 standard deviations above the mean. In the case of an abnormal value, repeat screening samples were requested. If the initial sample had a higher "alert" value, or if the second sample remained above the cutoff, the infant's local physician was contacted immediately. The possibility of a metabolic disorder was discussed and recommendations for follow-up were made. Infants were referred directly to a metabolic genetics center. If the elevated metabolite(s) did not signal a specific or life-threatening disorder, blood and urine samples were sent to the centers from the local physicians for follow-up testing.

The importance of appropriate cut-off values and adequate follow-up testing was illustrated by an infant with glutaric acidemia, type I (GA-I), initially detected on the basis of elevated glutaryl carnitine in the bloodspot.<sup>6</sup> Initial cut-off values for each metabolite are typically set by a statistical determination of 4 standard deviations above the normal mean, but must be adjusted up or down for some metabolites based on experience during newborn screening. Although the patient had an abnormal blood acylcarnitine profile at birth, the repeat specimen was normal; thus, newborn screening ultimately failed to indicate the diagnosis of GA-I. Newborn screening is a powerful tool to potentially diagnose presymptomatic infants; however, it should not be considered a diagnostic test. In order to allow a precise diagnosis and treatment of GA-I, we recommend a complete evaluation, including both a plasma acylcarnitine profile and a urine organic acid analysis of any patient with elevated glutaryl carnitine in a blood spot acylcarnitine profile. The North Carolina State Laboratory has adjusted the cut-off value for glutaryl carnitine to increase the sensitivity of the newborn screening test for GA-I and this is now

suggested as a general recommendation for laboratories screening for GA-I by MS/MS.

**Diagnosis.** The diagnoses of fatty acid oxidation disorders is established by testing urine organic acids and a plasma acylcarnitine profile; whereas, the diagnoses of organic acid metabolism disorders is confirmed by plasma amino acids +/- urine organic acids. Enzyme analysis is required to diagnose disorders where the elevations of metabolites in blood and urine do not provide a conclusive diagnosis.

Since the addition of MS/MS to the North Carolina Newborn Screening Program, 20 infants with elevated hydroxyl-isovalerylcarnitine (C5OH) levels were evaluated. Eight of these 20 infants had persistent elevations of both 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in their urine, highly suggestive of 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. Other enzyme deficiencies that could provoke elevated C5OH, including biotinidase and holocarboxylase synthetase deficiency, were eliminated from the differential diagnosis by confirmatory enzyme testing. In 4 of the remaining 12 infants, maternal 3-MCC deficiency was demonstrated. It is likely that the remaining 8 of these 12 infants for whom urine organic acids normalized also represented maternal 3-MCC deficiency; however, follow-up testing was not requested from the mother or she refused to provide her samples in each case. Infants and mothers with 3-MCC deficiency commonly have clinically significant carnitine deficiency, which motivated the detection and treatment of these individuals.

**Management.** The prompt referral of patients with confirmed or suspected life-threatening disorders of metabolism is critical to fulfill the mission of newborn screening. The successful treatment of inborn errors of metabolism provides justification for MS/MS newborn screening. For example, untreated MCAD deficiency presents as hypoketotic hypoglycemia and is commonly lethal, due to hepatic failure which often mimics Reye syndrome. Since the initiation of MS/MS newborn screening, there have been no deaths among confirmed MCAD deficiency and no cases of missed MCAD deficiency. Treatment consisted of early referral to a metabolic-genetics center, avoidance of fasting, L-carnitine supplementation, and prohibition of formulas containing medium-chain triglyceride (MCT oil). Likewise, nutritional and pharmacologic treatment is available for other disorders detected by MS/MS.

However, the treatment of other potentially detectable disorders of metabolism has been less than optimal, related to issues of detection or delays in detection. While tyrosinemia, type 1, can be effectively treated with



a life-saving enzyme inhibitor, tyrosine levels are not elevated during the newborn period to allow detection of that disorder. More frustrating has been the ineffectiveness of treatment in disorders with severe complications early in life, including glutaric acidemia, type II (GA-II) and maple syrup urine disease (MSUD). GA-II cannot be effectively treated when the presentation is severe, and MSUD can only be effectively treated when a formula lacking branched-chain amino acids is used prior to the onset of symptoms which usually occurs in the first 10 days of life. Although treatment is available for GA-I, MSUD and tyrosinemia, type I, these disorders are quite rare outside selected population isolates (eg. MSUD among the Amish). Consequently, aggressive, earlier detection by more specialized approaches to newborn screening is not practiced.

**Evaluation.** Newborn screening programs require periodic review and analysis of outcome measures to be successful. Adjustment of cut-off values is one important exercise in MS/MS newborn screening, since the cut-off values determine the likelihood of false positive or false negative results.<sup>7</sup> False negative results should be assiduously avoided. False positive results can hamstring a program. Specific causes of false positives are listed in Table 2.

Ratios of metabolites are helpful in the interpretation of elevations unrelated to a metabolic disorder, such as the ratio of C8:C10, which is elevated in MCAD deficiency but not in MCT oil supplementation. Age-specific cut-off values could potentially reduce the frequency of false positive results because the majority of spurious elevations are related to prematurity.<sup>7</sup> Until age-specific cut-off values are available, the newborn screening laboratory typically obtains serial specimens from premature infants until the postconceptual age approaches 40 weeks.

The effectiveness of modifying cut-off values was illustrated by the experience with C5OH. The initial cut-off for C5OH was determined statistically (4 standard

deviations above the mean); the cut-off was increased when the false positive rate was determined to be unacceptably high. Thereafter, the cut-off for C5OH was increased to 5 standard deviations. This adjustment of cut-off values for normal samples has reduced the number of initially elevated samples from 1 in 720 to 1 in 7,400 infants screened, and dramatically reduced the ratio of falsely positive initial screens to a truly positive test in affected infants from 65 to 1 to 3.3 to 1. There was no reduction in the rate of 3-MCC detection observed after the cut-off for C5OH was increased, and no infants with symptomatic 3-MCC deficiency have come to the attention of the North Carolina medical community since the MS/MS screening began.

## CONCLUSION

The difference in newborn screening brought about by MS/MS is the ability to detect more than 20 inborn disorders of metabolism from a single blood dot with a single test. The method detects a confirmed disorder in about 1 in 4,000 cases screened. The most common diseases are MCAD deficiency, PKU, and 3-MCC deficiency. Early diagnosis and treatment of these cases is preventing adverse outcomes, and screening programs are reporting a very low incidence of false positives and false negatives. About half of the states are either screening newborns by MS/MS or have made a decision to do so soon. Even so, there is controversy and debate regarding what is perceived to be a paradigm shift, since the testing equipment is expensive and some of the disorders it detects have no effective treatment. However, once a state decides to implement this method it must accept the responsibility of performing the test properly and of treating diagnosed patients. To do so means providing adequate professional support to include dietitians, genetic counselors, biochemical geneticists and appropriate mechanisms in place for follow-up testing. Pediatric Endocrinologists are often called to consult with infants with emergencies due to inborn errors of metabolism, a good review of the subject should be kept at hand.<sup>8</sup>

Table 2  
Causes for false positive results in MS/MS newborn screening

| Condition                | Metabolites affected   | False positive              |
|--------------------------|------------------------|-----------------------------|
| MCT oil supplementation  | C8, C10                | MCAD deficiency             |
| Prematurity              | C4, C5, C8             | GA-II & MCAD deficiency     |
| Prematurity              | Tyrosine               | Tyrosinemia                 |
| Carnitine supplementaion | C0, C2, C3, (+ others) | Propionic acidemia & others |



## ACKNOWLEDGMENT

We gratefully acknowledge the assistance of Dr. Diane Frazier and Dr. Joseph Muenzer of the University of North Carolina, and of Dr. Shu Chaing and Ms. Susan Weavil of the North Carolina State Laboratory of Public Health, for compilation of data and in the follow-up and evaluation of patients with abnormal MS/MS newborn screening in North Carolina.

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### Letter to the Editor:

Dear Dr. Blizzard and Editors of GGH:

I've been reading GGH for years, and have found it so useful. This month's timely release of the intersex review really "hit the mark". I work in a state birth-defects surveillance department. The non-physicians have expressed tremendous interest in the management of ambiguous genitalia, either as an isolated finding, or related to exstrophy. This review will serve as the focal point for our next monthly teaching session to be supplemented by your review (GGH Vol. 19, No. 1).

Angela E. Lin, MD  
Brigham-Women's Hospital  
MA Center Birth Defects Prevention

Dear Dr. Linn: Thank you!

Dear Other Readers:

Please let us know your positive and negative comments – and your agreements or disagreements regarding the abstracts and their comments and the lead articles. Your input is absolutely necessary for us to maintain, upgrade, and disseminate your agreements and disagreements. We encourage you to respond quickly after your thoughts and criticisms come to mind.

Robert M. Blizzard, MD

## Abstracts from the Literature

### Factors Determining the Pattern of the Variant Creutzfeldt-Jakob Disease (vCJD) Epidemic in Great Britain

**Editorial Preface:** Growth hormone (GH) extracted from human pituitaries obtained at autopsy was first given to children in 1958. Twenty-seven years later (1985), the first cases of Creutzfeldt Jakob Disease (CJD) resulting from such injections were observed in individuals who had received GH injections 8 to 10 years prior to that time. The fact that no cases of CJD were reported reflects the long latent period between exposure and the onset of symptomatic disease.

The exact number of the pituitary injections that may have been contaminated with the CJD prion is unknown. GH from only one of three laboratories in the U.S. extracting pituitaries has been associated with CJD. All three of the laboratories extracting GH used different procedural techniques. In retrospect, the GH extraction procedure of two of the three laboratories eliminated the active prion from the final product. From 1985 until April 2003 only 26 cases of CJD were recorded among several thousand (7,700) recipients in the U.S. who had received native human growth hormone. All U.S. patients with CJD received GH prior to 1977; afterward a new purification step was added to the GH extraction procedure.

The early symptoms of CJD consist of degenerative neurological function. Death unfortunately follows within a period of 6 to 36 months. The number of catastrophes to date in the United States have been relatively small, particularly in light of the number anticipated in 1985 when the first two deaths were reported within a month of each other. Postulation, with reasonable justification, was that the incubation period and susceptibility to the disease were influenced by the dose of contaminated material, possibly the age of the recipient, and possibly by an individual's genetic susceptibility. The latter was suspected on the basis of a few studies using scrapie disease in sheep as a prototype since CJD, occurring primarily in humans, is similar to scrapie disease in sheep. These diseases produce degenerative neurological alterations; although the histology of the pathological findings in the central nervous system are different. They are known as spongiform cerebral encephalopathies.

**Abstract:** In 1985 and 1986 a similar but different spongiform encephalopathy manifested itself in England when humans were first diagnosed with "mad cow

disease" or bovine spongiform encephalopathy (BSE). Cows had been infected by the ingestion of commercially prepared food for cows to which had been added a food enforcement consisting of bovine CNS and other organ components that were unmarketable to humans. Cows ingesting these ground up organ components, when the organs were contaminated, developed BSE after a prolonged incubation period. Infected cattle in the presymptomatic stage were often sent to the slaughter house. This meat was sold in the markets and subsequently infected humans. Thus, the mad cow disease was perpetuated and humans developed a variant of CJD (vCJD). The brain pathology of CJD and vCJD are distinguishably different even though both are spongiform encephalopathies. Over one million cows in the UK were believed to be infected. Identification of infected asymptomatic cows is not easy even though the prion accumulates in the lymphoid tissue as well as in the central nervous system.

Spongiform encephalopathies result from a replicating abnormal protein called a prion. The prions proliferate, destroy cell membranes, and accumulate as they are not destroyed themselves. Clinical symptoms develop when the abnormal protein is diffusely spread through the CNS. Transmission from mother to fetus occurs during pregnancy in the cow. It is not known whether prions are transmitted in cow's milk or colostrum. There are no data regarding transmission in humans by placenta, in human milk or colostrum.

At the end of 2001 in the UK there were 113 cases of vCJD, nine of whom were alive at that time. A few cases have occurred in other countries including France and Ireland and two cases in the United States. BSE crosses species barriers and consequently is found in squirrels and other mammals. The disease scrapie has been adapted to mice and genetic predisposition has been studied. Different strains of mice react differently to the exposure of the scrapie prion. Recently a genetic predisposition for susceptibility in humans has been demonstrated. At the time the referenced article was written, all of the human cases tested in the UK (87) shared a common genetic trait, being methionine homozygous (MM) at codon 29 of the prion protein (PrP) gene. Estimates in Caucasian populations are that 40% of the population share this trait. Of the other 60% of the population, 13% are valine homozygous (VV) and the remaining 47% heterozygous for methionine and valine (MV). The authors of the referenced article also refer to a report that there is a decreased risk of CJD in those with HLA-DQ7. This new finding, if correct, suggests complex multi gene determinations of patterns of susceptibility.

The authors discuss extensively the difficulty in predicting the potential magnitude of the UK epidemic.

Of significant importance, the authors believe that even in the worst case scenario in which over 8,000 cases will appear by the year 2080, it is unlikely that a very large increase in case numbers would be expected in the short term (2-5 years).

The epidemiological determinants of the cause of the epidemic which make projections complex include; (a) incubation period distribution, (b) possible age dependent susceptibility to exposure to infection, (c) the effectiveness of the specified bovine ban in the UK, and (d) the genetic susceptibility to infection. For each of these determinants the data used for calculation are nebulous. However the best current estimate (guesstimate) of (a) for mean incubation period is stated with trepidation to be ca. 7 years, (b) the age dependent maximum susceptibility for individuals is 10-20 years of age, (c) for effectiveness of the specified bovine ban, the authors are unable to utilize current data in the calculation, and (d) in respect to utilizing genetic susceptibility, recent studies have indicated that there may be substantial genetic variation in susceptibility, which prevents more than speculation.

The authors conclude that the main priority, in view of all the above stated difficulties, is to develop a diagnostic test that is able to both detect infection early in the incubation period and which can be applied to large population samples in humans, bovine and other species.

Ghani AC, et al. *Proc R Soc Lond B Biol Sci* 2003;270:689-698.

**Editor's Comment:** *Disease curses continue to befall mankind. These are often of our own making such as in the instance of man promoting "mad cow disease". Hopefully a test will be designed that permits identification early in the incubation period of the presence of the prions and thus make it possible to identify those animals affected. Much has yet to be learned about the prion and how it might be combated.*

*In respect to CJD in humans who received native pituitary growth hormone from autopsied bodies, we have suffered enough, even though only 26 of over 7,000 potentially infected subjects have died. A philosophical point, which hopefully we have learned, is that treatments which physicians prescribe today may not manifest their toxic effects for many years. As the Hippocratic Oath states, and as Lawson Wilkins practiced (Growth, Genetics & Hormones Vol. 19, No. 2) and taught, "do no harm to the patient". Unfortunately we do not have a crystal ball to assist us with the decisions we must make.*

Robert M. Blizzard, MD

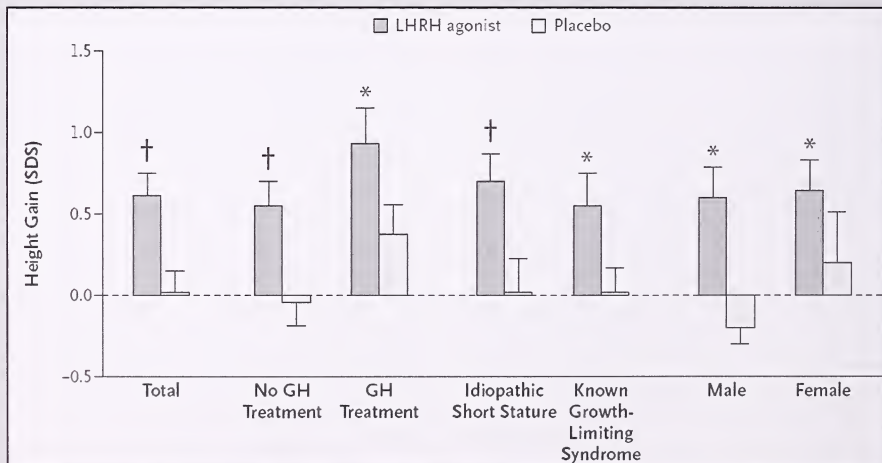
## Treatment with Lutenizing Hormone-Releasing Hormone Agonist in Adolescents with Short Stature

This study was performed to evaluate whether treatment with a lutenizing hormone-releasing hormone agonist (LHRHa) increases adult height in short adolescents with normally timed puberty. There were 32 girls and 18 boys with a mean predicted adult height of more than 3 SDS below the population mean who were administered an LHRHa or a placebo in a randomized double-blind fashion; 26 subjects received the medication and 24 were given placebo. There were a variety of growth limiting disorders, but principally idiopathic short stature. Three patients were also treated with growth hormone (GH) because they had a peak GH after stimulation of less than 7 µg/l. The treatment was started at approximately 12-13 years of age; mean bone age was 11.5-13.2 years, and mean Tanner stages were 2.8 to 3.2 in the two groups, respectively. The mean duration of the LHRHa treatment group was 3.5 years, and that of the placebo group was 2.1 years. Adult height was measured when the bone age exceeded 16 years in girls and 17 years in boys, and when the growth rate was less than 1.5 cm per year. Forty-seven subjects were followed until they attained full adult height.

At the end of the study, those treated with LHRHa were older and taller than those who received placebo (20 vs 18 years of age; and -2.2 vs -3.0 SD below the 50<sup>th</sup> percentile, respectively). Treatment with LHRHa resulted in a mean increase of 0.6 SDS in height (4.2 cm) over the initial predicted adult height in these short patients. The gain in height among the LHRHa treated group was independent of sex, concomitant GH treatment or presence of growth limiting syndromes (Figure). However, added GH treatment produced an apparent additive effect on growth (+ 0.4 SDS). The principal adverse event of this treatment was a decrease in bone accretion, with reduced bone mineral density below that attained in the placebo group. There were no apparent lasting effects on secondary sexual characteristics. The authors concluded that LHRHa increases adult height, but because of resulting decreased bone mineral density, it should not be routinely employed to augment adult height.

Yanovski JA, et al. *New Eng J Med* 2003; 348:908-917.

Figure



Standard-Deviation Score (SDS) for Gain in Height over the Initially Predicted Adult Height. The T bars indicate standard errors. Data are for all 47 patients with adult-height measurements. Asterisks denote  $P < 0.05$ , and daggers  $P < 0.01$  for the comparison with placebo. LHRH denotes lutenizing hormone-releasing hormone, and GH growth hormone.

Reprint with permission from Yanovski JA, et al. *New Eng J Med* 2003; 348:908-917.



**First Editor's Comment:** This very well controlled study clearly showed that there may be a small increment achieved in adult height (mean of 4.2 cm) with LHRHa treatment of short stature patients. Previous studies have also shown that there is a small gain in adult height with such therapy.<sup>1,2</sup> However, in this study the medication was given for more prolonged periods (mean 3.5 years) and it resulted in a significant reduction of bone mineral density. This is not surprising, since bone accretion at the time of adolescence is greatly dependent on the presence of adequate pubertal hormones which are suppressed by LHRHa. Of great concern is that this deficit persisted even after the LHRHa treatment ceased. It would have been of interest to ascertain calcium intake and determine if some of these detrimental effects could have been counteracted by an increased ingestion of this mineral. I agree with the authors that LHRHa treatment for augmentation purposes to increase height should not be routinely prescribed. The average cost of such treatment is \$10,000 to \$15,000 per year, and this should also be kept in mind.

Fima Lifshitz, MD

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**Second Editor's Comment:** While the current study may not be ideal in terms of the present approach to inhibition of hypothalamic-pituitary-gonadal function with LHRHa, it is unlikely that similar investigations will be conducted in the future. Furthermore, the preponderance of girls with intrinsic short stature (32/50) without gonadal dysgenesis is the reverse of that encountered in general pediatric endocrine experience. Thus, present data serve for future recommendations. This writer agrees with the conclusion of the authors and that of the first editor's comment; namely that routine administration of LHRHa is not to be recommended for subjects with intrinsic short stature. It is of interest that the increase in adult height was greatest in patients who received both GH and LHRHa. Nevertheless, in the absence of data demonstrating significant educational, social, and occupational benefit of relatively small increases in adult stature, such efforts cannot be routinely supported.

Allen W. Root, MD

## Reference

1. Carel JC, et al. *J Clin Endocrinol Metab* 2002;87:4111-4117.

# Do Growth Hormone (GH) Serial Sampling, Insulin-Like Growth Factor-I (IGF-I) or Auxological Measurements Have an Advantage Over GH Stimulation Testing in Predicting the Linear Growth Response to GH Therapy?

Reliable indices that are consistently able to predict the linear growth promoting effects of recombinant human growth hormone (rhGH) in short children have long been sought. The authors analyzed data from a National Cooperative Growth Study of the usefulness of IGF determinations, auxological measurements, and 12-hour serial GH measurements obtained every 20 minutes between 2000 and 0800 hours, in children who were treated with rhGH (0.29 mg/kg/week in 6± weekly injections) for a mean of 3.6-3.8 years. There were 825 prepubertal children with short stature studied (mean height -2.8 SDS; bone age delay ~2.3 years). The children were subdivided into one group of 300 (231 males, 69 females) with isolated GH deficiency (IGHD - peak GH response to provocative stimulation <10 ng/mL by unstated methods) and 525 (404 males, 121 females) with idiopathic short stature (ISS - peak stimulated GH response ≥10 ng/mL). The data were analyzed by the cluster program. In addition, a measurement of the "orderliness" or "regularity" of overnight spontaneous, endogenous GH secretion, which is termed "approximate entropy", was calculated.

As anticipated, mean and maximum spontaneous peak GH levels, pooled mean GH concentrations, and mean area under the GH peaks were significantly lower in subjects with IGHD than in those with ISS. Interestingly, pretreatment IGF-I concentrations were similar in the two groups (120 and 125 ng/mL, respectively). The increment in height SDS after treatment with rhGH was similar in the two groups (+1.2 to 1.3 SDS). Significant but weak correlations ( $r < 0.4$ ) related rhGH-induced height increment to height deficit prior to treatment, duration of treatment, and mid parental height SDS in both groups. Maximum stimulated GH values, spontaneous overnight GH measurements, and pre-treatment IGF-I levels were also inversely related to rhGH-induced growth, but again the  $r$  values were low ( $-0.15$  to  $-0.395$ ). By multiple regression analyses, only the peak GH response to secretagogue was inversely correlated to treatment related height increment; spontaneous GH measurements were not related. When data from children with "severe" IGHD (peak stimulated GH response <5 ng/mL) or "extreme" ISS (height <-3.3 SDS)



were isolated and examined, spontaneous GH measurements were inversely related to treatment induced growth but did not improve calculated height prediction models. Spontaneous GH secretion was more orderly in children with severe IGHD than those with "moderate" IGHD. In the ISS subjects, GH secretion was more orderly in those with "mild" ISS, and IGF-I concentrations were higher than in those with extreme ISS. The authors conclude that in general, serial measurements of spontaneous overnight GH secretion did not provide information helpful in the prediction of the linear growth response to rhGH, thus supporting the conclusion of several earlier studies of this question.

Rogol AD, et al. *Clin Endocrinol* 2003;58:229-237.

**First Editor's Comment:** Clearly, the diagnosis of IGHD is fraught with difficulty as 40-80 percent of such children will have normal GH secretion as adults. Thus, there must be overlap between the diagnostic categories of IGHD and ISS in this study. In this regard it is of interest that the "disorderliness" of GH secretion was greatest in those subjects with "moderate" IGHD and both "mild" and "extreme" ISS – implying a close relationship between these groups in the regulation of GH secretion. As the investigators suggest, it may be that a defect in the "orderly" secretion of GH is translated into decreased tissue responsiveness to GH even though the absolute amount of GH secreted may be normal. Although several factors were related to height increment on rhGH, none had the high  $r$  value we seek as an "absolute" predictor of response. Hence, the search goes on!

Allen W. Root, MD

**Second Editor's Comment:** I feel pressured to comment that a possible reason that many children appear to be GH deficient as children but not as adults is that the sex hormones stimulate GH release. We use the same threshold criteria for GH release to secretagogues in adults as children. How do we know that apparent isolated GHD children are not still partially GHD as adults? Studies are needed in this group of patients when they reach adulthood to evaluate comparison of GH response to secretagogues in adults who were not thought to be GHD as children. In such a study we might find that those diagnosed with GHD as children are still GHD as adults relative to others who were never short as children. The fact that many pediatric endocrinologists used to prime suspect GHD children with testosterone before administering secretagogues for the purpose of exaggerating the GH response in suspect GHD children supports my hypothesis.

Robert M. Blizzard, MD

**Third Editor's Comment:** In the previous issue of *Growth, Genetics & Hormones* (Vol. 19, No. 2) a paper by Lanes and Jacobowitz was reviewed.<sup>1</sup> These authors also showed that IGF-I and IGFBP3 were not useful in assessing the response to hGH therapy. Careful measurements and monitoring of growth are the gold standards.

Fima Lifshitz, MD

## Reference

1. Lanes R, Jacobowitz S. *J Pediatr* 2002;141:606-610.

## Is the Growth Hormone/IGF-I Axis Stimulatory or Inhibitory on the Aging Process?

Two recent articles published in *Science* identify the GH/IGF-I axis as playing a major role in the aging process of many species including humans. The data persuasively argue that components of this axis may negatively affect longevity. The majority of the data support the hypothesis that limited secretion of IGF-I promotes long life. A brief synopsis follows.

In yeast, down-regulation of intracellular signaling pathways that are dependent on glucose increases the life span of the organisms up to 300%. In worms (*C. elegans*), loss-of-function mutations of a gene called *Daf-2* encoding an ortholog of the IGF-I receptor extend survival up to 300%. In the fly, inactivation of the gene encoding the insulin receptor increases longevity up to 200%. Mice with homozygous inactivating mutations in *Prop-1*, *Pit-1*, or *Ghr* survive 25%-65% longer than do

wild-type mice. Since mice with a defect in the GH receptor have high serum GH, the decrease in IGF-I signaling probably is the common factor responsible for the extended life of the mutant animals, insects, etc. Partial caloric restriction in rodents and possibly in monkeys also increases life span. Decreased synthesis of IGF-I and lowering of serum concentrations of glucose and insulin occur simultaneously with caloric restriction.

Since IGF-I acts in part by increasing transcription through the mitogen-activated protein kinase pathway (MAPK),<sup>1</sup> which promotes cell division and growth, attenuation of this pathway possibly reduces the potential for lethal errors in this system. Since IGF-I decreases the activities of anti-oxidant enzymes such as superoxide dismutase and catalase, there is reduced ability in the presence of IGF-I to respond to stress and

thus enhanced susceptibility to cellular damage; accordingly, inhibition of this property of IGF-I would be expected to augment the stress response. Caloric restriction also increases the longevity of the *Prop-1* deficient or Ames mouse; thus, the mechanisms by which caloric restriction and IGF-I deficiency act to increase life span may differ. In rodents, partial caloric restriction increases the immune response to infectious agents and attenuates the destructive cellular immune changes of aging. This thereby decreases the incidence of degenerative and inflammatory diseases and tumor formation.

In adult humans, hypopituitarism is associated with abnormal lipid metabolism, atherosclerosis, and early death. Yet, acromegalic subjects with excess GH secretion also have a shortened life span, and critically ill patients who receive exogenous GH have a greater mortality rate than do those with similar illnesses not so treated. Since patients with *PROP-1* deficiency or patients with inactive GH receptors (who do not have ACTH deficiency), do not succumb at an early age and may even live exceedingly long,<sup>2</sup> Tatar et al suggest that perhaps ACTH rather than GH deficiency is responsible for early death in humans with pan-pituitary dysfunction. The authors further suggest that pharmacological agents designed to reduce IGF-I levels be explored as extenders of life span.

Longo VD, Finch CE. Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science* 2003;299:1342-1346.

Tatar M, et al. The endocrine regulation of aging by insulin-like signals. *Science* 2003;299:1346-1351.

**Editor's Comment:** The authors point out that glucose/

insulin/GH/IGF-I and their signaling pathways may actually decrease rather than prolong life span. While experimental findings cannot always be directly translated into analysis in humans, the authors' conclusions merit consideration when we prescribe GH for our adult patients. These findings also should cause those who claim that GH is an effective anti-aging agent in non-GH deficient elderly adults to reconsider this recommendation. Body weight control combined with an efficient exercise program is likely to be far more effective in lengthening life than is administration of GH to adults without GH deficiency. The need to maintain adequate glucocorticoid replacement therapy in adults with panhypopituitarism must also be emphasized to minimize stress.

Other papers in the *Science* series are also worth reviewing, particularly one by Hasty et al<sup>3</sup> describing aging defects due to genetic abnormalities in genome maintenance (transcription, DNA repair, DNA helicase activity). Blüher et al<sup>4</sup> recently reported that mice with loss of the insulin receptor only in adipose tissue had extended life span. In addition, an extensive review on caloric restriction and extension of life is available on the internet.<sup>5</sup>

Allen W. Root, MD

## References

1. Pearson G, et al. *Endocrine Rev* 2001;22:153-183.
2. Rosenfeld RG, et al. *Endocrine Rev* 1994;15:369-390.
3. Hasty P, et al. *Science* 2003;299:1355-1359.
4. Blüher M, et al. *Science* 2003;299:572-574.
5. Masoro EJ. *Science's SAGE KE* 2003; <http://sageke.sciencemag.org/cgi/content/full/sageke/2003/8/re2>

## Can Growth Hormone Prevent Aging?

Dr. Vance recently published an article by the above title. She concludes that antiaging therapy with human growth hormone (hGH) has not yet been proven safe or effective. Although not the first investigator to study GH in relation to body composition, Dr. Dan Rudman<sup>1</sup> in 1990 authored the first publication concerning the use of hGH in 12 elderly men. Dr. Vance summarizes the data in that report; the administration of hGH at approximately twice the dose of hGH used in adult growth hormone deficiency (GHD) for six months resulted in a 4.7 kg increase in lean body mass, a 3.5 kg decrease in adipose tissue, and an increase of 0.02 gm/cm in lumbar spine density, and significant increases in BPs and in fasting glucose concentrations. There were no assessments of exercise endurance, muscle strength, or quality of life. Vance points out that the follow-up to this study does not include any substantiation that hGH in elderly men does more than

confirm an increase in lean body mass and a reduction of body fat (with no change generally in total body weight).

Vance appropriately criticizes the proliferation of commercial "antiaging" clinics which promote the sale of inappropriate and ineffective agents such as arginine and other agents to release growth hormone and of hGH itself. Vance chastises those who for monetary gain are so dishonest and potentially destructive of their customer's health.

The use of long-term administration of hGH in adults with no established growth hormone deficiency is appropriately deprecated as it is not known whether the effect of long-term administration of hGH in the elderly is potentially harmful. Cancer of various organs is of particular concern. The work of Chan et al is cited.<sup>2</sup> In 152 healthy men, the relative risk of the subsequent development of prostate cancer was increased by a

factor of 4:3 among men who had serum concentrations of IGF-I in the highest quartile as compared with those men with concentrations in the lowest quartile.

The author's complete conclusion is that there is no "current" magic bullet medication that retards or reverses aging.

Vance ML. *N Eng J Med* 2003;348:779-780.

**Editor's Comment:** This editor agrees with Dr. Vance's conclusions. I concur having initiated in 1982 the first study of the effect of hGH in elderly individuals. I and four other male subjects over the age of 55 received native hGH daily for 2.0 - 2.5 years at a dose that raised IGF-I levels from GHD concentrations to levels above the 50th percentile for young adult males. In myself there was an increase in lean body mass and decrease in free fat mass. The same occurred in two other subjects but not in subjects 4 and 5. Some element of hyperinsulinemia and glucose intolerance occurred but not overt diabetes mellitus. No overt changes in gross body configuration occurred. Subjectively there were no changes in self image, sense of well-being or libido and no changes in psychological mood. There were no

changes in hair color, the rate of hair or nail growth, or disappearance of wrinkles. The study was stopped in 1985 when native hGH was no longer extracted from human pituitaries because of the development of Creutzfeldt Jakob disease in some GHD patients having received hGH. On the basis of all reports in the literature and my scientific observations among the five normal elderly patients in the study cited, I agree with Dr. Vance and most other pediatric endocrinologists, "to give hGH for purposes of attempting to alter aging in individuals who secrete GH normally for age is unacceptable unless administered under a rigidly controlled double blind study".

Reference to the role of IGF-I in shortening or lengthening life in animals is presented in the abstract immediately preceding this one (page 42). Theoretically longevity can be shortened by the indiscriminate use of GH in mammals.

Robert M. Blizzard, MD

## References

1. Rudman D et al. *N Engl J Med* 1990;323:1-6.
2. Chan, et al. *Science* 1998;279:563-566.

## High Dose Growth Hormone Treatment Induces Acceleration of Skeletal Maturation and an Earlier Onset of Puberty in Children with Idiopathic Short Stature

Kamp et al report on the experience of their multicenter European randomized trial of high dose (0.07mg/kg/week) recombinant growth hormone (GH) in prepubertal idiopathic short stature (ISS) children with baseline heights less than - 2SDS. Forty children (ages 4 -10 years) were recruited and 12 completed 4 years of study while 8 completed 5 years of treatment. Inclusion criteria, in addition to pre-pubertal status and age <8 years for girls and < 10 years for boys, were normal responses to GH stimulation testing (GH >10µg/l). Subjects were measured and Tanner staging performed every three months; bone age determinations were made yearly. During the first year of treatment all subjects randomized to GH treatment participated in a "GH responsiveness" study where GH was administered at two different doses for three months each, separated by three-month washout periods. High dose GH treatment was continued until the first signs of puberty.

In the second and subsequent years of treatment, height SDS for chronological age increased significantly and there was a significant difference in bone age advancement compared to controls. Indeed, height SDS for bone age was not different between the two groups at five years. Eighty-five percent (11/13) of boys in the high dose GH group entered puberty at a median age of 12.2 years during the study, compared with 54% (7/13) of

controls at a median age of 13.9 years. Similar findings for girls included 50% (2/4) of treated children entering puberty at a median age of 10.2 years versus 20% (1/5) of controls at a median age of 9.9 years. The age and sex adjusted relative risk of entering puberty earlier was 4.7.

The authors conclude that there is no evidence that young children with ISS benefit from high dose GH in the pre-pubertal period. They point out that their study differs from previous studies in that they sought to treat younger pre-pubertal children with ISS for a longer period of time with high dose GH, and that they discontinued GH at the onset of puberty so as to separate the influence of GH from that of sex steroids in pubertal growth. They are critical of other studies that did not include randomized ISS control groups, but used reference data for pubertal onset and GH dose.

Kamp GA, et al. *Arch Dis Child* 2002;87:215-220.

**Editor's Comment:** This is an interesting and well-conceived study. The use of high dose GH in ISS remains controversial, and well-controlled studies using different GH doses in different age groups are important aids in helping the endocrinologist decide whom to treat and for how long. The data from this manuscript suggest that early high dose GH treatment may improve height



SDS for CA, but that there may be a price to pay in final height gain by entering puberty earlier. We await the data on final heights of the subjects in this study.

In an accompanying "Commentary", Clayton<sup>1</sup> summarizes and reiterates previous data which demonstrate that the response to GH in ISS, whether short, mid- or long-term is variable, that overall reported gains in final heights range from 3 – 9 cm in various studies, and that pre-pubertal improvements in growth

velocity are dose dependent. He reemphasizes the importance of matched contemporaneous control groups and the current lack of information regarding the dose response for GH in conditions where it is currently being used.

William L. Clarke, MD

## Reference

1. Clayton PE. *Arch Dis Child* 2002;87:219-220.

## Low Nutrient Intake and Early Growth for Later Insulin Resistance in Adolescents Born Preterm

In this potentially very important paper the investigators study the effects of various diets in the newborn period of premature infants versus the presence at ages 15-16 years of a plasma marker for the development of insulin resistance and non-insulin dependent diabetes in adults. The marker is known as 32/33-SPI (split proinsulin). Plasma concentrations were measured in 216 mid to late adolescents (13-16 years of age) who had been delivered prematurely (mean gestational age of 31 weeks and mean birth weight of 1.4 kg). Of these preterm infants, 110 had received a low nutrient formula and 106 had received a high nutrient formula.

Not surprising, the preterm newborns fed the lower nutrient formula gained less weight prior to discharge compared to those receiving the higher nutrient formula. The specific formulas were stopped when the infants were discharged from the neonatal unit or had reached a weight of 2000 gms. At 16 years of age the children were re-evaluated and fasting serum concentrations of insulin, proinsulin, and 32/33-SPI were determined in specific assays. As adolescents, the low nutrient group had lower levels of 32/33-SPI than the levels in the high nutrient group. Levels of insulin, proinsulin and glucose were similar in the two groups.

After statistically adjusting for the effects of gender, gestational age, birth weight, neonatal morbidity, and other variables, the relationship between neonatal diet and concentrations of 32/33-SPI remained significant. Further analysis revealed that high rates of weight gain in the neonatal period (basically a surrogate for higher caloric intake) - specifically within the first two weeks after birth - were most closely related to elevated levels of 32/33-SPI in adolescence which were independent of birth weight. There was no association between values of 32/33-SPI and weight gain between two weeks of age and discharge from the nursery, discharge and 18 months, 18 months and 9 years, 9-12, and 13-16 years. Preterm adolescents, fed a low nutrient diet at birth, did not differ in stature, weight, BMI, or sum of skinfold thickness compared with premature infants who

were fed the high nutrient formula or from the control group of adolescents born at term.

The investigators conclude that premature infants who were fed a low nutrient formula (albeit one that impaired neonatal weight gain) for several weeks after birth resulted in lower concentrations of 32/33-SPI in adolescence, and by inference these subjects may be less likely to develop insulin resistance. They hypothesize that the risk for developing insulin resistance in low birth weight neonates is not necessarily programmed by the intrauterine environment, but also by the immediate post partum extrauterine environment as exemplified by the high nutrient formula and more rapid weight gain that accompanies this diet. They suggest that altering current feeding practices of preterm infants by lowering their caloric intake and decreasing their early rate of weight gain may prevent later development of insulin resistance, cardiovascular disease, and the dysmetabolic syndrome without adversely affecting their long-term growth.

Singhal A, et al. *Lancet* 2003;361:1089-1097.

**Editor's Comment:** Low birth weight infants are at risk for future development of the dysmetabolic syndrome (X) of dyslipidemia, insulin resistance, and type 2 diabetes mellitus.<sup>1</sup> It has been hypothesized that intrauterine factors that affect the fetal response to decreased blood flow or nutrient availability "program" the subsequent development of this syndrome - primarily by inhibiting tissue responsiveness to insulin. However, there is no specific explanation that explains the cellular and molecular mechanisms by which low birth weight predisposes to insulin resistance. The current work is of interest because it points to the possibility that postnatal factors, in this instance rapid growth secondary to increased nutritional intake in very early life, contribute to the later development of insulin resistance. Thus, this observation affords the possibility of an intervention that may prevent this long-term complication without



negatively impacting the overall growth of the low birth weight subject. Considered in the context of the findings is that partial nutrient restriction and growth hormone deficiency extend life in many species<sup>2,3</sup> including perhaps primates. Since the level of 32/33-SPI is only a marker of insulin resistance, it will be necessary for Singhal et al to continue to follow these subjects and to document the development of insulin resistance and

other adverse events as, and if they occur.

Allen W. Root, MD

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1. Goran MI, et al. *J Clin Endocrinol Metab* 2003;88:1417-1427.
2. Longo VD, Finch CE. *Science* 2003;299:1342-1346.
3. Tatar M, Bartke A, Antebi A. *Science* 2003;299:1346-1351.

## Genetics, Chondrodysplasias and Other: New Potpourri

Skeletal dysplasias are distinguished by what part of the skeleton and/or bone is involved in various types of short stature. Metaphyseal dysplasia (MCD) refers to a group of skeletal disorders in which the diagnostic findings primarily involve the metaphyses of the tubular bones. Other bones are usually normal or only slightly affected. The metaphyseal involvement may be mild (as in Schmid's MCD) or more severe (as in Jansen's MCD). Some MCD syndromes have associated extra-skeletal features (e.g. MCD – McKusick type which is also known as Cartilage Hair Hypoplasia). There appears to be a new type of chondrodysplasia with a distinctive pattern of involvement, as described by Lee et al. An eight-year-old boy with a distinctive form of metaphyseal chondrodysplasia and a previously described family with 4 generations affected are the focus of Lee's report. The child had short stature and the birth weight was 3 kg. Bilateral genu varus and wrist swelling were first noted at 4 years of age. The mother had mild wrist flaring. She was not disproportionate by U/L ratio. At 8 7/12 years the boy's height was -2.9 SD below the mean and his U/L ratio was 1.21 (normal 1.0). No significant differences were noted in the length of the upper versus the lower part of each extremity, the spine, the facial configuration or the hair. Skeletal survey revealed metaphyseal abnormalities affecting proximally and distally the tibiae, fibulae, femurs, humeri, radii and ulnar bones and the hands, but the spine was unaffected. The physical and radiological findings did not fit the Schmid, McKusick, or Jansen types of MCD. A very rare autosomal dominant 4 generation affected condition described by Rosenberg and Lohr (*Eur J Pediatr* 1986;145:40-45) has features similar to those of this patient, except the patients in this family reportedly had a wedge deformity and platyspondyly of the spine which Lee et al believed to be within the range of normal variance. No molecular studies were reported in the four patients reported by Rosenberg et al or in this 8-year-old boy.

Another example of describing a chondrodysrophy by the sites where skeletal abnormalities occur is rhizomelic chondrodysplasia punctata (RCP). This rare autosomal recessive disorder has severe shortening of the proximal long bones (rhizomelia), bilateral cataracts and severe growth and psychosocial delay. White et al report the natural history of rhizomelic chondrodysplasia punctata. Radiographic evidence of stippled epiphyses is present and MRI examination of the cervical spine is often abnormal (kinking without compression of the cord and/or compression of the cord). All children with RCP are born with severe joint contractures that improve with time although not before many of the patients (40-85%) die by one year of age. Less than 10% of the 48 cases described in respect to death were alive by 12 years of age.

Biochemical analysis and complementation studies allowed separation of the 97 patients whose data were tabulated to be differentiated on the basis of peroxisomal enzymes into three types: (Type 1) a spectrum of PEX7 gene mutations, (Type 2) mutations in the acyl-CoA:diOHacetonePO4 acyltransferase (DHAPAT) gene, and (Type 3) mutations in the ADAPS (alkyl-diOHacetonePO4 synthesis) gene.

The value of this article by White et al is that there has been a sincere attempt to delineate the natural history of RCP. The authors systematically address health concerns that arise in infants and children with RCP. The intent of White et al is to present evidence-based guidance to care providers so they can better help families understand and cope with this diagnosis. For example, 90% of infants survive for the first year and 50% survive until 6 years. Previously, death was believed to almost always occur early in infancy or childhood. Medical personnel or parents concerned and/or involved with patients with suspect or proven diagnosis of RCP are strongly encouraged to read the complete article.

Lee YS, et al. A distinctive type of metaphyseal chondrodysplasia with characteristic thickening of the distal ulna and radius: Possible MCD – Rosenberg. *Am J Med Genet* 2003;119A:50-56.

White A, et al. Natural history of rhizomelic chondrodysplasia punctata. *Am J Med Genet* 2003;118A:332-342.

Other examples of chondrodystrophies are those in the subgroup known as spondylo-epi-metaphyseal dysplasia (SEMDs) which includes a number of disorders each defined by the combination of vertebral, epiphyseal, and metaphyseal anomalies present.

One such entity is the Dyggve-Melchior-Clausen Syndrome (DMCS) which is characterized by short trunk dwarfism (<4SD) with specific radiological appearances most likely reflecting abnormalities of the growth plates including platyspondyly (flattened peripheral bodies) with notched end plates, metaphyseal irregularities, laterally displaced capital femoral epiphyses, and small iliac wings with lacy iliac crests. Mental retardation is an inherent part of the syndrome. DMCS is progressive and clinical features are reminiscent of a storage disorder, specifically Morquio's disease, but the two conditions can be differentiated by the absence of corneal clouding, deafness, valvular disease and/or mucopolysacchariduria, all of which are characteristic of Morquio's disease.

Ghouzzi et al have used a positional cloning strategy to identify the DMC gene. They detected 7 deleterious mutations within a gene predicted from a human transcript (FLJ20071) in 10 DMC families. The DMC gene transcript is widely distributed but appears abundant in chondrocytes and fetal brain. The authors cannot explain the function of the gene product at this time, but conclude that the DMC syndrome results from loss of function of a gene that they propose to name Dymeclin, which may have a role in the process of intracellular digestion of protein.

Ghouzzi VE, et al. Mutations in a novel gene dymeclin (FLJ20071) are responsible for Dyggve-Melchior-Clausen syndrome. *Hum Mol Genet* 2003;12:357-364.

A fourth example of types of chondrodysplasia and how they are designated is the entity called acrocapitofemoral dysplasia which is characterized by short stature of variable degrees with short limbs and brachydactyly. It is included in the differential diagnosis of hypochondroplasia. These patients also have large heads and often have pectus deformities. Epiphyseal changes are present at the shoulders, knees, ankles, hands, hips and proximal femurs. The latter are egg shaped with very short femoral necks. Shortened tubular bones characterize the brachydactyly. Congenital anomalies are limited to the skeletal system and intelligence is characteristically unaffected.

Homozygosity mapping by descent was performed in two consanguineous families. The Indian hedgehog gene (IHH) was found to be mutated in affected individuals. The nucleotide changes are seen in the amino terminal signaling domain, which is responsible

for short and long range signaling. Thus, it appears to affect the regulation and proliferation of the hypertrophic chondrocytes in the growth plate. The authors postulate that the mutations cause an increased rate of chondrocyte differentiation by diminished Indian Hedgehog signaling in the growth plate.

Hellems J, et al. Homozygous mutations in *IHH* cause acrocapitofemoral dysplasia, an autosomal recessive disorder with cone-shaped epiphyses in hands and hips. *Am J Med Genet* 2003;72:1040-1046.

**First Editor's Comment:** *The genome project has made identification of mutated genes relatively easy to identify. The effects of different mutations of the same gene have been particularly evident among the chondrodystrophies, both in relating two different entities to different mutations of the same gene and differentiating and identifying different gene abnormalities for what used to be thought the same disease entity. Unfortunately descriptive names are often misleading because there is tremendous overlap among these entities. The most recently updated classification of skeletal dysplasias can be found at [www.csmc.edu/genetics/skeldys](http://www.csmc.edu/genetics/skeldys).*

Judith Hall, OC, MD

**Second Editor's Comments:** *One is struck by the clinical resemblance of acrocapitofemoral dysplasia (ACFD) to achondroplasia. The phenotype is not identical, but the rhizomelic shortening of limbs, large head with prominent forehead, narrow thorax, bowing of the knees and even overgrowth of the proximal fibula on X-ray are similar. The reason for this resemblance may lie in the relationship of *Ihh* to *FGFR3*, which is mutated in achondroplasia, in the growth plate. Both regulate chondrocyte proliferation: *Ihh* positively and *FGFR3* negatively. In ACFD the positive effect on proliferation is lost; however in achondroplasia the mutations are activating in nature so that they enhance the anti-mitotic effects of *FGFR3*. In other, both lead to reduced chondrocyte proliferation. A consequence of the anti-mitotic effects of *FGFR3* mutations in achondroplasia is a reduction in the number of terminally differentiating chondrocytes. Since these cells are the source of *Ihh*, the achondroplasia mutations secondarily reduce the production and local effects of *Ihh*. Thus, these two disorders look alike to clinicians because they involve disturbances of the same regulatory pathways in the growth plate.*

William Horton, MD

## Size at Birth and Early Childhood Growth in Relation to Maternal Smoking, Parity & Infant Breast-Feeding: Longitudinal Birth Cohort Study and Analysis

The relationship between maternal smoking, parity and early breast or bottle feeding to size at birth and childhood growth were evaluated. A large representative birth cohort was studied between 0 and 5 years of age. A total of 1335 normal infants had weight, length, height and head circumference measured at birth and subsequently up to ten occasions until they were 5 years of age. Multilevel modeling was used to analyze the longitudinal growth data. Infants of maternal smokers were systematically small at birth when compared with infants of non-smokers. However, these infants showed complete catch-up growth over the first 12 months of life. Infants of primiparous pregnancies were thin at birth and showed dramatic catch-up growth, and were heavier and taller than infants of nonprimiparous pregnancies from 12 months onwards. Breast-fed infants were similar in size at birth to bottle-fed infants, but grew more slowly during infancy; differences in weight and length persisted throughout the study period. Among infants who showed catch-up growth, males caught up more rapidly than females. The authors concluded that early postnatal growth rates are strongly influenced by a drive to compensate for antenatal restraint or enhancement of fetal growth by maternal uterine-factors.

Ong KKL, et al. *Pediatr Res* 2002;52:863-867.

**Editor's Comments:** *This very interesting paper provides unique longitudinal growth data from a large prospective birth cohort. Some of the factors studied are well known to alter growth, such as maternal smoking which inhibits growth in utero, and/or breast milk which is known to be to be associated with lower growth rates in infancy as compared with cow-milk*

*formula fed children. However, little data existed for long-term measurements of these types of infants up to 5 years of age. This paper contributes significantly with strong data. Although it is reassuring to note that infants born to mothers who smoke during pregnancy exhibit catch-up growth with no long-term consequences in height, the negative effects of smoking should not be overlooked as they transcend growth. These were not studied in this paper.*

*Of great interest is the long-term growth divergence in breast-fed infants as compared to bottle-fed infants. This difference in growth progression persists after infancy with significant differences throughout the first 5 years of life. Both weight and height were decreased in the breast-fed group as compared to the bottle-fed group. It is now known that the way infants grow in utero, as well as during the first year of life, might have very important consequences for the development of adult-onset disease. Similarly, the rate of weight accretion during infancy and childhood might play a role in the development of obesity later in life. These data provide evidence that human milk feedings are best for feeding infants, allowing them rates of weight gain for the first 5 years of life that may be more compatible with a more appropriate body weight later in life. In light of the current epidemic of obesity, any factor that may contribute to it should be seriously considered. The growth charts for breast-fed infants developed by the CDC (<http://www.cdc.gov/growthcharts/>) and by the Eurogrowth study ([www.Eurogrowth.org](http://www.Eurogrowth.org)) are very useful in monitoring the growth of such children, but these do not extend until 5 years of age; such would be highly desirable in light of these data.*

Fima Lifshitz, MD

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# GROWTH

## Genetics & Hormones

Vol. 19 No. 4 [www.gghjournal.com](http://www.gghjournal.com) December 2003

### DR. ROBERT M. BLIZZARD - A LEGACY

**Fima Lifshitz, MD**

*For the Editorial Board*

*Growth, Genetics and Hormones (GGH)* has been published without interruption for the past 19 years. This journal was conceived and founded in 1984 by Dr. Robert M. Blizzard; the first issue appeared in March 1985. The goal set by him and the editorial board was to integrate reports of current advances in the fields of growth, genetics, endocrinology, metabolism and nutrition by bringing the most pertinent papers, with erudite editorial comments, to the attention of pediatricians, internists, pediatric endocrinologists, geneticists, nutritionists, nurses, and to others interested in these fields.

As Editor-in-Chief, Dr. Blizzard has worked tirelessly since the inception of the journal. He has been personally responsible for selecting, recruiting and stimulating the editorial board. He has elicited the best from all of us. Initially the editorial board consisted of Drs. David L. Rimoim, Fima Lifshitz and Alan Rogol from the United States, Judith G. Hall from Canada, and Dr. Jürgen R. Bierich as a European representative. Subsequently other distinguished Pediatric Endocrinologists from Europe joined the editorial board, including Drs. Jean-Claude Job and James Tanner. The

current editorial board members, serving *GGH* since 1993, are Drs. William Clarke, William Horton, and Allen Root, plus founding members Judith G. Hall and Fima Lifshitz. Dr. Blizzard has spearheaded all aspects of the publication including the content, quality, and format.

Throughout the last 19 years *GGH* has exceeded his goals and has become a well established resource for all 6,000 of its current readers, many of whom cherish the journal and keep each issue in their reference libraries. As well, Dr. Blizzard has made sure that as the cycle of life continues there would be a positive and productive transition for *GGH*. During the past two years he has fostered a smooth passage to ensure that upon completion of his tenure as Editor-in-Chief the journal will continue to serve the needs of our colleagues and continue to grow. He personally has overseen all transitional aspects and bestowed responsibility for the future of *GGH* to me as Editor-in-Chief.

Dr. Blizzard requested that a short announcement be inserted about his retirement in this his last issue Vol. 19 No. 4. He wished to see that the many readers who have read *GGH* throughout the years were thanked and appreciation was expressed to all those who have contributed to *GGH* by writing lead articles and to those who have been consistent readers. We pass this message along for him, and the editorial board joins him in saying "thank you".

The editorial board, wishing to acknowledge the many years of service and the most important contributions of Dr. Blizzard, has prepared a brief outline of the accomplishments of this founding editor, teacher, pediatric endocrinologist, clinician, scientist, and man described below. This tribute to him is but a token way to bid him farewell and to imprint his legacy, so that future generations of our colleagues also may be inspired by him.

First and foremost, Dr. Blizzard will be remembered and recognized as a teacher and educator. He is an accomplished teacher, and his competence as an educator and preceptor is well known. He was trained (1955-1957) by Lawson Wilkins and he was "trained to

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train", when there were only approximately 20 pediatric endocrinologists in the country. He prides himself in being a pediatric endocrinologist for 48 years (1955-2003) and throughout his career he set the course for his students. Over 50 fellows, including myself and other members of the editorial board, undertook and completed their training with him. Forty-five of these are now in academic positions. Many are full professors including three deans, an associate vice president for health affairs, several chiefs of staff of children's hospitals, and several pediatric department chairpersons in the U.S. and abroad. He is proud of the fact that most of his fellows have established their own pediatric endocrinology training programs, and thus provided an ongoing transmission of the teaching of Lawson Wilkins and himself to second and third generations of pediatric endocrine fellows. He has received multiple teaching awards including those from the Johns Hopkins Hospital, the University of Virginia, and other prestigious universities. Very possibly the teaching award of which he is most proud is his election to alpha omega alpha in 1970 by the members of the Johns Hopkins Alpha Omega Alpha (AOA) Society. His accomplishments as a student had not qualified him for AOA membership, and, therefore, his election by the student membership was particularly gratifying, since only one faculty member per year was elected to the society.

He also is proud of the opportunity to have served as Acting Chairman of the Department of Pediatrics at the Johns Hopkins University School of Medicine (1972 and 1973) and as Chairman of the Department of Pediatrics at the University of Virginia, School of Medicine (1974-1987). At these institutions he fostered 15 generations of pediatric residents who sought and attained their pediatric training in his departments. Most of them are now in academic and/or clinical practice in the US. A significant number are abroad. For his educational activities he has been honored with other prestigious awards. Among them are the Ayerst (1973) and Williams (1994) distinguished service and leadership awards bestowed by the American Endocrine Society. Recently he has been honored to be elected to the Johns Hopkins Society of Scholars (2002) and honored by the establishment (2002) of the Robert M. Blizzard Annual Lectureship at the annual meetings of the Lawson Wilkins Pediatric Endocrine Society (2002). He also has been honored by invitations to deliver over 150 named lectureships and visiting professorships at many national and international academic institutions. He was elected to the prestigious Hall of Fame of Miami Children's Hospital in 1997. Those who attended the teachings of Dr. Blizzard have always recognized his talents in teaching, and most have asked for more!

However, his contributions as an educator transcend

the traditional teaching role through which he personally touched so many individuals and imparted his knowledge. Dr. Blizzard made major contributions to continuing medical education by serving on multiple editorial boards of journals, editing and publication of several textbooks, and by his 19 years of editorship of *GGH*. The number of physicians and other scientists whom he reached via this journal through the years cannot be easily counted nor measured, but *GGH* is currently read regularly, as previously stated, by over 6,000 colleagues world-wide. Thus, the impact of Dr. Blizzard as an educator can be summarized as "the teacher par excellence".

In the field of endocrinology, he is particularly known for his contributions in the areas of growth and in autoimmunity, with over 200 original peer-review papers published in the literature. His picture is a clear testimony to his legacy as a clinician. He has always, in the Wilkins' style, promoted accurate measurements of children in assessing growth. This is still the gold standard in the evaluation of children with short stature. Dr. Blizzard was a pioneer in this field, publishing his first studies on the action of human growth hormone in 1959, one year after the first publication by Dr. Raben of the use of human growth hormone in growth hormone deficient individuals. His interest continues in this field to this day. Dr. Blizzard, along with Dr. Joanne Brasel and Dr. James Wright in the early 1960s published several important papers that changed the approach to the diagnosis and treatment of growth hormone deficiency. Included were the observations that growth hormone deficiency can be manifested by delayed growth even in the first year of life, previously not thought to be the case, and that the



Robert M. Blizzard, MD

acute metabolic response to human growth hormone did not correlate with its growth promoting effects when growth hormone deficient children were treated. The search for reliable indicators to predict a quantitative response to growth hormone is still ongoing.

Subsequently, Dr. Blizzard authored or co-authored 56 publications in peer-review journals pertaining to growth hormone or growth factors. These studies clarified the role that growth hormone played in producing the adolescent growth spurt, and the phenomenon of growth hormone production and its relationship to steroid production during this stage of life. A series of articles published under his tutorage unequivocally demonstrated that growth hormone increases at the time of adolescence when testosterone is produced in males. These studies showed that growth hormone and testosterone each have separate mechanisms of action in promoting growth, as well as permissive actions in the relationship to the secretion of each other.

In 1971 Dr. Blizzard stimulated his associates to design a pump that would permit a constant withdrawal of blood over a 24-hour period, that would permit the measurement of integrated concentrations of circulating hormones. Dr. Avinoam Kowarski was successful in this endeavor, and he and Dr. Robert Thompson, Dr. Claude Migeon, and Dr. Blizzard first reported the determination of integrated concentrations of human growth hormone and true secretion rates of human growth hormone. The importance of pulsatility and the intricacies of growth hormone production at various stages of life were subsequently delineated using this technique in studies with Dr. Alan Rogol, Dr. Paul Marthia, Dr. Nelly Mauras, Dr. Kathleen Link, and others at the University of Virginia.

While being a leader throughout his life and an innovative initiator of investigative protocols, he appropriately was appointed Director of the Clinical Research Center at the University of Virginia (1980-1983), while serving simultaneously as Department Chairman. He collaborated extensively with his colleagues in the Divisions of Endocrinology in Internal Medicine (Dr. Michael Thorner in particular among others). He coauthored 15 papers concerning the effect of growth hormone releasing hormone in humans - both as a diagnostic and therapeutic agent.

Although not as well known, Dr. Blizzard initiated and significantly contributed in elucidating the possible role of decreased growth hormone production during adult life in the aging process. He, his associate Dr. Ann Johanson, and his group initially demonstrated that older males secrete less growth hormone than do young males, and that older males receiving growth hormone retain nitrogen, comparable to that seen in growth hormone deficient young adults. They also reported that

growth hormone administered to older males generated insulin-like growth factor I, comparably to that generated in growth hormone deficient children. He subsequently described the changes in pulsatility of growth hormone secretion in older men and women as compared to younger subjects.

These studies led to the involvement of Dr. Blizzard in the first study to evaluate the effect of chronic growth hormone administration in older males. His research was not only at the intellectual/research level; he was the first of five males in a study which he initiated to receive growth hormone every day over a period of 30 months. He and his colleagues demonstrated that growth hormone had no significant effect upon skin collagen and its amino acid composition. He was obliged to stop the study in 1985 because of the report of possible contamination of native pituitary extracts by the prion producing Creutzfeldt-Jakob disease. However, the results of this project undoubtedly stimulated other investigators to assess the effect of growth hormone in the elderly.

Another major contribution of Dr. Blizzard was the concept that psychosocial dwarfism (also called emotional deprivation, maternal deprivation, the garbage can syndrome, and reversible hyposomatotropism) resulted from transient growth hormone deficiency. He insists that major credit in the concept be accepted by Dr. Dagfinn Aarskog, Dr. Gerald Powell, Dr. Salvatore Raiti, and others. The demonstration of the pathophysiology of such alterations gave great impetus to studying how the hypothalamus and its neurotransmitters are controlled by higher cerebrocortical centers which has been the subject of countless studies. To date Dr. Blizzard continues to be considered a world authority on psychosocial dwarfism or reversible hyposomatotropism.

Although currently known by young pediatric endocrinologists and academicians more for his work in the field of growth, he contributed significantly in other fields of endocrinology. In 1959 he initiated a study to determine aldosterone excretion in virilizing adrenal hyperplasia. He was the lead author of an article in the *Journal of Clinical Investigation* demonstrating that salt-losing congenital virilizing adrenal hyperplasia was due to decreased aldosterone secretion.

Between 1955 and 1980 Dr. Blizzard was a leading international investigator and authority on autoimmune endocrine diseases. He suggested that many endocrine diseases characterized by glandular atrophy, including adrenal insufficiency, hypoparathyroidism, premature ovarian failure, and insulin dependent diabetes mellitus, were of autoimmune origin. He studied this model in his laboratory over the next 25 years and applied the

findings in the clinic setting which led to publications of 27 papers in peer-review journals.

In his laboratory with the assistance of Dr. Robert Chandler, he was one of the first investigators to demonstrate that Addison's disease was frequently of autoimmune origin and the first to elucidate the physical and biochemical characteristics of the antigens involved. In 1966 he reported that hypoparathyroidism was also related to antibody formation. In 1960 he had demonstrated that there was a high incidence of antibodies against thyroid microsomes and thyroglobulin in the serum of mothers of athyreotic cretins. Dr. Blizzard postulated that autoimmune thyroid disease in the pregnant woman might be the etiology of at least some cases of congenital athyreotic cretinism. At the Pediatric Endocrine Research Meetings in May 1987, Dr. Dussault of Canada presented confirmatory evidence of this hypothesis, and acknowledged that the concept and early data had been presented by Dr. Blizzard years previously.

In the early 1960's he proposed that some cases of insulin dependent diabetes mellitus were probably of autoimmune origin with destruction of the beta cells of the pancreas. This observation was based on his earlier papers reporting the associations of diabetes mellitus with Addison's disease and hypoparathyroidism. In 1961, he submitted a grant to the National Institutes of Health (NIH) proposing to study this concept. The grant was rejected stating that the concept was preposterous; subsequently it was demonstrated that indeed many patients with insulin dependent diabetes mellitus had antibodies to beta cells and the role of autoimmunity in insulin dependent diabetes mellitus was firmly established.

Even subsequent to 1979, when Dr. Blizzard was devoting the majority of his investigative time to problems of growth, he published major papers concerning autoimmunity. These papers further elucidated the associations of various types of autoimmune diseases, and particularly clarified the associations of the various types of polyglandular autoimmune adrenal disease with other endocrine disorders. At an international autoimmune conference held in Pisa, Italy, in 1979, Blizzard proposed a classification of polyglandular autoimmune diseases, which was accepted internationally and continues to be used today with only minor modifications.

Blizzard has recorded many other "firsts" in the field of pediatric endocrinology, including, with the collaboration of Dr. Ann Johanson and Dr. Harvey Guyda and other fellows, the elucidation of the intricacies of luteinizing hormone and follicle stimulating hormone secretion during childhood and puberty, and the abnormalities

found in sexual precocity. In his laboratory, along with Dr. Robert Penny, he demonstrated that gonadotropin levels were elevated in hypothyroid children who have associated sexual precocity. He reported with Dr. Johanson that patients with gonadal agenesis or Turner syndrome grew significantly when treated with anabolic agents.

Other firsts included a description and report of the Johanson-Blizzard syndrome of congenital anomalies in congenital hypothyroidism and a description of the syndrome of congenital adrenal cortical-unresponsiveness to ACTH with Dr. Claude Migeon. In addition, Dr. Blizzard actively contributed to and participated in the treatment and research of patients with central sexual precocity utilizing gonadotropin releasing hormone agonists (GHRHa) to block pubertal development. Dr. Blizzard was proud of his capability to work collegially and collaboratively with others to promote multicenter investigation. An example was his collaboration over several years with Dr. Paul Boepple, Dr. William Crowley, and others in Boston in studying the role of GnRH analogues.

In 1961, in association with Dr. Alfred Wilhelmi, Chairman of the Department of Biochemistry at Emory University, the National Pituitary Agency was established. The purpose of this agency was to collect human pituitaries at autopsy examination, extracting their hormones, and to distribute these hormones on a national basis for investigation and therapy. He organized this collection and distribution program under the auspices of the (NIH), and was the Director of the agency until 1967. Dr. Blizzard inspired and led a lay group of individuals to develop an organization of parents and others to assist in the collection of human

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pituitary glands. Their success led to the establishment of the Human Growth Foundation in 1965. The scope of this organization grew and eventually became a support source for families of children with growth disorders with chapters across the country and with an ability to fund research in the area of growth disorders. After the National Pituitary Agency and The Human Growth Foundation were firmly established, Dr. Blizzard was followed by Dr. Salvatore Raiti, one of his former fellows, as director. It was this program that led to, and made possible, all of the investigation pertaining to pituitary hormones that occurred in humans in the subsequent 24 years (1961-1985) before synthetic growth hormone became available.

In 1993 he was asked to establish the Genentech Foundation for Growth and Development, a grant awarding organization separate from Genentech Inc., with an independent board and decision making authority. In the 8 ½ years of its existence under his leadership this foundation provided more than \$18 million dollars in grants to clinical investigators, to basic science investigators, to physicians receiving training in the fields of growth and development, and to support professional and personal education of growth and development in these fields.

Discussing the many contributions of Dr. Blizzard to

pediatrics and to science is an easy and enjoyable endeavor, particularly because he always attempted to recognize the contributions of those with whom he worked professionally. Examples of his appreciation for professional collegiality and recognition are cited in the text above. A major professional colleague of Dr. Blizzard and contributor to the success of Growth, Genetics & Hormones for 19 years is Ms. Juanita Bishop, his trusted and dependable assistant of over 20 years.

Describing the human qualities of Dr. Blizzard also is an easy and enjoyable endeavor. He is an exceptional human being, and it is worth noting the comforting way he talked to his patients and families and his ability to put them at ease despite their difficult problems. He has a special skill to develop closeness with others lasting a lifetime, and to nourish and support his patients, students, fellows, and associates. This is what I and his other associates appreciate the most!

The cycle of life continues, with the publication of this issue Dr. Robert M. Blizzard has officially retired from the editorship of *GGH*. He has had a most prestigious and distinguished career with enough accomplishments for many lifetimes. He now plans to enjoy more time with his family. We anticipate he will continue that which he does best, inspiring and teaching. As he has thanked so many of us, we thank him for all!

#### Abstracts from the Literature

### Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry

Newborn screening for inborn errors of metabolism has been in place in many countries for many years. Strong arguments have been made for screening not only for improving care of patients identified through screening, but also for reducing the cost of this care. Indeed, there are numerous examples, PKU most notably, of how early diagnosis and treatment have prevented serious illness or death from these disorders. However, as Wilcken and colleagues point out, formal evidence for the clinical effectiveness of screening is lacking, especially for rarer diseases, such as inborn errors of metabolism. Randomized, controlled trials of screening have been very limited because of the rarity of these disorders and also because of the strong conviction based on clinical experience that there is a benefit from early diagnosis.

Against this backdrop Wilcken et al compared the effectiveness screening for inborn errors of metabolism in all newborns with tandem mass spectrometry from 1998 to 2002 to conventional biochemical screening performed because of clinical suspicion from 1974 to 1998. The study population lived in New South Wales (Australia) and the Australian Capital Territory and totaled six million. Thirty-one disorders were selected for study. PKU and pterin disorders were excluded

because effective screening by other methods had been in place for many years; also excluded were disorders known to be benign or of maternal origin.

The diagnosis rates were reported in four-year brackets, i.e., 1974-1978, 1978-1982 ... 1998-2002, etc. During the six four-year periods preceding the implementation of tandem mass spectrometry screening, 22-34 cases were diagnosed per period giving rates from 6.6 to 9.0 cases per 100,000 births. Diagnoses were made at different ages depending on the age of clinical presentation. There were no trends toward increased overall rates of diagnosis between 1982 and 1998 even though some of the 31 disorders were first recognized during these periods.

Between 1998 and 2002, when all infants were tested between 48 and 72 hours after birth, 57 infants were diagnosed with one of the 31 inborn errors or 15.7 diagnoses per 100,000 births. Of these, 48 infants were diagnosed by screening, while six were diagnosed clinically before or at the same time as the screening result became available, usually within 24 hours of testing. Two patients, siblings with ornithine transcarbamylase deficiency born to a mother with known risk, did not undergo screening. Seven disorders



whose diagnoses were made later on clinical grounds had negative results on newborn screening.

Although results showed an increase in the rate of diagnosis following the introduction mass spectrometry screening in newborns, most of the increase could be accounted for by the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency and to a lesser extent by the diagnosis of other disorders of fatty acid oxidation.

The authors calculated the cost of establishing a diagnosis. The incremental cost of the tandem mass spectrometry screening was \$0.70 (USD) per newborn. The cost of confirmatory testing was \$217 and the cost per relevant disorder detected was \$3,939 if PKU was excluded or \$2,519 if it was included. They concluded that their approach provides a rapid and inexpensive way to screen for a wide range of very rare metabolic diseases and that it identifies more cases than are diagnosed clinically. However they caution that it is not yet clear which patients identified through newborn screening would have become symptomatic if screening had not been performed.

Wilcken B et al. *New Eng J Med* 2003;348:2304-2312.

**Editor's Comment:** *This paper brings to the fore the debate over the extent to which tandem mass spectrometry technology should be used to screen for a growing number of inborn errors of metabolism. As*

*noted in a recent article by Marshall,<sup>1</sup> the debate pits parents and often physicians who advocate the application of this technology against ethicists with concerns over costs and public health officials with concerns over how the potentially large amount of genetic data will be managed. The Wilcken study demonstrates the successful implementation of the technology in a public health setting. It documents that the technology leads to an increased rate of diagnosis at low cost, especially for disorders of fatty acid oxidation, although acknowledges the possibility that some patients diagnosed as newborns may not have become symptomatic if screening had not been performed. Readers should note that metabolic screening by tandem mass spectrometry was highlighted by a recent lead article in GGH.<sup>2</sup> This article explains how technology works, provides guidelines for its use and describes its successful application in North Carolina. Together, these articles provide support for advocates of wider use of tandem mass spectrometry for newborn screening.*

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2. Millington D, Koeberl D. *GGH* 2003;19:32-38.

William A. Horton, MD

## The Effect of Clitoral Surgery on Sexual Outcome in Individuals Who Have Intersex Conditions with Ambiguous Genitalia: A Cross-Sectional Study

It is estimated that intersex conditions occur in one per 2,000 live births. In the past, treatment had been based on the assumption that infants were gender neutral at birth, and that assignment of sex of rearing in early years which is reasonably compatible with the appearance of the external genitalia would provide a normal gender identity and partner orientation in adulthood. Subsequently, it has been recognized that there is a complex interaction between prenatal and postnatal factors that lead to the development of gender and sexual identity.

In the United States and in most western European societies, female rearing was most frequently recommended to parents whose infant had ambiguous genitalia. When the decision to raise the child as a female was made, surgery was usually undertaken to remove any ambiguity of the genitalia and to feminize the external appearance. This was done with the hope of a good psychosocial outcome.

Minto et al undertook a study involving individuals with several intersex conditions which included ambiguous genitalia, and who were living as adult females. Individuals were recruited from the Androgen

Insensitivity Syndrome Support Group, the Adrenal Hyperplasia Network and the Intersex Clinic at University College in London Hospital.

Questionnaires were distributed and individuals could respond anonymously or identify themselves, in which case, their records would be examined with their permission. The self-administered questionnaires included the Golombok-Rust inventory of sexual satisfaction (GRISS) for women. Of the 39 patients included in this study, 11 had no clitoral surgery and 28 had had clitoral surgery. Almost all individuals who had undergone gonadectomy were taking hormone replacement therapy. Historical trends were noted in that most individuals seen before 1979 had undergone clitorrectomy, while those operated on since 1980 usually underwent nerve-sparing clitoral reduction surgery. Many individuals also had vaginal reconstructive surgery.

The authors did multiple types of analysis of the data; however, the bottom line is that of the 39 participants, 13 individuals had never been sexually active and the 28 sexually active individuals had below normal scores in terms of sexual function. A low score on sensuality

was evident in the clitoral surgery group when compared to the non-surgical group. Both groups had difficulty with orgasm, which is relatively rare in a sexually healthy population. Of the 28 who had clitoral surgery, 18 found it impossible to have orgasm, compared with none among those who had not had clitoral surgery.

It was difficult to determine exactly why most of the study individuals were having difficulty with sexual function because only a questionnaire was used to obtain the data. There did not appear to be a difference among those patients recruited from the clinic versus those in support groups.

It would appear that genital surgery at a young age did not lead to satisfactory gender identity and sexual activity. However, it is not clear what the most appropriate approach should be. The authors encourage debate about the ethical issues, the development of reliable information, support of research in this area and how important it is to share this information with parents and patients who are considering clitoral surgery.

Minto CL et al. *Lancet* 2003;361:1252-1257.

**First Editor's Comment:** *The outcomes of the management of intersex are not perfect. This study following up on previously treated individuals suggests that clitorrectomy does not lead to sexual satisfaction, however, neither does clitoral reduction. Clearly, more research and discussion are needed in this area.*

Judith G. Hall, OC, MD

**Second Editor's Comment:** *As the authors acknowledge, interpretation of their study is hampered by the small number of study subjects and the possibility that those electing to participate were among the more*

*dissatisfied patients contacted initially. Quite interesting are the data that indicate that clitoromegaly itself is associated with sexual dysfunction. In addition to the concept that clitoral recession will permit the child to more readily accept her female sex assignment, the procedure is performed to ease parental acceptance of their newborn child. Those who have dealt on a personal and daily basis with parents of children with ambiguous genitalia know the need to assure and reassure parents is a paramount goal which is difficult to attain. Early clitoral recession by a skilled surgeon is most often recommended by this writer in those neonates with more severe degrees of genital ambiguity.*

*Because of widespread neonatal screening for CAH, there is an increasing number of females with the most severe form of genital ambiguity known as Prader V or complete incorporation of the urethra into the phallus/clitoris. In the opinion of this writer and many others it is inappropriate to rear these genotypic and potentially fertile girls as males, thus necessitating genital surgery. Since both clitoromegaly and clitoral surgery impede sexual satisfaction, the challenge is to devise a corrective procedure that does not do so.*

*It would have been of interest to learn whether in those women with ambiguous genitalia who did not undergo clitoral surgery, clitoromegaly during childhood and young adulthood was a matter of significant concern. Counseling girls with ambiguous genitalia, whether operated upon or not, needs to begin in mid-childhood and to be conducted by individuals skilled in the management of this problem, as mentioned by Slipher in an excellent commentary regarding this article, in the same issue of *Lancet* (2003;361:1236-1237).*

*Minto's article also provides further support for the antenatal treatment with glucocorticoids of women bearing female CAH offspring at risk for development*

Table  
Sexual function of 28 participants, according to GRISS

|                      | Subscale scores (%)           |               |                      |                                  |               |                      |
|----------------------|-------------------------------|---------------|----------------------|----------------------------------|---------------|----------------------|
|                      | Clitoral surgery group (n=18) |               |                      | No clitoral surgery group (n=10) |               |                      |
|                      | Normal*                       | Difficulties† | Severe difficulties‡ | Normal*                          | Difficulties† | Severe difficulties‡ |
| Frequency            | 28%                           | 72%           | 33%                  | 30%                              | 70%           | 30%                  |
| Communication        | 28%                           | 72%           | 17%                  | 20%                              | 80%           | 20%                  |
| Satisfaction         | 61%                           | 39%           | 0%                   | 80%                              | 20%           | 0%                   |
| Avoidance            | 28%                           | 72%           | 22%                  | 20%                              | 80%           | 10%                  |
| Sensuality           | 22%                           | 78%           | 22%                  | 80%                              | 20%           | 10%                  |
| Vaginal penetration§ | 33%                           | 67%           | 33%                  | 33%                              | 67%           | 22%                  |
| Orgasm               | 39%                           | 61%           | 28%                  | 60%                              | 40%           | 0%                   |

\*Score of 1-4. †Score 5-9. ‡Score of 8 or 9. §Four individuals chose not to answer the question on vaginal penetration.

Adapted from Minto CL et al. *Lancet* 2003;361:1252-1257.

of ambiguous genitalia. It will be of great interest to assess the psychosexual development, orientation, and sexuality of these subjects as adult women. With the observations collected to date the impression is that they are normal little girls.

Allen W. Root, MD

**Third Editor's Comment:** The topic of intersex management, outcome, and research has received much attention in the past 2-3 years. The reader should be aware of publication of a collection of excellent papers

presented in May 2002 at a conference entitled "Genetic and Hormonal Basis of Sexual Differentiation Disorders" (*The Endocrinologist* 2003;13:175-287) and of a "Summary of a Research Workshop on Intersex" held in sequence with the above conference (to be published in *The Endocrinologist*). Furthermore an excellent review entitled "Management of Children with Intersex Conditions: Psychological and Methodological Perspectives" by S. Berenbaum was presented in *GGH* 19:1.

Robert M. Blizzard, MD

## Neonatal Exendin-4 Prevents the Development of Diabetes in the Intrauterine Growth Retarded Rat

Intrauterine growth retardation (IUGR) has been shown to be associated with significant adult morbidity, including insulin resistance, reduced pancreatic  $\beta$ -cell mass, and subsequent type 2 diabetes. Uteroplacental insufficiency, a cause of IUGR, limits the availability of substrates, growth factors, and hormones to the fetus. A rat model of IUGR can be induced with bilateral uterine artery ligation at 19 days of the 22 day gestation period. In rats during the newborn period there is extensive remodeling of the pancreas brought about by  $\beta$ -cell replication, neogenesis and apoptosis. A second wave of neogenesis occurs during weaning.

The incretin hormone glucagon-like polypeptide-1 (GLP-1) stimulates pancreatic neogenesis and increases  $\beta$ -cell mass. Therefore its administration to rat pups who have undergone 90% partial pancreatectomy results in an increase in both  $\beta$ -cell mass and improved glucose homeostasis. Exendin-4 is a long-acting GLP-1 which in addition to the aforementioned activities stimulates expression of Pancreatic Duodenal Homeobox (PDX) protein in the pancreas. PDX is critical for the early development of both the endocrine and exocrine pancreas and mediates glucose responsive stimulation of transcription of the insulin gene.

Stoffers and colleagues treated IUGR rat pups with exendin-4 during the early postnatal period to study its effects on the subsequent development of type 2 diabetes. Four groups of rat pups were studied: (1) control pups given vehicle injection, (2) control pups given exendin-4 injections, (3) IUGR pups given vehicle injections, and (4) IUGR pups given exendin-4 injections. Injections were administered on postnatal days 1 through 6. Glucose tolerance,  $\beta$ -cell mass,  $\beta$ -cell proliferation and PDX gene expression were measured at 14 days and 3 months of age. Glucose tolerance was also determined at 7 weeks and 8 months of age.

Exendin-4 decreased weights in both control and IUGR pups (Groups 2 and 4) at 2 weeks. This decrease persisted into adulthood (Table). At day 14, glucose

tolerance in the IUGR pups treated with exendin-4 was similar to that in control animals. The treated animals remained euglycemic at 8 months. Vehicle-treated IUGR pups (Group 3) developed diabetes by 3 months and died by 8 months of age. Exendin-4 treated IUGR pups (Group 4) had normal  $\beta$ -cell mass comparable to that in Group 1 as the result of normalized replication rates. While Pdx-1 mRNA levels were reduced by 60% in IUGR rats not receiving exendin-4 at 14 days, those treated with exendin-4 had normal levels.

The authors state their major finding is that a short treatment with exendin-4 during the early newborn period prevents the development of diabetes in the IUGR rat. It is not clear whether this effect is through the stimulation of Pdx-1. However, the effect is independent of  $\beta$ -cell mass, since its effects were observed prior to any reduction in the IUGR pancreatic mass. They suggest that the permanent improvement in maintenance of  $\beta$ -cell mass by exendin-4 may mean that similar drugs could be effective in reducing the risk or preventing type 2 diabetes mellitus in individuals born with IUGR. The negative part of the study was the growth inhibiting effect of exendin-4.

Stoffers DA, et al. *Diabetes* 2003;52:734-740.

| Table                               |                      |                       |
|-------------------------------------|----------------------|-----------------------|
| Body weight at 2 weeks and 3 months |                      |                       |
| Treatment group                     | 2 weeks (g)<br>(n=9) | 3 months (g)<br>(n=7) |
| Control vehicle                     | 27.7 $\pm$ 0.3       | 331.7 $\pm$ 7.0       |
| Control Ex-4                        | 22.2 $\pm$ 0.6*      | 305.3 $\pm$ 12.7*     |
| IUGR Ex-4                           | 13.8 $\pm$ 0.7†      | 311.0 $\pm$ 4.0†      |
| IUGR vehicle                        | 17.2 $\pm$ 0.7‡      | 351.7 $\pm$ 26.2‡     |

Data are means  $\pm$  SE. \*P < 0.05 control Ex-4 vs. control vehicle; †P < 0.05 IUGR Ex-4 vs. IUGR vehicle; ‡P < 0.05 control vehicle vs. IUGR vehicle.

Adapted from Stoffers DA, et al. *Diabetes* 2003;52:734-740.



**Editor's Comment:** These fascinating data suggest that possibly there may be a treatment available in the future for the prevention of type 2 diabetes mellitus in IUGR individuals, if treated early in the neonatal period. Stoffers and colleagues have shown using an IUGR rat model that exendin-4 given for a short period of time postnatally can prevent glucose intolerance by restoring Pdx-1 function and normalizing  $\beta$ -cell proliferation rates. One cannot read this study without thinking about other

morbidity associated with IUGR and how other treatments administered in the neonatal period might someday become available to treat those as well. The obvious example would be treatment given early to restore normal growth velocity. These authors have presented data that opens up a whole new world of possibilities.

William L. Clarke, MD

## Morbid Obesity and Mutations in Appetite Controlling Genes

It is known that the loss of function mutations of the melanocortin 4 receptor (MC4R) gene lead to severe obesity in humans and in mice. These genetic mutations disrupt the appetite control centers in the hypothalamus and lead to severe obesity. In the March 20, 2003 issue of the *New Eng J Med*, two papers were published which clarify the clinical syndrome resulting from the mutations in the appetite controlling MC4R gene.

In the first paper, Farooqi et al determined the nucleotide sequence of the MC4R gene, which is known to be a cause of a monogenic form of obesity. They studied 500 probands with severe obesity. In these families they examined the cosegregation of identified mutations, and in the subjects who were found to have MC4R deficiency they performed a metabolic-endocrine evaluation and characterized their clinical phenotype. The results were correlated with the signaling properties of mutant receptors. Twenty-nine probands (5.8%) had mutations in MC4R; 23 were heterozygous and 6 were homozygous. Mutation carriers were severely obese; their mean percentage of body fat was 43% of their body composition. Excess body weight gain was evident since the first year of life. They also presented increased lean body mass, increased linear growth, hyperphagia and severe hyperinsulinemia. The serum leptin and lipid levels, the metabolic rate, and thyroid, adrenal and reproduction function were normal. Homozygous individuals were more severely affected than the heterozygous ones. The subjects with mutations who retained some residual signaling capacity had a less severe phenotype than those with a totally absent signaling capacity. MC4R mutations resulted in a distinct obesity syndrome inherited in a co-dominant manner. The authors concluded that MC4R alterations play a key role in the development of a distinct form of severe obesity commencing in early childhood.

In the second paper, Branson et al studied the interactions of genetic and environmental factors which may have a bearing on the development of obesity in MC4R affected individuals. Four hundred sixty-nine severely obese white subjects with an average age of 42 years and with a mean body-mass index of 44, and

25 control subjects with normal weight and no history of obesity or dieting were included in this study. They sequenced (1) the complete MC4R coding region, (2) the proopiomelanocortin gene (POMC) region which encodes the  $\alpha$  melanocyte-stimulating hormone (MSH), and (3) the binding domain of the leptin receptor (LEPR) gene. They also obtained detailed data concerning phenotypes, resting energy expenditure, diet-induced thermogenesis, serum leptin levels, and eating behaviors. Twenty-four of the 469 obese subjects (5.1%) and one of the 25 controls (4%) had MC4R mutations, including 5 novel variants. All mutation carriers reported binge-eating behavior, defined as repeated rapid consumption at least twice per week of an unusually large amount of food in the absence of hunger, causing the subject to feel embarrassed, depressed or guilty and out of control. This 100% prevalence of binge eating in MC4R mutation patients was compared with a 14% frequency of such behavior in obese subjects without genetic mutations. The prevalence of binge eating was similar among carriers of mutations in the LEPR as among that of non carriers. No mutations were found in the region of POMC encoding  $\alpha$  MSH. The authors concluded that *binge eating* is a major phenotypic characteristic of subjects with a mutation in MC4R, a candidate gene for the control of eating behavior.

Farooqi IS et al. Clinical Spectrum of Obesity and Mutations in the Melanocortin 4 Receptor Gene. *New Eng J Med* 2003;348:1085-1095.

Branson R et al. Binge Eating as a Major Phenotype of Melanocortin 4 Receptor Gene Mutations. *New Eng J Med* 2003;348:1096-1103.

**Editor's Comment:** These two papers simultaneously published in the *New Eng J Med* are landmark studies. They contribute greatly to the understanding of the pathogenesis of obesity in humans. Farooqi and colleagues determined what proportion of obesity is attributed to a mutated gene of MC4R. They found that about 6% of severely obese individuals who had obesity since early childhood had these mutations. These patients carrying MC4R mutations constituted 3-



important subgroup of the severely overweight population. Given the high prevalence of observed MC4R deficiency, it appears that this condition represents the most common form of monogenic obesity in humans. Pediatricians and pediatric endocrinologists should be on the look out for this, especially in children who gain excess weight beginning in early childhood. Clinically, these patients differ from those with Prader Willi syndrome, who also have another form of monogenic severe obesity, by the normal stature and muscle development which are abnormal in Prader Willi syndrome.

The second study showed that overweight people who are binge eaters are more likely to harbor genetic mutations of MC4R than overweight people who constantly overeat. Until now, binge eating was considered a psychological phenomenon or disorder. For the first time a genetically driven characteristic was demonstrated. MC4R mutations appeared to disrupt brain signals governing satiety.

Both studies clearly document that there are severely obese individuals who overeat, not because of lack of will power, but because they have a genetically determined pathological syndrome.

However, these data also demonstrate that there are some individuals who have genetically determined mutations, yet are not obese. The reverse also occurs; specifically, binge eating behavior may occur and not be found to be associated with genetic mutations of MC4R. Thus, these two reports also support the thesis that the etiology of obesity is multifactorial, even in individuals who have a genetically determined alteration in the appetite control centers in the hypothalamus. In these patients, as well as in other obesities, excess

energy intake over energy expenditures must occur for obesity to develop.

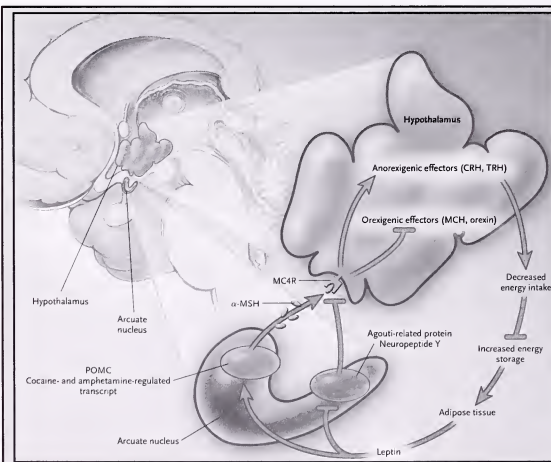
The reader is encouraged to review these two papers in detail, as well as to study the accompanying editorial by List and Habener<sup>1</sup> who clearly described the model of the homeostatic circuit regulating energy balance via the MC4 receptor. These authors point out that several hormones are known to influence the appetite control centers in the hypothalamus (Figure). MC4R deficiency is clearly implicated in the etiopathogenic mechanisms in some cases of severe obesity and binge eating, through short-circuiting the regulation of appetite in the hypothalamus. MC4R deficiency decreases the signals of anorexigenic pathways, such as CRH and TRH; and prevents the inhibition of orexigenic pathways, such as MSH and orexin. The result is increased food intake. The melanocortin agonist  $\alpha$  MSH is a peptide that is produced by the POMC, and is an agonist of MC4R. On the other hand, leptin reduces food intake through stimulation of the expression of POMC and the production of MSH, while inhibiting MC4R antagonists such as the agouti-related protein.

The abnormal molecular physiology demonstrated in MC4R deficient patients constitutes an important discovery of a missing link between genes and behavior. However, there is a lot more to be uncovered before we fully understand satiety in individuals with MC4R gene mutations, as well as in other obesity syndromes, and in normal individuals.<sup>2</sup>

Fima Lifshitz, MD

## References

1. List JF, Habener JF. *New Eng J Med* 2003;348:1160-1163.
2. Godola T. *N Eng J Med* 2003;349:606-609.



Figure

**Model of Homeostatic Circuit Regulating Energy Balance through the Melanocortin 4 Receptor (MC4R).** Increased adiposity leads to increased leptin production in fat tissue. Leptin stimulates neurons in the arcuate nucleus of the hypothalamus that coexpress the anorexigenic hormones a melanocyte-stimulating hormone ( $\alpha$ -MSH), a cleavage product of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript. Leptin also inhibits neurons in the arcuate nucleus that coexpress the orexigenic hormones agouti-related protein and neuropeptide Y. The neurons in the arcuate nucleus project to other regions of the hypothalamus (including the paraventricular nucleus and the lateral hypothalamic area—parafornical area), where  $\alpha$ -MSH binds to its receptor, MC4R, resulting in an up-regulation of anorexigenic effectors such as corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) and a down-regulation of orexigenic effectors such as melanin-concentrating hormone (MCH) and orexin. Agouti-related protein acts as an antagonist of MC4R.

Reprint with permission from List JF, Habener JF. *New Eng J Med* 2003;348:1160-1163.

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## Hypogonadism and Pubertal Development in Prader-Willi Syndrome

Genital abnormalities are common in Prader-Willi Syndrome (PWS) and are one of the eight major clinical criteria for diagnosis. Previous reports of the type and frequencies of these abnormalities were not necessarily from individuals with genetically confirmed PWS. Crino and associates report data from patients evaluated by the Genetic Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology. Eighty-four patients (42 males), mean age  $15.8 \pm 8.2$  years were studied. Sixty-three percent were over 14-years-old. All satisfied the Holm and Cassidy clinical criteria for the diagnosis of PWS and the methylation test was positive in all subjects. Microdeletion of chromosome 15(15q12-13) was demonstrated in 66%, while uniparental disomy or an imprinting defect was suspected in the others.

All males showed cryptorchidism (86% bilateral). Small testes and scrotal hypoplasia were observed in 76% and 69%, respectively. Micropenis was seen in 36%. Twenty-two of 29 males had spontaneous onset of puberty at  $14.0 \pm 3.2$  years but it was incomplete in all cases. Specifically, pubertal changes past Tanner 2-3 genital stages were rarely observed.

In females there was hypoplasia of the labia minora and/or of the clitoris in 71% and 69% of cases. Thirty-four of 39 females had spontaneous onset of puberty at  $12.6 \pm 2.7$  years, with very slow progression. Menarche occurred at a mean age of  $17.3 \pm 5.2$  years in 44% of cases over 14 years of age. Primary amenorrhea was diagnosed in 56%. Menstrual cycles were seldom regular and secondary amenorrhea occurred in 33% who had spontaneous menarche. Of note, premature

pubarche occurred in 12 subjects (6 males) and true precocious puberty in 3. It is suggested that premature pubarche might have been related to obesity. Genital and pubertal abnormalities were evenly distributed among subjects with microdeletion and UPD-imprinting defects. Treatment of various types for hypogonadism was discussed, including the use of dihydrotestosterone transdermally. However, no systematic trials on treatment with sex hormone treatment in adolescents or adults are available.

Crino A et al. *Eur J Pediatr* 2003;162:327-333.

**Editor's Comment:** *This paper provides interesting information concerning genital abnormalities in individuals diagnosed with PWS, confirmed with genetic testing. The large number of subjects in this descriptive study and the careful presentation of the findings should assist all who work with these patients and who must counsel them and their families in regard to expectations for pubertal development and fertility. It is interesting that sexual precocity was observed at a frequency that should be considered high in this group. This suggests that examination of the genitalia should be performed at each clinical visit. Whether or not current treatment with exogenous GH, which has been shown to significantly alter body composition in PWS, will affect pubertal development remains to be shown.*

William L. Clarke, MD

## Growth and the Tyrosine Kinome

Tyrosine kinases (TKs) add phosphate moieties to tyrosine residues on proteins that typically serve as docking sites to recruit other molecules that bind and propagate signals. As such, they function as central regulators of signaling pathways that control transcription, cell cycle progression, differentiation, apoptosis and other processes that are highly relevant to growth of cells and tissues. Given this central position in regulation of growth, Bardelli et al raised the question: why have mutations in TK genes been found in only a small number of instances including certain human cancers? They speculated that mutations do exist, but have yet to be detected because the vast number of TK genes is only now becoming apparent as the human genome project unfolds. To test this idea, they took advantage of high-throughput sequencing and bioinformatics from the human genome project to search

for TK mutations in a select group of cancers, colorectal cancers.

A recent analysis organized the protein kinase complement of the human genome (the "kinome") into a dendrogram containing nine broad groups or branches of genes. Bardelli et al selected one major branch, which contained three groups including 90 TK genes, 43 TK-like genes and 5 receptor guanylate cyclase genes. Mutation analysis of 813 exons from the genomic database carried out on DNA from 35 colorectal cancer cell lines yielded 14 mutations. Further analysis of DNA from 147 tumors identified 46 novel mutations in 14 genes. All of the mutations were somatic in nature based on comparison of DNA from tumor to matched normal tissues.

The authors suggested that mutations found in seven genes, which were detected in more than one tumor

were functional rather than coincidental. Based on the specific locations of the mutations, they further suggested that many of the mutations were activating in nature, i.e., they resided in key regions of the TK, such as the autoinhibitory activation loop. The authors concluded that at least 30% of colorectal cancers contain at least one mutation in the tyrosine kinase. They emphasized that an important reason to study TK genes is that they provide attractive targets for therapeutic intervention for growth disorders, noting the convincing success of targeting BCR-ABL tyrosine kinase in leukemia (Druker BJ. *Cancer Cell* 2002;1:31).

Bardelli A et al. Mutation Analysis of the Tyrosine Kinome in Colorectal Cancers. *Science* 2003;300:949.

**Editor's Comment:** While this paper specifically

addresses cancer, it does not take too much imagination to see its potential relevance to growth of other tissues, such as the skeleton. Indeed, achondroplasia is due to activating mutations of the FGFR3 tyrosine kinase. Given the scope of regulation necessary to orchestrate and coordinate events in a growing bone, it seems highly probable that there are other members, perhaps many, of the tyrosine kinome involved. Accordingly, mutations of these as of yet undefined TKs may underlie disorders of skeletal growth. Considering the remarkable success of Gleevec in treating chronic myelogenous leukemia by inhibiting the BCR-ABL TK, it is not inconceivable to dream of using pharmacologic manipulation of growth-plate TKs to therapeutically manage certain bone growth disturbances in the future.

William A. Horton, MD

## What do Craniosynostosis and Kallmann Syndrome Have in Common? *FGFR1*

Kallmann syndrome is characterized by loss of the sense of smell, anosmia and hypogonadotropic hypogonadism. The anosmia results from absence or hypoplasia of the olfactory bulbs and tracts. The hypogonadism is due to a deficiency of GnRH, probably the result of failure of GnRH-synthesizing neurons to migrate from the olfactory epithelium to the forebrain along the olfactory nerve pathway. Kallmann syndrome occurs mainly in males and most often is inherited in an X-linked recessive fashion; the gene responsible for this form has been identified, *KAL1*. However, there are instances, such as failure to detect a *KAL1* mutation, that suggest an autosomal form of Kallmann syndrome.

Through segregation analysis of polymorphic markers and FISH chromosomal analysis, Dodé et al identified two *de novo* deletions of about 11 Mb at chromosome 8p11.2-p12 in two individuals affected by different contiguous gene syndromes that included Kallmann syndrome. The overlapping region of about 540 kb contained three genes, one of which, *FGFR1* (fibroblast growth factor receptor 1) was considered a strong candidate for causing Kallmann syndrome because of its known interaction with the *KAL1* gene product, anosmin-1. Southern blot analysis of 43 individuals with familial or sporadic Kallmann syndrome failed to detect additional deletions of *FGFR1*. However, sequencing of *FGFR1* in 129 unrelated patients with Kallmann syndrome revealed heterozygous mutations in four familial and eight sporadic cases. The mutations, including nonsense, frameshift and splice-site mutations, predicted loss of *FGFR1* function.

These observations suggest that Kallmann syndrome can result from haploinsufficiency or reduced dosage for *FGFR1*. The authors point out that anosmin-1 binds

to heparin sulfate proteoglycans which are required for FGF ligands to bind to FGF receptors and that *KAL1* and *FGFR1* are expressed in many of the same areas in the embryo including the region of olfactory bulb development. They offer a possible explanation for the higher prevalence of Kallmann syndrome in males even in families with autosomal inheritance which is based on the assumption that the local concentration of anosmin-1 is important to FGF signaling, and the observation that *KAL1* partially escapes X-inactivation. Accordingly, females with two *KAL1* alleles synthesize higher amounts of anosmin-1 than do males with a single *KAL1* allele. The authors propose that this may be enough in some cases to maintain FGF signaling above a critical threshold with regard to *FGFR1* signaling in the context of olfactory bulb and tract development.

Dodé C, et al. Loss-of-function Mutations in *FGFR1* Cause Autosomal Dominant Kallmann Syndrome. *Nat Genet* 2003;33:1-3.

**First Editor's Comment:** *FGFR1* joins a small group of genes for which both gain and loss of function mutations are known and associated with disease. It is not surprising that gain and loss of function mutations lead to quite different clinical consequences. Gain of *FGFR1* function causes craniosynostosis, especially Pfeiffer syndrome, while loss of *FGFR1* function results in Kallmann syndrome. Thus, these two syndromes are technically allelic disorders. One wonders how common this phenomenon actually is. Indeed, those of us with interest in *FGFR3* have pondered if some individuals with tall stature have loss of function mutations of this gene in contrast to the gain of *FGFR3* mutations that cause achondroplasia.



The paper also illustrates the importance of gene dosage. In some instances, the precise dosage of a gene or its product does not seem to matter so much. Examples include, metabolic disorders in which half the normal amount of enzyme is more than enough to prevent disease and mutations of structural proteins, where inclusion of variable amounts of abnormal gene product can disrupt the formation of multimeric molecules containing the products of both mutant and normal alleles. When mutations involve regulation, such as mutations that affect signaling or formation of transcription factor complexes, small differences may have large effects on the outcome of the regulated events, especially if they involve thresholds as proposed for FGFR1 signaling in this report.

William A. Horton, MD

**Second Editor's Comment:** The authors have identified a second gene involved in the pathogenesis of Kallmann syndrome. The large number of subjects with Kallmann syndrome (N=116) in this study in whom mutations in neither KAL1 or FGFR1 were found indicates that there are (many) more genetic mutation which lead to this disorder. Search for involved genes might be directed toward those that encode products known to be important in neural cell migration and upon the intracellular proteins that are phosphorylated and the downstream genes whose transcription is regulated by FGFR1. It is interesting (curious?) that gain-of-function mutations of FGFR1 are associated with the Pfeiffer syndrome of craniosynostosis, but that inactivating mutations of this gene have not been linked to delayed closure of cranial sutures.

Allen W. Root, MD

## Clinical, Autoimmune, and HLA Characteristics of Children Diagnosed With Type 1 Diabetes Before 5 Years of Age

Little is known about auxologic, autoimmune, and HLA characteristics specific to children with early-onset diabetes (EOD). In this paper 40 children with a mean CA of 2.6 years who developed diabetes mellitus before 5 years of age were studied. These patients were compared with a matched subgroup of children of mean age of 9.9 years, therefore, with later onset diabetes mellitus (LOD). Auxologic data and antibody radioimmunoassay data from medical records were retrospectively analyzed. HLA diabetes related class II alleles were typed and evaluated for comparison between "whites" and "Hispanics". The frequencies of glutamic acid decarboxylase (GAD) and islet cell antibodies (ICA) were significantly lower in the EOD group than in the group developing diabetes at an older age. No significant differences were detectable for insulin auto-antibodies (IAA), thyroid peroxidase, and thyroglobulin antibodies. None of the patients of the EOD group developed hypothyroidism, whereas 20% of the

LOD patients did. There was a negative correlation between GAD antibodies and the predisposing haplotype DR3/DQ2. None of the EOD patients had either of the protective alleles (DRB1\*1501 or DQB1\*0602) for diabetes. There were significant differences in the frequencies of some diabetes related HLA alleles between EOD patients and a large non-age stratified type 1 diabetes group. The pertinent clinical information, frequency of autosomal markers and HLA data among ethnic groups are below (Tables 1-3). The authors concluded that children with EOD have different diabetes related autoimmune and genetic characteristics from those diagnosed later on in life.

Hathout EH et al. *Pediatrics* 2003;111: 860-863.

**Editor's Comment:** Very young children with diabetes mellitus are known to have a more severe course than those diagnosed later in life. The difficulties in the control

Table 1  
Clinical Information on Study Children With Type 1 Diabetes

|                                  | Early-Onset Group<br>(Diagnosis Age<br><5 Years) | Late-Onset Group<br>(Diagnosis Age<br>>5 Years) | P<br>Value |
|----------------------------------|--|---|------------|
| Concomitant illness at diagnosis | 72.73%   | 31.80%  | <.01       |
| Documented honeymoon period      | 16.70%   | 42.10%  | .24        |
| DKA at diagnosis                 | 80.80%   | 36.40%  | <.01       |
| Hemoglobin A1C at diagnosis      | 10.33 ± 2.20                                     | 11.27 ± 2.57                                    | .21        |
| No. of ICU days at diagnosis     | 1.82 ± 2.09                                      | .53 ± .70                                       | .07        |

DKA indicates diabetic ketoacidosis; ICU, intensive care unit.



Table 2  
Frequency of Autoimmune Markers in Study Children With Type 1 Diabetes

| Marker | Early-Onset Group<br>(Diagnosis Age <5<br>Years; %) | Late-Onset Group<br>(Diagnosis Age >5<br>Years; %) | P<br>Value |
|--------|---|--|------------|
| TpoA   | 6   | 9  | 1.00       |
| TGA    | 9   | 11   | 1.00       |
| IAA    | 50  | 65   | .43        |
| GAD    | 32  | 77   | <.01       |
| ICA    | 29  | 68   | <.01       |

TpoA indicates thyroid peroxidase antibody; TGA, thyroglobulin antibody

Table 3  
HLA Data in the 2 Major Ethnic Subgroups of Study Children With Onset of Type 1 Diabetes Before 5 Years of Age

| HLA Allele(s)               | Percentage of Whites<br>With Allele(s) (%) | Percentage of Hispanics<br>With Allele(s) (%) |
|-----------------------------|--|---|
| DRB1*0401-DQA1*03-DQB1*0302 | 70.6                                       | .0  |
| DRB1*0402-DQA1*03-DQB1*0302 | .0   | 92.9  |
| DRB1 0401                   | 35.3                                       | .0  |
| DRB1 0405                   | .0   | 21.4  |

Tables 1-3 are adapted from Hathout EH et al. *Pediatrics* 2003;111: 860-863.

of the disease among the younger patients may account for more frequent and more severe complications of the disease occurring earlier in life. However, the data in this paper are suggestive that there are autoimmune and genetic differences among type 1 diabetic patients according to age (early vs late onset), and these may account for the differences in the control and the outcome of the disease. Chromosomal abnormalities (parental isodisomy of chromosome 6) also have been described among patients with the transient form of

neonatal diabetes.<sup>1</sup> Studies like these suggest that EOD probably is not classic type 1 diabetes mellitus, and thus may require unique approaches for prevention and therapy.

Fima Lifshitz, MD

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1. Metz C et al. *J Pediatr* 2002;141:483-489.

## The Thyrotropin Receptor Autoantigen in Graves Disease is the Culprit as well as the Victim

The thyrotropin (TSH) receptor (TSHR) is the only 7-transmembrane G-protein coupled receptor (GPCR) for glycosylated hormones that undergoes cleavage after its primary formation; the amino terminal extracellular domain is cleaved at/near amino acid 289 (*subunit A*) leaving a short residual extracellular amino acid sequence, the 7 transmembrane domains and extracellular and intracellular connecting loops, and the intracellular carboxyl terminal domain (*subunit B*). *Subunit A* then circulates and can serve as an immunogen. The role of *subunit A* of the TSHR in the pathogenesis of autoimmune hyperthyroidism and the development of TSHR stimulating immunoglobulin (TSIg) was examined by the present investigators. They constructed within adenovirus cDNA transcripts of the

amino terminal 289 amino acid sequence (*subunit A*), the wild-type (wt) *TSHR* from which amino acids 317-366 had been removed rendering the truncated TSHR resistant to cleavage, and the intact wt *TSHR*.

Adenoviruses expressing different forms of the TSHR were then administered to female mice who subsequently developed abnormalities of thyroid function and antibodies of variable biologic activity in response to these proteins. In animals receiving TSHR 1-289, clinical, biochemical, and thyroid histologic evidence (thyromegaly, hyperthyroxinemia, and follicular hyperplasia) of thyrotoxicosis developed. These animals also developed TSig (assessed by increase in cyclic AMP formation in CHO cells expressing TSHR). In only a few mice receiving cleavage resistant TSHR or wt

TSHR were serum thyroxine levels increased and thyroid follicular hyperplasia present. In contrast, all mice, regardless of the form of TSHR received, developed high but approximately equal titers of immunoglobulins that bound to TSHR or inhibited radiolabeled TSH from binding to TSHR. TSlg did not develop in animals receiving cleavage resistant TSHR, but did appear in 30% of those injected with wt TSHR. Higher titers of thyroid blocking antibodies (assessed by their effect on TSH mediated increase in cyclic AMP generation in CHO cells expressing TSHR) were present in mice receiving the cleavage resistant form of the TSHR than in those receiving TSHR 1-289. The authors conclude that it is the extracellular segment of the TSH receptor that is ordinarily shed that serves as the immunogen for the development of TSlg in this experimental model of hyperthyroidism (and by analogy in patients with Graves disease).

Chen C-R, et al. *J Clin Invest* 2003;111:1897-1904.

**First Editor's Comment:** This extremely interesting manuscript provides significant insight into the pathogenesis not only of thyrotoxicosis, but of autoimmune thyroid disease itself. Thus, when the ectodomain of the TSHR is cleaved, it provokes the production of TSHR stimulating immunoglobulins (as well as low titers blocking antibodies) in genetically susceptible individuals. In other at-risk patients, the intact TSHR (or perhaps other sequences or epitopes of the TSHR) or TSH itself, serves as the immunogen for development of TSHR function-blocking antibodies. Other components of the thyroid gland serve as immunogens for antibodies that are injurious to the thyroid cell. A human monoclonal antibody has been recently isolated from a patient with Graves disease, but the epitope of the TSHR to which it is directed has not been identified to date.<sup>1,2</sup> It would be of interest if it were directed to the ectodomain of the human TSHR.

While a number of tyrosine kinase receptors shed their extracellular domains (growth hormone binding protein, prolactin binding protein, many cytokines), it is apparently unusual for G-protein coupled receptors to do so. This is an area that merits further examination.

Allen W. Root, MD

**Second Editor's Comment:** In Dr. Root's editorial comment, he refers to the recent identification of a monoclonal antibody that stimulates the TSH receptor in the thyroid cell to release thyroxin.<sup>1,2</sup> This also was no small accomplishment in helping us understand Graves' disease more fully. As pointed out by Dayan, who states:

"So, is the final proof of the existence of thyroid-

stimulating immunoglobulin after a journey of 47 years of anything more than academic interest? Almost certainly the answer is "yes." First, this finding might lead to a new generation of assays for thyroid-stimulating immunoglobulin in which competition for labeled TSH is replaced by competition for specific monoclonal antibodies. If a sensitive assay can be developed, it should have close to 100% specificity for Graves' disease and replace all other antibody tests, such as antithyroid peroxidase and antithyroglobulin, in this condition. Second, it should finally allow us to understand how such antibodies, even in the monomeric Fab form, can activate the TSH receptor. Such understanding of the biology of glycoprotein-hormone receptors may lead to new small-molecule agonists and antagonists not only for thyroid disease but also for hypogonadism and infertility (via the closely related receptors for luteinising and follicle-stimulating hormones). And it may prove possible to clone a potent human TSH-receptor-blocking antibody which might provide a rapid initial treatment for thyrotoxicosis. Third, the finding may lead to a better understanding of the pathogenesis of Graves' disease. How is it that the spontaneous development of such agonist antibodies, unique in autoimmune diseases, occurs so frequently (almost 1 in 100 of the population)? Does the agonist activity itself, once it appears, promote autoimmunity in a positive feedback loop? Most intriguingly, cloning of agonist TSH-receptor autoantibodies might reveal antibodies that contribute to thyroid eye-disease, the most mysterious manifestation of Graves' disease, and perhaps lead to inhibitors for these antibodies. And finally, agonist antibodies may prove a useful therapeutic agent in their own right, such as to enhance iodine-131 uptake in thyroid cancers. Many of the holy grails of biological science, from the structure of DNA to the nature of the T-cell antigen receptor, have been found. Thankfully, once in hand, they change into pointers to the many more waiting to be discovered."

The findings of Chen and those of Sanders et al are linked closely and the almost simultaneous reporting of these factors which are linked should permit a logarithmic advance in our understanding of how antibodies and receptor structure and function can relate and, consequently, provide better therapy of immunological diseases.

Robert M. Blizzard, MD

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## GHRELIN A NEW HORMONE IMPLICATED IN THE REGULATION OF GROWTH HORMONE SECRETION AND BODY ENERGY HOMEOSTASIS

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### INTRODUCTION

Growth hormone (GH) has a complex regulation with two antagonistic hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin, as well as the liver-derived hormone IGF-I. Perhaps the old name of somatotrophic hormone (STH) is more coherent than GH, as this hormone is tightly regulated by the metabolic milieu; additionally, this regulation appears to be superimposed over the classical regulation by peptide hormones. For example, metabolic signals such as glucose, amino acids, free fatty acids and their by-products, such as keto-acids, as well as the energy balance status regulate the secretion of GH in a very relevant form. In turn, GH causes complex actions on the general metabolism of a given individual.

### Highlights In This Issue

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### From The Editor's Desk

This issue marks the beginning of a new era for *Growth, Genetics & Hormones (GGH)*; Dr. Robert M. Blizzard retired and I became the Editor-in-Chief. This opportunity is an honor and the task is a major challenge, as it will be hard to fill the shoes of my mentor. In this issue you will find some modifications in the format and appearance of the journal and some changes in the editorial board. Hopefully, you will also note and appreciate the continuous high quality of the publication, papers reviewed and editorial comments. The lead article on Ghrelin and the abstracts along with editorial comments are very pertinent and timely, all together we are very pleased with this issue.

Dr. Judith G. Hall who had been with this journal since its inception has retired from *GGH* and will be sorely missed. I bid her farewell and thank her for all of her many contributions. On the other hand, I welcome Adda Grnberg, MD and David E. Sandberg, PhD whose abbreviated biographical sketches are posted on our web site ([www.GGHjournal.com](http://www.GGHjournal.com)). Two years ago we launched *GGH* on the internet and this has allowed us to reach a larger group of colleagues worldwide. Thus, I want to welcome a new group of international consulting editors who will help us project the journal internationally: Yoshikazu Nishi, MD in Japan, Raphael Rappaport, MD in France, and Alfonso Vargas, MD representing Latin America.

The last issue of 2003 included a survey which helped us renew our subscribers list and profile the readership. Over 60% of readers are pediatric endocrinologists and 15% are geneticists. A majority of our readers (78%) reside in the USA. Over two-thirds of the subscribers access the journal via the internet, usually after they are notified via e-mail announcing the publication of a new issue. Sixty percent view the journal and download it to keep as a reference. I thank everyone who responded to the survey and for the wonderful comments received; 81% gave *GGH* a very high/high ranking. The details of the survey are posted at [www.GGHjournal.com](http://www.GGHjournal.com).

This sets the challenge for the future - to reach more colleagues, to continue to improve the journal and to bring to our readers the most current reviews, updated information and advances in the field along with erudite editorial comments. Only through the internet with the on-line version of *GGH* can this be accomplished; within the budgetary constraints, a printed publication would not allow us to meet these goals. Thus we will continue to limit the printed subscriptions and phase out the printed journal by the end of 2004 while we further expand the readership through the internet.

Finally, I want to express my deep appreciation to our sponsors for the generous unrestricted, no strings attached educational grant for the publication of *GGH*. Please be sure you tell them how much you appreciate *GGH*. We also request that you keep us up-to-date with your current e-mail address so you continue to receive *GGH*. I thank you in advance for your consideration and comments ([editor@GGHjournal.com](mailto:editor@GGHjournal.com)).

Fima Lifshitz, MD, Editor-in-Chief



The upshot of this picture is of one hormone whose actions are implicated in a dual action on somatic growth and in the regulation of general metabolism, and which is in turn, regulated by the energetic homeostasis of the individual.<sup>7</sup> The recently discovered hormone, ghrelin, may well be the bridge connecting somatic growth with general metabolism.

## HISTORICAL BACKGROUND

Ghrelin is the result of the so called "reverse pharmacology", which started with the development of artificial compounds named growth hormone secretagogues (GHS), followed by the cloning of their receptor and finally the identification of the natural hormone. In fact, in the late 1970s the first highly potent GH-releasing hexapeptide (GHRP-6), was developed. This was followed by other GHS compounds such as hexarelin, or MK-0677.<sup>2</sup> These GHSs were found to be potent releasers of GH *in vitro* and *in vivo*, by acting on specific receptors at the pituitary level not related to GHRH or somatostatin. Furthermore, they were active by any route of administration, including oral, and active in all the species tested. Later GHSs were used for the cloning of the GHS-receptor.<sup>3</sup> The GHSs were not discovered, but invented, as no similar compounds existed in nature. Obviously, the new receptor must have a natural endogenous ligand. The orphan-receptor strategy was then employed by the group of Kojima and Kangawa<sup>4</sup> to screen different tissue extracts. The highest expression of GHS-receptor activating factor was found in the stomach. This endogenous ligand was named ghrelin. Ghrelin was found to be a potent releaser of GH and in addition, actively participate in controlling energy balance and the regulation of food intake.<sup>5</sup> Reverse pharmacology permitted identification of this natural ligand, ghrelin.

## DISTRIBUTION OF GHRELIN-SECRETING CELLS

Two cellular areas in the body were found to be relevant in the production of ghrelin. One was an area in the gastric fundus where ghrelin is predominately expressed and secreted. Specifically, plasma ghrelin originates in the oxyntic gland where A-like cells exist.<sup>6</sup> Lower concentrations have also been reported in the remainder of the bowel including the colon. Ghrelin positive cells are positioned close to the capillaries and have no contact with the lumen of the oxyntic gland, indicating that secretion occurs into the plasma and not into the intestinal tract.

The second area was found in the central nervous system where neuronal cell groups release ghrelin in a synaptic transmission. Since ghrelin was determined to be implicated in the regulation of appetite, it was not surprising to find abundant ghrelin in the arcuate

nucleus of the hypothalamus which also is a region rich in GHRH neurons.<sup>4</sup> Elsewhere, in the CNS, ghrelin was also present. Immunoreactive neurons were observed in a continuum filling the internuclear space between the paraventricular, arcuate, ventromedial, and dorsomedial hypothalamic nuclei, the perifornical region, and the ependymal layer of the third ventricle.<sup>7</sup> Interestingly, these novel cell groups of ghrelin immunoreactive neurons did not overlap with any of the known cell populations implicated in energy homeostasis, thus suggesting new functions. In addition to their role in the regulation of energy balance, whether these neuronal groups also participate in the regulation of GHRH or somatostatin neurons is an open question.

Ghrelin has also been identified in the placenta,<sup>8</sup> an organ that contains all the main regulatory components of the somatotrope axis, i.e., GH, GHRH, SST, IGF-I, and ghrelin. Although, placental expression of ghrelin changes significantly throughout pregnancy,<sup>8</sup> and is involved in the decidualization of human endometrial stromal cells,<sup>9</sup> the physiological function of this new hormone in the placenta is unknown. The pituitary, heart, kidney, endocrine pancreas, gonads, lungs, and lymphocytes all express ghrelin in low amounts.<sup>10-15</sup>

## MOLECULAR BIOLOGY

The human ghrelin gene is located in chromosome 3. It is made up of 4 exons and 3 introns. The mature protein is encoded in exons 1 and 2 (Figure 1).<sup>16</sup> The genetic structures of the ghrelin genes in the human and rat are identical and very similar to that gene in the mouse. The 5'-flanking region of the gene contains a non functional TATATAA box, as well as a ghrelin promoter which is activated by glucagon and c-AMP, although no AP1 site or CRE element is present.<sup>17</sup> Some gastric tumor cell lines express the promoter, however others do not, suggesting that human ghrelin promoter may have cell-specific activity. The hnRNA of the gene transcript is processed by alternative splicing to yield two different mature mRNAs; one produces the ghrelin precursor and the second yields des-Gln 14-ghrelin.<sup>18</sup> Ghrelin provides the first example of the production of two different mature biologically active peptides resulting from the alternative splicing of a peptide coding region.

The human ghrelin precursor (prepro-ghrelin) is composed of 117 amino acids, and the ghrelin sequence of 28 amino acids immediately follows the 23-residue signal peptide. Before being secreted, the ghrelin molecule undergoes an enzymatic process at the cytoplasm, an n-octanoyl addition at Ser 3. This esterification by n-octanoic acid, which is essential for the biological activity of ghrelin, yields the finally secreted peptide of 3315 mw. This process of acylation



ghrelin reportedly does it with less effectivity.<sup>22</sup> No doubt exists that ghrelin administration activates *fos* and *Egr-1* proteins in neurons of the arcuate, paraventricular and dorsomedial nuclei, and the area postrema of the hypothalamus, while deamidated ghrelin in these studies was devoid of action.<sup>23</sup> The debate is whether peripheral ghrelin acts by directly activating CNS receptors located inside or outside the BBB, or if these actions are mediated peripherally through activation of vagal nervous structures.<sup>24</sup> The latter point is of extraordinary interest as several reports state that in rats, vagotomy abolishes ghrelin-induced feeding and GH discharge. This suggests that the gastric vagal nerve is the major afferent pathway conveying ghrelin's signals to the brain.<sup>24</sup> Regardless, direct neuronal activation occurs after the activation of the ghrelin receptors, which are located on GHRH and NPY neurons, as well as in additional neurons, as was previously demonstrated for GHRP-6.<sup>23</sup> Somatostatin, cortistatin, thyroid hormones and insulin powerfully reduce gastric ghrelin secretion,<sup>25,26</sup> while cholecystokinin (CCK) and gastrin stimulate it (Figure 2).<sup>27</sup> There is no information on the regulation of ghrelin discharge by hypothalamic neurons.

Ghrelin activates the GH secretagogue receptor called GHSR-1a, a G protein coupled receptor. It activates the phospholipase C signaling route leading to an intracellular  $Ca^{2+}$  rise.<sup>9</sup> An active cross-talk at the somatotrope cell is maintained between the GHRH and the ghrelin receptors in order to coordinate and potentiate the ulterior cell response.<sup>28</sup> There is an ongoing controversy about whether the cloned secretagogue receptor is truly the receptor or just one of the receptors for that family of compounds. GHSs have specific receptors in a wide range of endocrine and non-endocrine human tissues. Most probably, different receptor subtypes exist for GHSs, with different tissue distributions.<sup>29</sup>

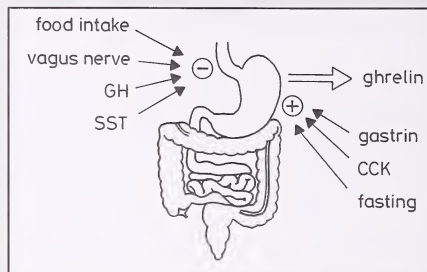
### GHRELIN ROLE IN THE REGULATION OF SOMATOTROPE CELL FUNCTION AND GH SECRETION

Ghrelin is a potent GH releaser in humans (Figure 3). No side-effects have been reported after the administration of large doses of this compound.<sup>30</sup> The potency of ghrelin as measured by its GH releasing capability is higher than for GHRH and comparable to synthesized GHS.<sup>9</sup> Thus, for ghrelin to be operative, the normal functioning of the GHRH receptor is necessary, as GHRH antagonists prevent or diminish the GH releasing possibilities of ghrelin.<sup>31</sup> Ghrelin is able to release GH *in vivo* when administered intravenously (IV), as well as when infused directly via the intracerebroventricular (ICV) route;<sup>27</sup> since it is able to enter the CNS from the periphery,<sup>22</sup> it is possible that

stomach-derived ghrelin may physiologically participate in GH regulation, although this has not yet been demonstrated. An important point is that ghrelin's mechanism of action is route dependent, as the vagus nerve and the arcuate nucleus are in the loop when

Figure 2

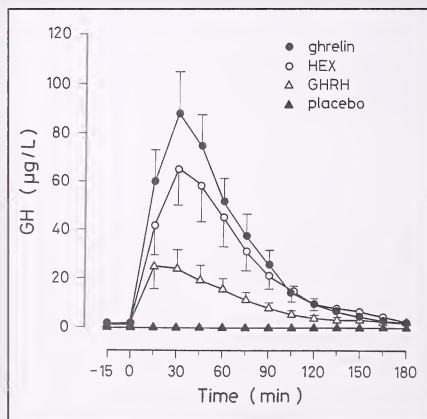
#### Regulation of gastric-derived ghrelin by different signals



SST= somatostatin; CCK = cholecystokinin.

Figure 3

#### GH secretion



GH secretion in normal subjects after the administration of ghrelin, the GHS hexarelin, and GHRH (all at 1 µg/Kg intravenously).

Redrawn from: Arvat E, et al. *J Clin Endocrinol Metab* 2001;86:1169-1174. Reprinted from: Casanueva FF, Dieguez C. *Rev Endocrinol Metab Dis* 2002;3:325-338.

ghrelin is administered peripherally, but not when administered ICV.<sup>24</sup> Ghrelin-mediated GH secretion is partially insensitive to the inhibitory action of somatostatin and of metabolic compounds such as glucose or free fatty acids.<sup>25</sup> Ghrelin and GHRH showed a strong potentiation of their GH secretory capability when injected together in humans.<sup>30</sup> This peculiar activity occurs due to a simultaneous ghrelin activation of pituitary and hypothalamic structures.<sup>31</sup> There is some evidence suggesting that hypothalamic ghrelin may participate in the physiologic regulation of pulsatile GH secretion.<sup>32</sup> Contrasted with the *in vitro* data, ghrelin *in vivo*, administered in what were probably pharmacological doses, induced a significant secretion of prolactin and ACTH/cortisol without altering the secretion of LH, FSH or TSH.<sup>9,30</sup> It remains to be determined what happens in respect to these responses when more physiological ghrelin doses and long-term administration are tested.

To show that IV pharmacological doses of ghrelin raise GH levels suggests, but is not proof, that ghrelin participates in the physiologic regulation of GH. A negative point is that rodents with knockout of the GHSR-1a did not show significant alterations in somatic growth, although a compensatory mechanism during fetal development may explain the lack of such results. Inferential evidence favoring a regulatory role for ghrelin, are from one side, the report of a simultaneous increase in GH and ghrelin in states of negative energy balance, and from the other the simultaneous decrease in GH and ghrelin in states of positive energy balance and obesity.<sup>9</sup> In the fetus, ghrelin mRNA is undetectable, but starts rising progressively after delivery to reach a peak at 3 weeks post-partum and it decreases thereafter.<sup>33</sup> The general pattern of ghrelin changes reminds one of similar patterns of growth rate, and GH and IGF-I secretion. Furthermore, ghrelin mRNA level increases rapidly during the early phase of rapid growth (in the 2-3 first weeks of life), a phase which is GH insensitive,<sup>34</sup> and a high level is maintained prior to and during the pubertal growth spurt which is GH sensitive (Figure 4).

In trying to understand the participation of this new hormone in the regulation of the somatotrope axis, it is worth mentioning that adult patients with GH deficiency or GH excess (i.e. acromegaly) have ghrelin levels similar to control subjects.<sup>35,36</sup> However, it may be that ghrelin plays a contributing role in the gender based differences in the pattern of GH secretion, as women in the late follicular stage have higher ghrelin levels than men.<sup>36</sup> In addition to its regulatory role on GH secretion, ghrelin has recently been reported to activate *pit-1* expression in anterior pituitary cells, an action that appears to be developmentally regulated as it is observed only in infant rats but not in adult rats.<sup>37</sup>

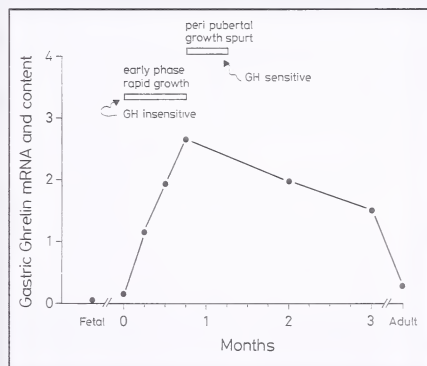
## GHRELIN AND THE REGULATION OF ENERGY HOMEOSTASIS

Ghrelin administration in humans powerfully induces a sensation of hunger in 75% of the subjects tested.<sup>30</sup> In rodents, ghrelin stimulates food intake while reducing fat utilization by a metabolic switch that increases the consumption of carbohydrates.<sup>38</sup> Different mechanisms than those involved in GH regulation<sup>38-40</sup> control the activity of ghrelin over food intake. Its action seems to be the exact opposite of leptin. Ghrelin is the most powerful appetite stimulant of all the known peptides; it is the unique gastrointestinal peptide that stimulates food intake. All other peptides affecting appetite are anorexigenic. Ghrelin also stimulates food intake in rodents when administered either centrally or peripherally. Other orexigenic peptides are devoid of action with peripheral administration. CNS peptides such as NPY, orexin, and agouti-related protein (AGRP) partially mediate the ghrelin action.<sup>41,42</sup>

Relevant changes in plasma levels of ghrelin appear to endorse the hypothesis that *gastric derived circulating ghrelin* regulates central appetite mechanisms. For example in rodents, ghrelin mRNA in stomach and ghrelin levels in plasma are increased by fasting and reduced by feeding, actions unrelated to gastric volume

Figure 4

### Ghrelin expression and content



Ontogenetic changes in ghrelin expression and content in mice gastric tissue.

Redrawn from: Liu JL, LeRoith D. *Endocrinology* 1999;140:5178-5184.



changes.<sup>38,43</sup> Passive immunoneutralization with ICV ghrelin antibodies inhibited starvation-induced as well as natural food intake in rodents, clearly indicating a tonic ghrelin action at hypothalamic receptors.<sup>44</sup> However, as blockade of the vagus nerve inhibits ghrelin-induced feeding in rodents,<sup>24</sup> perhaps peripheral ghrelin does not need to cross the BBB to activate central structures. These data do not preclude that the CNS neuronal groups secreting ghrelin may play a role, perhaps one even more relevant in the physiological regulation of appetite.

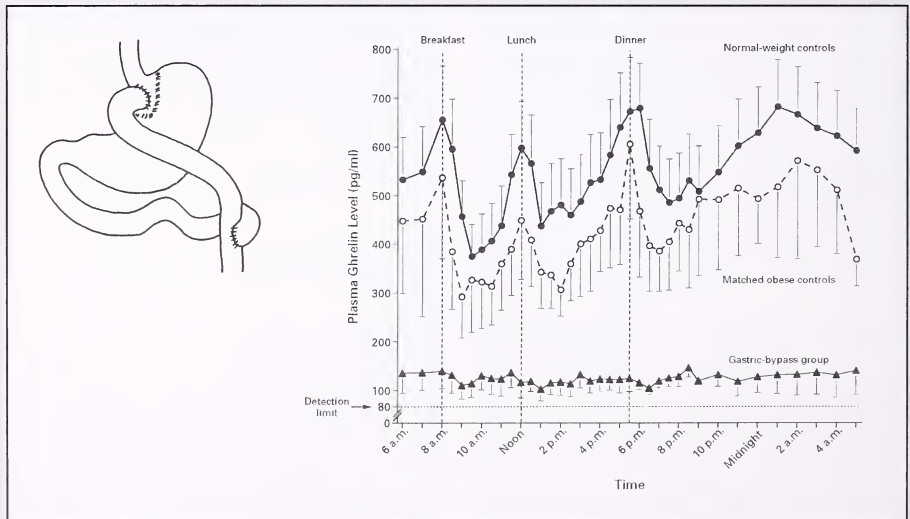
Ghrelin levels are decreased in obese subjects while elevated in states of malnutrition such as cachexia and anorexia nervosa. In the latter, weight recovery normalizes ghrelin plasma values.<sup>45</sup> In respect to the etiology of human obesity, no solid information supports its association with polymorphisms in the ghrelin gene. Circulating ghrelin undergoes relevant changes in relation to food intake, it is elevated before and decreased after feeding in a reciprocal pattern with insulin, and with intermeal changes that are in phase

with leptin.<sup>20</sup> Such results suggest that the preprandial ghrelin rise has a role in initiating meal consumption in humans. Interestingly, obese subjects who lose weight show an increase in plasma ghrelin. This fact may explain the facility of obese individuals to recover weight after dieting on the classic low-calorie diets.<sup>46</sup> Patients who have undergone bariatric surgery as treatment for obesity show a reduced ghrelin level, probably due to the absence of direct food stimulation on the gastric fundus (Figure 5).<sup>46</sup> It is a well known fact that bypass bariatric surgery is more effective over the long-term than other techniques, and that patients often refer to an absence of appetite after the surgical intervention.

Although they need to be replicated by different groups, the above results open new ways of understanding the regulation of energy homeostasis. Furthermore, the linear correlation in humans between hunger sensation and ghrelin levels, and the supranormal levels of plasma ghrelin in patients with uncontrolled hunger, such in Prader-Willy patients,<sup>47</sup> directly links ghrelin with hunger control.

Figure 5

### Circulating ghrelin levels in controls and in obese subjects



The action of gastric bypass surgery decreases ghrelin levels.

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## GHRELIN ACTION ON OTHER HORMONAL SYSTEMS AND NON ENDOCRINE STRUCTURES

Ghrelin may also be involved in the neuroendocrine and behavioral response to stress,<sup>48</sup> and in reducing LH secretion.<sup>49</sup> Ghrelin and its functional receptor have been shown in testicular tissue to inhibit testosterone secretion, as well as in both the rat and human ovary, suggesting that ghrelin may be responsible in part for the energy homeostasis associated with control of reproduction.<sup>17,50</sup>

Ghrelin mRNA and ghrelin receptor mRNAs are expressed in gastric, thyroid, breast and lung neoplasias.<sup>15,51</sup> This opens potential new routes of treatment. Also recent data suggests that ghrelin may be an endogenous factor to promote sleep.<sup>52</sup>

In a totally different perspective, a most promising report is that both ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells.<sup>53</sup> These data support the protective actions of ghrelin on the cardiovascular system, and possibly more importantly, that there may be biological actions for the deacylated molecule.

## SUMMARY AND SPECULATION

As ghrelin anticipates the initiation of meals and releases GH, one could share the teleological view that ghrelin integrates anabolic changes in the body. In catabolic situations, raised ghrelin levels may induce a combination of enhanced food intake, increased gastric emptying and food assimilation coupled with GH levels which promote a prompt nutrient incorporation into muscles and to fat. These actions of ghrelin are the opposite of leptin which reduces food intake and selectively eliminates fat mass. Thus, both peptides may act as physiological regulators of energy balance. Interestingly, each comes from a peripheral organ (stomach and white adipose tissue, respectively). Furthermore, with conceptual incorporation of ghrelin into the group of physiological regulators of GH (i.e., GHRH, somatostatin, IGF-I), we may be on the verge of understanding better aspects of the regulation of secretion of GH that previously were not understood.

The clarification of these and other speculations are eagerly awaited. For example, it is not known if ghrelin participates in a physiological way in regulating GH secretion and energy homeostasis. If it does, it needs to be clarified whether stomach-derived circulating ghrelin and/or neuron secreted ghrelin regulate CNS food intake and GH secretion. Similarly, it is unknown whether circulating ghrelin acts after crossing the BBB, or alternatively through an unexpected mechanism related to the structure of the vagus nerve. Finally, the

part played by the scattered neuronal systems which secrete ghrelin at both hypothalamic and extrahypothalamic sites have been largely ignored for both food intake and regulation of GH secretion. Such studies will provide better knowledge of the intricate regulation of GH secretion and appetite. It can be foreseen that important new physiological insights and contributions will be provided in the future.

## ACKNOWLEDGMENTS

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## Letter to the Editor: Preterm Birth and Insulin Resistance at Adolescence

In the September issue of *GGH* (Vol 19, No 3) you reviewed an interesting publication by Singhal et al<sup>1</sup> who studied the relation between infant feeding, early growth and insulin resistance at age 13-16 years in individuals with a birth weight below 1,850 grams (which the authors labeled preterm). In their study, insulin resistance was not associated with birth weight but with growth in the first two weeks of postnatal life; thus, they concluded that Barker's hypothesis "can be reinterpreted as a postnatal event". In our opinion, their data should be interpreted more cautiously, for the following reasons.

The first point is that selection bias is quite likely. The application of birth weight instead of gestational age as inclusion criterion (< 1,850 grams) suggests that severely growth-retarded individuals born at term are also included. Another point of our concern is that in both experimental groups there were considerable numbers lost to follow-up (65-68%).

Secondly, conclusions with respect to insulin resistance in later life were drawn from a population aged 13-16 years. In this age period there is a wide variation in pubertal stages, and during pubertal development insulin sensitivity is decreased.<sup>2</sup> Moreover, girls born small for gestational age have a tendency towards early and rapid progression of puberty,<sup>3</sup> and hyperandrogenism,<sup>4</sup> which is accompanied by decreased insulin sensitivity. It is likely that many infants in the experimental groups had a low weight for gestational age at term; thus, it is conceivable that they may have shown abnormalities in pubertal onset and tempo, as well as in androgen metabolism.

Thirdly, although an earlier study of this research group (in the same population at age 7.5-8 years) suggested that suboptimal nutrition, which may result in poor early postnatal growth, adversely affects neurodevelopmental outcomes, little emphasis is put on the possible beneficial effects of nutrient-enriched preterm formulas.<sup>5</sup> This suggests that discouraging early postnatal catch-up growth by restricted food intake in infants with a birth weight below 1,850 grams is hard to justify.

The last and major point is that Singhal and colleagues have extrapolated their findings in individuals born preterm to conclusions about the general population. The first two postnatal weeks of the experimental groups took place halfway into the third trimester. In our opinion it cannot be automatically assumed that postnatal growth taking place at an age that is normally spent *in utero*, can be considered equivalent to postnatal growth of a term infant.

In conclusion, this interesting study has shown that slow early postnatal growth of preterm infants is associated with low insulin resistance at adolescence. In our view it is uncertain whether these effects persist into adulthood and whether early postnatal catch-up growth predisposes to insulin resistance only in preterm infants or also in those born at term.

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**Response:** The comments of Dr. Finken and colleagues are welcome as they point to several possible methodologic and interpretive flaws in the work of Singhal et al. Although we do not know the exact number of small for gestational age neonates included in the cohort of subjects reported, it is likely that the majority were preterm and appropriate for gestational age. If there is a potential way in which to prevent the development of insulin resistance and the dysmetabolic syndrome, it should be explored. However, clearly, one would not want to jeopardize optimal neural development under any circumstances.

Allen W. Root, MD

## ABSTRACTS

## Low-Carbohydrate Diet, Weight Loss and Cardiovascular Risk

The prevalence of childhood obesity continues to rise to epidemic proportions, with adolescents beginning to show significant signs of developing cardiovascular risk factors. A variety of weight-loss diets have been tested in adult populations, but the assessment of these diets in children, especially those with decreased carbohydrate (CHO) or fat remains limited. Sondike and colleagues report on the use of a low carbohydrate (LC) versus a low fat (LF) diet in a group of adolescents (ages 12-18) with a BMI >95<sup>th</sup> percentile. Thirty-nine adolescents participated in the 12-week randomized controlled study. The LC diet consisted of a daily CHO intake of <20g/d for the initial 2 weeks and then up to 40g/d. There were no restrictions on protein, fat or calories. The control group was assigned to a LF diet (<30% energy from fat, <40g/d) with 5 servings of starch (15g CHO each serving) daily. There were no restrictions on calories. Thirty minutes of exercise 3 times a week was encouraged, but not monitored. Subjects were weighed every 2 weeks and dietary adherence was monitored at those visits by a dietitian who reviewed 3-day food records. Lipid profiles including fasting total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were measured along with electrolytes and liver function studies at baseline and at 12 weeks. Ketonuria was monitored and recorded by the subjects daily.

Thirty subjects completed the study (LC=16, LF=14). Subjects in the LC group lost significantly more weight than those in the LF group ( $9.9 \pm 9.3\text{kg}$  vs  $4.1 \pm 4.9\text{kg}$ ,  $p < 0.04$ ) despite having consumed more daily average calories ( $1830 \pm 615$  vs  $1100 \pm 297$ ,  $p < 0.03$ ). BMI improvement was significantly greater in the LC vs LF group as well ( $p < 0.05$ ). LF group subjects had significantly lower LDL cholesterol levels at 12 weeks than at baseline, whereas there was no change in these levels in the LC group. HDL cholesterol rose significantly in both groups and triglycerides fell significantly in the LC group. The authors state their results were consistent with those from previous weight-loss studies employing strict calorie control (protein-sparing modified fasts). Their de-emphasis on calorie control may reduce the concern for the effects of dieting on linear growth velocity. The authors also suggest that the LC diet may not be appropriate for adolescents with significant baseline elevations in LDL cholesterol. The palatability of the LC diet may be one reason that 8 of the LC subjects voluntarily remained on the diet for a year.

Sondike SB, et al. Effects of low-carbohydrate diet on weight loss and cardiovascular risk factors in overweight adolescents. *J Pediatr* 2003;142:253-258.

**First Editor's Comment:** This is an important study and hopefully it is but the first in a series of weight-loss studies designed to improve fitness and cardiovascular risk among obese children. The authors refrained from overstating their findings. As pointed out in an accompanying editorial by Daniels,<sup>1</sup> the long-term effects of LC diets on bone density, body composition, insulin resistance, and glucose metabolism remain to be defined. Sondike and colleagues do not, and because of the short 12-week duration of their study, could not address these issues. But these will need to be addressed, as will the metabolic and pathophysiologic abnormalities associated with obesity; none of these are trivial. It is anticipated that pieces of this complex "biopsychosocial" disorder will become more evident over the next few years as more and more investigators begin to study obesity and develop effective treatment regimens.

William L. Clarke, MD

**Second Editor's Comment:** The first low-carbohydrate diet for weight loss was described in 1863<sup>2</sup> and was popularized by Dr. Atkins<sup>3,4</sup> in the modern era. However, the efficacy and safety of such diets are still being debated. A systematic review of 107 articles recently concluded that there is insufficient evidence to make recommendations for or against its use.<sup>5</sup> The first randomized trial conducted for up to 12 months of such dietary therapy showed that LC diets initially induced more weight loss than the low-calorie high-carbohydrate LF diets. However at the end of one year the differences were no longer evident.<sup>6</sup> Differences in weight loss were principally associated with energy intake.<sup>7</sup> A calorie is a calorie no matter its source.

Fima Lifshitz, MD

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## Interactions in Gene Encoding Mutations Leading to Cortisone Reductase Deficiency

Draper et al studied a virilized 6-year-old boy with gonadotropin-independent isosexual precocious puberty and two adult women with polycystic ovarian syndrome (PCOS); subjects had low ratios of urinary tetrahydrocortisol to tetrahydrocortisone excretion. These findings were consistent with an autosomal recessive deficiency of cortisone reductase - the enzyme complex that interconverts cortisone (E) and cortisol (F). Cortisone reductase has dual dehydrogenase and oxo-reductase activities depending on the availability of a cofactor - NADP/NADPH. There are two isozymes of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) - hepatic (and adipose tissue) type 1 (E $\rightarrow$ F) and renal type 2 (F $\rightarrow$ E). In previous studies, as in the present patients, the nucleotide sequence of the 6 exons of 11 $\beta$ HSD1 (chromosome 1q32-q41, OMIM 604931) was normal. However, in the three subjects in this report, mutations were found in intron 3 of 11 $\beta$ HSD1. One woman with PCOS was homozygous for double mutations - insA @ NT 83557 and T $\rightarrow$ G substitution @ NT 83597, while the second woman and the virilized boy were heterozygous for these mutations. Heterozygous carriers (parents, siblings, general population) of these linked mutations were clinically and biochemically normal. Further examination of the importance of these mutants (or polymorphic variants) revealed that the linked mutations impaired expression of 11 $\beta$ HSD1 and biologic activity of the enzyme product. Thus, the investigators concluded that intron 3 of 11 $\beta$ HSD1 served as an "intronic enhancer" of the expression of its gene.

Because the activity of 11 $\beta$ HSD1 requires a co-factor (NADPH) the authors examined NADPH generating systems and identified two mutations in the gene (*H6PD*, chromosome 1pter-p36.13, OMIM 138090) encoding the enzyme - hexose-6-phosphate dehydrogenase - that is the principle generator of NADPH in the endoplasmic reticulum in which 11 $\beta$ HSD1 is located. One mutation in *H6PD* - heterozygous 29 bp insertion between NTs 620 and 621 was present in the woman who was homozygous for the double mutation in 11 $\beta$ HSD1; a homozygous mutation - Arg453Gln - was present in the other woman with PCOS and the virilized youth. Both mutations resulted in products with substantially decreased H6PD functional activity.

The investigators concluded that inactivating mutations in both 11 $\beta$ HSD1 and *H6PD* (a total of 3 mutated alleles) must be present in order to result in sufficiently decreased 11 $\beta$ HSD1 activity to lead to the syndrome of cortisone reductase deficiency. Thus, this disorder is another example of a digenic-triallelic pattern of inheritance as are some forms of the Bardet-Biedl syndrome (OMIM 209000).<sup>1,2</sup>

Draper N, et al. Mutations in the genes encoding 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nature Genet* 2003;34:434-439.

**Editor's Comment:** This manuscript presents yet another cause of gonadotropin-independent pseudoisosexual precocity in boys - cortisone reductase deficiency - indicating the need to measure cortisol, cortisone, and their urinary metabolites in patients with otherwise unexplained hyperandrogenic states. One wonders why females with a similar enzymatic defect do not manifest signs of hyperandrogenism until adulthood. Might there be yet another factor (gene product?) present/absent in young females that preclude early disease expression? It has been suggested that enhanced reductase activity in visceral adipose and perhaps other tissues, with consequent local hypercortisolism, might be associated with the development of visceral obesity and the "dysmetabolic syndrome".<sup>3,4</sup> Loss-of-function mutations in the gene 11 $\beta$ HSD2 (chromosome 16q12, OMIM 218030) encoding renal 11 $\beta$ HSD2 lead to hypertension in the presence of subnormal mineralocorticoid values (the syndrome of "apparent mineralocorticoid excess") because unmetabolized cortisol occupies and activates the mineralocorticoid receptor leading to renal tubular reabsorption of sodium and water and hypervolemia.

Allen W. Root, MD

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## Anorectic Effects of PYY in Obesity

The gut hormone fragment peptide YY<sub>3-36</sub> (PYY) is known to reduce appetite and food intake when given to subjects of normal weight as well as to rodents. The authors investigated whether obese subjects were also sensitive to the anorectic effects of PYY. They compared the effects of this peptide by infusing it into 12 obese

and 12 lean subjects in a double-blind, placebo-control, crossover study, and measured the effects on appetite, food intake as well as plasma levels of PYY, ghrelin, leptin and insulin. Caloric intake during a buffet lunch two hours after the infusion of PYY was significantly decreased by 30% in the obese and by 31% in the lean

subjects. PYY infusion also caused a significant decrease in the cumulative 24-hour calorie intake in both obese and lean subjects. The average decrease in the food ingestion was about one-third of the calories, as compared to the amount consumed the day prior to the infusion. However, food intake from 0-12 hours following PYY administration was more markedly reduced than that ingested from 12-24 hours after the infusion. The administration of PYY also reduced plasma levels of the appetite stimulatory hormone, ghrelin. Endogenous fasting and postprandial levels of PYY were significantly lower in obese subjects as compared to the non-obese group. Furthermore, the fasting PYY levels correlated negatively with BMI. The authors concluded that obese subjects were not resistant to the anorectic effects of PYY and suggested that a deficiency of PYY may contribute to the pathogenesis of obesity in humans.

Batterham RL, et al. Inhibition of food intake in obese subjects by peptide YY<sub>3-36</sub>. *N Engl J Med* 2003;349:941-948.

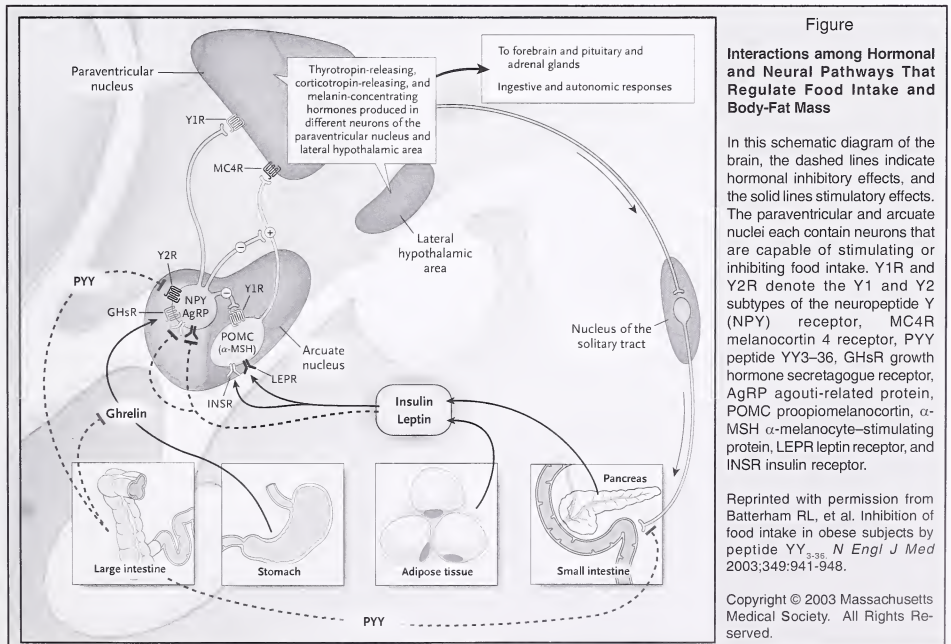
**Editor's Comment:** PYY is secreted postprandially, in proportion to the calories ingested, by endocrine L cells lining the distal small bowel and colon. PYY leads to a decrease food intake by inhibiting gut motility and increasing satiety. In this study, PYY infusion reduced hunger in both the obese and the lean individuals. These effects were directly related to the action of PYY, as there were no effects on the palatability of meals, feelings of

well being, or the presence of nausea. This peptide is one of the many signals that have been recently identified providing short-term information to the hindbrain and hypothalamus regarding hunger and satiety. Other gut hormones, such as cholecystokinin and ghrelin, also play a role in communicating with the hypothalamus and brain stem to stimulate or reduce the appetite. In this issue of GGH there is a review of ghrelin, the hunger hormone, acting on growth hormone secretagogue receptors and its pathophysiologic role in obesity related diseases.<sup>1</sup> However, the regulatory controls of food intake are more complex and involve other endocrine functions of adipose tissue, principally leptin, and appetite controlling genes, as previously reviewed.<sup>2</sup> However, PYY signal in satiety appears to play a role in obesity in humans and could be thought of as a therapeutic agent; a hope that was not realized by leptin, as in obesity there is marked resistance to the actions of this hormone. A graphic depicting the complex interactions among hormonal and neural pathways that regulate food intake and body fat mass<sup>3</sup> is shown below (Figure).

Fima Lifshitz, MD

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## A Gene Regulator of Puberty

While evaluating a Saudi family with several first cousin marriages in which many offspring had "idiopathic hypogonadotropic hypogonadism" transmitted as an autosomal recessive trait, the authors identified a locus on chromosome 19p13.3.<sup>1,2</sup> This locus had a homozygous mutation of *GPR54* (chromosome 19p13.3, OMIM 604161, encoding an orphan G-protein receptor termed GPR54) at codon 148 in which serine was substituted for leucine (Leu148Ser). An unrelated patient was demonstrated to be a compound heterozygote with mutations in both alleles of *GPR54* - Arg331Stop leading to a truncated product and Stop399Arg - the latter resulting in an elongated protein product. *In vitro*, all mutations were found to decrease signal transduction through phospholipase C in response to the natural ligand of this receptor - kisspeptin-1 - sequence 112-121 (encoded by *KISS1*, chromosome 1q32, OMIM 603286). Kisspeptin-1 [sequence 68-121] suppresses metastases of melanoma and breast carcinoma experimentally. This 54 amino acid peptide, termed metastatin, is secreted by the placenta. In the compound heterozygotic subject, there were low basal concentrations of LH and testosterone that increased during pulsatile administration of exogenous GnRH; interestingly, this patient was more sensitive to the gonadotropin stimulating effects of GnRH than were comparable patients with hypogonadotropic hypogonadism without this specific genetic mutation.

The investigators extended these studies by developing a "knock-out" mouse model of *GPR54*<sup>-/-</sup> that reproduced the clinical picture. The *GPR54*<sup>-/-</sup> heterozygous mice had normal growth and fertility. The *GPR54*<sup>-/-</sup> deficient animals of both genders were hypogonadotropic with small gonads, hypotrophic internal genitalia, and absence of secondary sexual characteristics. Interestingly, the adrenal glands of the *GPR54*<sup>-/-</sup> animals were immature as well. Serum gonadotropin and sex hormone levels were low in *GPR54*<sup>-/-</sup> animals, but LH and FSH values increased following administration of exogenous GnRH, but the hypothalamic concentrations of GnRH were normal. The authors conclude that the kisspeptin-GPR54 system is

important in the regulation of GnRH processing or secretion in the hypothalamus rather than in the movement of GnRH secreting neurons from their embryologic site of origin in the olfactory placode (the error in Kallmann syndrome) or in the synthesis of GnRH itself.

Seminara SB, et al. The *GPR54* gene as a regulator of puberty *N Engl J Med* 2003;349:1614-1627.

**Editor's Comment:** This exciting report exemplifies the best of clinical investigation employing the most up-to-date technology in a multi-institutional collaborative that should serve as a model for future studies. The identification of a G-protein receptor (and its aptly named endogenous ligand - kisspeptin) that are involved in the regulation of GnRH release opens an entirely new control system of the reproductive endocrine axis,<sup>3</sup> a finding analogous in importance to the discovery of the role of ghrelin in the regulation of growth hormone secretion<sup>4</sup> and energy metabolism. Elucidation of the mechanism(s) by which this unit regulates GnRH secretion is eagerly anticipated. One can envision many future studies of the kisspeptin-GPR54 axis. Perhaps it is involved in the development of normal puberty. Might polymorphisms of its component genes or signal transduction system account for variations in the early or delayed onset of adolescence? Are gain-of-function mutations in *GPR54* present in some children with idiopathic central precocious puberty? Does the development of gonadotropin secreting tumors involve this pathway? Since metastatin is secreted by the placenta, this suggests that it has a physiologic role during gestation - possibly in regulation of fetal gonadotropin secretion. Future studies are eagerly and impatiently awaited.

Allen W. Root, MD

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## Growth Hormone Effects on Quality of Life of Young Adults

The investigators' goals were to document changes in quality of life (QoL) over the course of the first year post-growth hormone (GH) withdrawal, and to subsequently assess the psychological effects of reinstating GH. Participants in the GH discontinuation study were recruited from a Dutch outpatient clinic and comprised of 14 males, 8 females (ages 15 to 22 years, mean = 19 years), 11 with isolated GH deficiency (IGHD), and 11 with multiple pituitary hormone deficits (MPHD). All had

achieved adult height and were receiving adequate replacement of other hormones. Although all tested GH deficient (GHD) as children, 8 of 11 IGHD retested GH-sufficient as young adults. In contrast, all MPHD patients retested as GHD in early adulthood.

During the first six months of discontinuation of GH, a statistically significant increase in psychiatric symptoms (assessed by Hopkins Symptom Checklist) was observed, with no further increases between 6 and



12 months. There were no differences in symptoms between IGHD and MPHD, or between GHD and non-GHD. These findings corresponded temporally with a decline in IGF-I. IGF-I concentrations did not differentiate the MPHD and IGHD groups. Depressive symptoms, assessed by the Profile of Mood States (POMS), increased in both IGHD and MPHD groups by 6 months of GH discontinuation and thereafter increased further for the IGHD, but decreased within the MPHD group. The opposite pattern was observed for the POMS Tension scale, which increased across the 12 months for the MPHD group, but declined for those with IGHD. Lower IGF-I concentrations were associated with more negative mood states and somatic complaints for the combined group, whereas higher IGF-I was associated with greater 'vigor'.

Nine of 14 patients (64%; 4 males, 5 females; 2 with IGHD and 7 with MPHD) from the GH discontinuation study who remained GHD when retested as adults subsequently participated in the GH treatment study. This sample was augmented with an additional 11 patients (6 males and 5 females; 3 IGHD and 8 MPHD) who were GHD both as children and adults, had not been treated with GH in the past year, and had not participated in the GH discontinuation study. Upon reintroduction of GH to only those patients meeting adult criteria for GHD, IGF-I levels increased between 0 and 6 months in both IGHD and MPHD, but without further change by 12 months. Accompanying this increase, scores on the insecure and depression scales (of the SCL-90) decreased across the entire 12 months for both IGHD and MPHD groups, whereas anxiety (assessed by the State-Trait Anxiety Scale) decreased significantly only from baseline to 6 months. QoL scores showed a significant improvement from 0 to 6 months of GH treatment. IGF-I levels were negatively correlated with negative mood states, but positively correlated with vigor, QoL, and short-term memory. The investigators concluded that GH-modulation of IGF-I concentrations is responsible both for deteriorating mood states during GH discontinuation and improved psychological status during the return to treatment.

Stouthart PJ, et al. Quality of Life of Growth Hormone (GH) Deficient Young Adults During Discontinuation and Restart of GH Therapy. *Psychoneuroendocrinology* 2003;28:612-626.

**Editor's Comment:** As recognition has grown that the actions of GH extend beyond linear growth, the practice of treating GHD in adulthood has become more widely accepted. Unlike most studies assessing the benefits of adult GH replacement, these outcome variables were psychological rather than metabolic. In this study, both the IGHD (73% of whom retested GH-sufficient by adult criteria) and MPHD subgroups exhibited similar deterioration in emotional state upon discontinuation of GH with improvement after reinstating GH therapy. The investigators related these psychological changes to lower and subsequently improved IGF-I concentrations.

Several methodological features of this study should be taken into account before factoring them into clinical management algorithms. For instance, the investigators provide no indication of how representative study participants were of those in this clinic in meeting diagnostic and age criteria. Were those who agreed to participate more emotionally symptomatic? Research suggests considerable variability among patients in responsiveness to the QoL benefits of adult GH replacement.<sup>1,2</sup> The potential contribution of a placebo effect to mental health indices also needs to be considered. A meta-analysis suggests that placebo effects are stronger in small trials with continuous subjective outcomes.<sup>3</sup> The investigators may be attributing some psychological benefits to GH that are potentially due to response bias or placebo effect. Nonetheless this study is of great interest and provides important information.

David E. Sandberg, PhD

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## Non-Hormonal Genetic Influence on Brain Development

Current dogma holds that differences in brain development and behavior between males and females depend primarily on gonadal steroid hormones, especially testosterone and its metabolites that induce the masculine pattern and inhibit the female pattern of brain development. However, there is also evidence that genetic factors may act directly on the developing brain contributing to these differences. Until recently, this alternative view has been difficult to document, but Dewing et al provide new and convincing evidence for non-hormonal genetic effects.

Their work was done in a mouse embryo 10.5 days

after conception. This is just before the first sign of sexual differentiation of the genital ridges occurs, thus the influence of gonadal hormones could be excluded. Their strategy was to harvest whole heads from the embryos, isolate RNA into separate pools for males and females and then analyze for differential gene expression in the male and female brains. For screening analysis, they used gene (microarray) chip (Affymetrix) technology which allowed the relative expression of nearly 10,000 characterized mouse genes and over 3,000 less well defined expressed sequences (Expressed Sequence Tags – ESTs) to be determined. The normalized gene



chip results reported as fold change or difference between male and female brain RNA revealed 36 genes or ESTs with enhanced expression in females and 18 genes or ESTs with enhanced expression in males. These genes exhibited a significant fold difference of greater than 1.1 and 7 genes or ESTs for each sex displayed a fold difference of 2.0 or more. The gene showing highest differential expression in females was Xist, which was 18.5 fold higher in females, while genes showing the highest differential expression in males included DEAD box peptide (Dby) and eukaryotic translation initiation factor 2,Y (Eif2s3Y) with fold differences of 10.0 and 8.8, respectively. Xist maps to the X chromosome, while the latter two genes reside on the Y chromosome.

Real-time quantitative analysis (RT-PCR) of littermate-matched male and female embryonic brain RNA confirmed and validated the results of the gene chip screening for a small number of genes based on their potential roles in brain development. The authors concluded that developmental differences in male and female brains in mice are due in part to the differential expression of genes before gonadal secretion starts.

Dewing P, et al. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Mol Brain Res* 2003;118:82-90.

**First Editor's Comment:** This is an important paper that documents the differential expression of genes in the male and female brain prior to any influence from gonadal hormones. If confirmed, it will have a substantial impact on understanding how genetic factors influence brain development. The design of the study allows for the identification of non-hormonal factors that act before the gonads are formed. However, there is no reason to think that genes act through mechanisms that do not involve gonadal hormones after gonadal hormone secretion begins, although other investigational approaches will be needed to demonstrate this. Dewing and colleagues provide no insight into the nature of the non-hormonal mechanisms through which genes may act before the appearance of gonadal hormones, although they could presumably be multiple and diverse.

One should note that the most dramatic differences were found for genes whose expression is expected to be limited to one sex or the other. For example, one would expect genes located on the Y chromosome to be expressed only in the male brain and Xist mRNA, which is expressed only by the inactive X chromosome in XX females, to be detected only in the female brain. That they were detected at all, seemingly reflects how the assays distinguish negative results from background signals. When these results are excluded the differences were diminished. Microarray gene chip and related approaches for studying gene expression are relatively new and evolving rapidly as is bioinformatics, the discipline that deals with analysis of the vast amounts of data this technology generates. Its novelty combined

with the complexity of its data has led to a certain amount of caution in the biomedical field with regard to the biological significance of microarray results. Initially, a 2-fold difference in expression was considered an informal threshold for biological significance. Many of the results in this study fall below this level and therefore would not be considered significant by this criteria even though they are statistically significant. However, as the analytical methods advance, the threshold is being progressively lowered such that a cut-off, such as the 1.1-fold difference used in this paper, is becoming acceptable. It is still probably wise, however, to view small differences in gene expression with caution until they are confirmed by others and placed in a biological context.

William A. Horton, MD

**Second Editor's Comment:** The findings of this study are important and exciting, and will likely contribute to a transformation of the dominant conceptual model regarding sexual differentiation of somatic phenotype, brain, and behavior. There is a risk that the findings may be misinterpreted in a manner potentially harmful to the clinical decision-making process in cases involving intersexuality. The findings force us to rethink the classic view of brain sexual differentiation and behavior which posits that the role of genes in the development of sex differences is restricted to the process of sex determination, i.e., the development of a bipotential and undifferentiated gonad into either an ovary or a testis. Evidence of a direct role of genes (not mediated by sex hormones) may lead clinicians to question the flexibility in decision-making they may currently exercise when sex assignment is in question. But should they?

The basic finding of the study is that over 50 candidate genes are differentially expressed in the brains of male and female mice, ostensibly prior to gonadal production of sex hormones. Although a remarkable observation, these findings are not necessarily relevant for one psychological outcome variable of great importance in intersex cases, that is the stability of gender identity across the lifespan. (Gender identity refers to the individual's self identification as either girl/woman or boy/man.) Readers of media reports of this article will likely draw different conclusions. The headline of one well-publicized report of this study states "Sexual Identity Hard-Wired by Genetics." Quotes within the article imply that gender identity springs directly from our genome. If so, then how do we account for the consistent finding in the literature that 46,XY individuals with complete androgen insensitivity syndrome develop an unambiguous gender identity as girls, and later women?

The conflict between research findings and their interpretation is likely more apparent than real and is promoted by an oversimplification of the process of psychosexual differentiation in humans. An individual's

gender identity need not be congruent with their gender-role (which refers to behaviors that differ in frequency or level between males and females in this culture and time such as toy play or maternal interest), and sexual orientation (the pattern of sexual arousal). At the present time, the clinical research literature suggests that gender identity generally conforms with the gender of rearing, even when gender assignment is discordant with genetic sex. The picture is quite different, however, with respect to the variables of gender-role behavior and sexual orientation. It is clear that many new findings will stem

from the line of research described in this report. However, it would be unfortunate if these data were to be interpreted as suggesting that gender assignment must conform with genotype to foster a stable gender identity.

David E. Sandberg, PhD

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## IGF, Learning & Memory

Lupien et al tested the following hypotheses: (1) IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory; (2) IGF-I can support cognitive function across the blood-brain barrier; (3) IGF can preserve brain function in diabetes independently of hyperglycemia; and (4) brain IGF contributes to hippocampal-based cognitive functions.

The first three hypotheses were tested by comparing normal rats versus streptozocin (STZ) diabetic rats. Four weeks after STZ, minipumps were implanted to deliver continuous infusions of 20 µg/day IGF-I or vehicle (10 mM acetic acid, pH 6.0) for 7.5 weeks. (For reference, daily IGF-I production by the adult rat liver is about 31 µg/day.) The hidden platform or "place" test was performed to assess spatial learning and memory; the "probe" test to examine memory; and the "cued" test to detect sensorimotor deficits. Following these tests, the mean blood glucose levels were 125.0±11 mg/dl in the non-diabetic rats versus 515±73 in the STZ + vehicle and 495±99 in the STZ + IGF rats. Body weights of both STZ groups were about half that of the non-diabetic rats.

All 3 groups decreased their latency times to escape the hidden platform, but there was a 3-day lag before latencies began to decline in the STZ + vehicle group. STZ-IGF performed similarly to the non-diabetic rats, and both groups decreased their latencies by shortening their search paths. The STZ + vehicle group decreased their latencies by increasing their swim velocity; their paths did not shorten. The average latency was more prolonged in the STZ + vehicle, than in the STZ + IGF rats. The STZ + vehicle rats also swam the furthest distance; STZ + IGF were again like the non-diabetics. Swim velocities were not significantly different, thus motor or proprioceptive disturbances were not the cause of the poorer performance of the STZ + vehicle rats. IGF infusion improved learning/memory performance without ameliorating the hyperglycemia or the catabolism of the STZ rats. Total brain weight and hippocampal weight were significantly reduced in the STZ rats, and these were not attenuated by IGF infusion. The second experimental design tested IGF's contribution to normal learning/memory by passive avoidance of electric shocks after two-weeks of continuous infusion into the lateral ventricle of either 40% anti-IGF-II

antisera or 40% preimmune serum. Whereas the latencies of the preimmune serum rats increased, those of the IGF-II antisera rats were significantly diminished. The authors concluded that IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory in experimental diabetes in rats.

Lupien SB, et al. Systematic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. *J Neurosci Res* 2003;74:512-523.

**Editor's Comment:** The authors integrated their results into a review of prior studies of the effects of diabetes and IGF on neurologic function. Experimentation in rats allowed controlled manipulations that cannot be made in humans, like the examination of brain tissues and the continuous intraventricular infusion of IGF antisera. These data add to the evidence supporting IGF benefits for neurologic function. Aleman and colleagues demonstrate significant associations between circulating IGF-I concentrations and performance on perceptual-motor performance and mental processing speed in healthy men aged 65-76 years.<sup>1</sup> Although it is tempting to attribute the better performance to the higher IGF-I levels, associations are NEVER sufficient to prove causation and require corroborative evidence.

While the associations between high circulating IGF-I concentrations and increased cancer risk have garnered a lot of attention, the neurologic effects of IGF should be considered, particularly pertaining to diabetes-induced learning/memory impairments and increased risk of dementia. Gasparini and Xu recently reviewed IGF-I and insulin as it related to the pathophysiology of Alzheimer's disease.<sup>2</sup> It appears that there may also be risks to having low IGF-I levels; IGF-I does more than promote somatic growth.

Adda Grimberg, MD

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## Beta Cell Capacity and Insulin Sensitivity in Prepubertal Children Born Small for Gestational Age

The association between intrauterine growth retardation (IUGR) and the development of type 2 diabetes mellitus (T2DM) in adulthood has been demonstrated in several studies. Veening et al studied beta cell capacity and insulin sensitivity in 28 children born small for gestational age (SGA) and 22 children born appropriate for gestational age (AGA). All were Caucasian, born at term, and pre-pubertal (mean age 9.1 and 9.0 years, respectively). Insulin sensitivity was determined using a hyperinsulinemic-euglycemic clamp, while beta cell capacity was determined using a hyperglycemic clamp combined with arginine infusion. Anthropometric studies were obtained and relationships between catch-up growth, change in BMI, and clamp findings were determined.

Family history of T2DM and hypertension was not different between the two groups and at the time of the studies, mean actual length and BMI were similar in both groups. Insulin sensitivity was significantly lower in the SGA group. However, arginine-stimulated insulin secretion, a measure of beta cell capacity, was similar in both groups. Changes in BMI values between 0 and 1 year, 0 and 2 years, and 2 to 9 years, were categorized into tertiles. In SGA children, insulin sensitivity was significantly lower in those with the highest BMI change between years 2 to 9, compared to those with the smallest BMI change. Insulin secretion was significantly higher in SGA children with the highest BMI change in years 2 to 9, compared to those with the lowest BMI change during those years. No similar changes were seen among the responses in the AGA children.

The authors conclude that insulin sensitivity, but not beta cell capacity, is reduced in children born SGA. Thus, insulin sensitivity is the primary effect promoting the

development of T2DM in later life. But studies have shown that insulin resistance is not by itself sufficient to cause T2DM. SGA children whose BMI was greater during childhood had more insulin resistance. Thus, being overweight is clearly an important factor in the insulin resistance of SGA children and adults. The authors suggest that SGA children with excessive gain in BMI after the second year of life should be screened for the development of T2DM and associated cardiovascular risk factors.

Veening MA, et al. *Diabetes* 2003;52:1756-1760.

**Editor's Comment:** *This is an important paper. These investigators have performed complex studies in a large group of SGA and AGA children and showed that insulin sensitivity rather than beta cell capacity is abnormal in the SGA children. Since we know that the risk for T2DM is increased among adults who were born SGA, and we know that T2DM requires both insulin resistance and reduced beta cell capacity, this paper implies that reduced beta cell capacity must occur later than 9 years of age. Whether reduced capacity occurs at a later age or is related in some way to increasing BMI remains to be demonstrated. The findings with regard to BMI tertiles support the need for weight control among these individuals. What role exogenous GH administration will pay in this complex metabolic process also remains to be seen. It is clearly very important that careful metabolic studies be performed in children born SGA before and during treatment with exogenous GH. Such studies should be an important part of every database that records the effects of such treatment with these children.*

William L. Clarke, MD

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## CLINICAL FEATURES IN SHOX HAPLOINSUFFICIENCY: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

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### INTRODUCTION

The distal end of Xp and Yp is composed of 2.6 Mb DNA sequences that are identical between the X and the Y chromosome.<sup>1</sup> This particular region is named the short arm pseudoautosomal region (PAR1), where the X and the Y chromosomes recombine during male meiosis.<sup>1</sup> Since Xp terminal deletions invariably result in short stature irrespective of the breakpoints,<sup>2</sup> and small Yp terminal deletions lead to short stature,<sup>3</sup> it has been suggested that a growth gene escaping X-inactivation resides in the PAR1, and that haploinsufficiency of the growth gene causes short stature in both sexes as a dominant phenotype.<sup>2</sup>

In 1997, Rao et al.<sup>4</sup> successfully cloned a novel gene at the position roughly 500 kb from the Xp/Yp telomere, and named it SHOX for short stature homeobox containing gene. SHOX consists of 7 exons and produces 2 transcripts

### From The Editor's Desk

The miracle of the Internet has allowed the readership of *Growth, Genetics & Hormones* to grow very rapidly. We have recently added to our subscribers a substantial number of pediatric endocrinologists worldwide. Members of the Pediatric Endocrine Societies from Europe, Latin America, Colombia, and Japan who have email addresses will now be receiving *GGH* on an ongoing basis. It gives me great pleasure to welcome these pediatric endocrinologists to the family of *GGH*. Surely, our European, Latin American & Japanese contemporaries will help us broaden our perspectives and apprise us of advances in the field for publication in *GGH*. I am looking forward to contributions from our colleagues; an example of such is the lead article in this issue.

This second issue of 2004 contains a review of the clinical features of the short stature homeobox gene, so called SHOX. This important factor is implicated in the etiology of short stature and, in particular, features that characterize patients with this abnormality. This paper addresses a complicated subject, presents it in a clear easy-to-read manner, and brings the state of the art in the field to the readers of *GGH*. Drs. Tsutomu Ogata and Maki Fukami from Tokyo, Japan authored this lead article, emphasizing aspects of particular interest to pediatric endocrinologists and geneticists. The authors deserve our congratulations and thanks for their erudite writing.

This issue also contains abstracts of recent articles published in the literature that were considered of importance by our editorial board; each article is reviewed with editorial comments. Unfortunately, we have limited space and cannot publish all articles of importance in the field, nor do we attempt to do so. We limit our scope to bring value by publishing only articles that attract the interest of the editorial board and that meet our editorial standards. The high value that *GGH* has received from the readership indicates we have met our objectives, and we want to surpass them. The report of the December 2003 survey is posted at [www.GGHjournal.com](http://www.GGHjournal.com) (click on survey results). We appreciate your comments so we may continue to serve your needs.

Fima Lifshitz, MD  
Editor-in-Chief

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generated by alternative splicing of its 3' exons (SHOXa and SHOXb). SHOXa and SHOXb proteins consist of 292 and 225 amino acids, respectively. SHOXa appears to have a major biological function, although it remains to be determined whether SHOXb has some biological role.<sup>5</sup> SHOX is expressed from an inactive X chromosome as well as an active X and a normal Y chromosome, indicating that SHOX exerts the dosage effect in sex chromosome aberrations.<sup>4</sup> Furthermore, expression analysis in human embryos has shown that SHOX is exclusively expressed in the developing distal limbs and in the first and second pharyngeal arches where Turner skeletal features are observed postnatally.<sup>6</sup>

Extensive clinical and molecular studies have demonstrated that SHOX haploinsufficiency is implicated in 2% of short stature individuals and is the predominant factor in Turner skeletal features and Léri-Weill dyschondrosteosis (LWD) characterized by Madelung deformity (shortening and bowing of the radius with dorsal subluxation of the distal ulna and partial foreleg anomalies).<sup>7</sup> In this paper, we summarize current knowledge about SHOX haploinsufficiency.

**Table 1. Skeletal features in the distal limb region in 36 patients with SHOX haploinsufficiency**

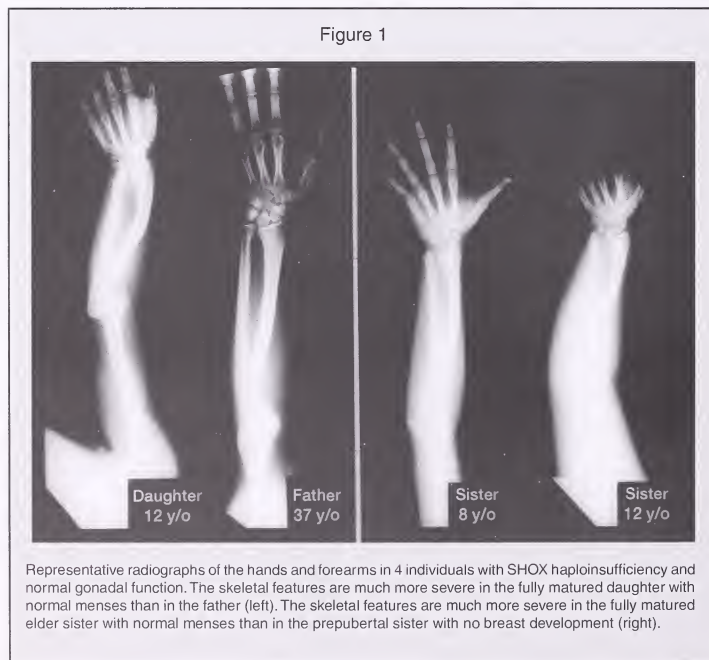
| Short metacarpals and/or cubitus valgus | Madelung deformity and/or mesomelia | Prepubertal boy | Pubertal to adult male | Prepubertal girl | Pubertal to adult female |
|---|-------------------------------------|-----------------|------------------------|------------------|--------------------------|
| No                                      | No                                  | 1               | 3                      | 2                | 0                        |
| Yes                                     | No                                  | 0               | 0                      | 0                | 1                        |
| No                                      | Yes                                 | 0               | 1                      | 2                | 5                        |
| Yes                                     | Yes                                 | 1               | 1                      | 6                | 13                       |

The actual number of patients is shown.

## LIMB SKELETAL FEATURES

Intragenic SHOX mutations, or pseudoautosomal microdeletions involving SHOX as the sole disease gene, have been identified in a large number of patients with normal karyotype and normal gonadal function<sup>7</sup> (also, Ogata, unpublished data). Skeletal features in the distal limb region of such individuals are classified into 4 groups on the basis of the combination of short 4th metacarpals and/or cubitus valgus which appears in 40-50% of Turner females, and Madelung deformity and/or mesomelia characteristic of LWD occurs in only approximately 7% of Turner females.<sup>8</sup> The prevalence of these features in 36 Japanese short-stature patients is shown in Table 1 (for representative roentgenograms, see Figure 1 in reference 7). These data indicated that SHOX haploinsufficiency is implicated in short stature and in the limb skeletal features of Turner and LWD patients.

Most people with SHOX haploinsufficiency have LWD features of variable extent, although there may be an ascertainment bias since patients with LWD are preferentially identified. In addition, genu valgum and relatively short lower limbs were clinically discernible in most patients with overt LWD, and tibial or fibular exostosis was occasionally detected. For the pseudoautosomal microdeletions in the telomeric part of Xp/Yp, no other features have been identified, suggesting that haploinsufficiency of pseudoautosomal genes other than SHOX has no clinical effects.<sup>9</sup>



SHOX haploinsufficiency also occurs in cytogenetically discernible Xp or Yp terminal deletions. In this context,

distal limb skeletal features in 43 female patients with various types of Xp deletions involving SHOX have been summarized as follows<sup>10</sup>: (1) the prevalence of the wrist abnormality suggestive of mild Madelung deformity was significantly higher in females with spontaneous puberty than in those without spontaneous puberty; (2) the severe Madelung deformity, often detected in pubertal or adult females with normal karyotype, was not identified in these patients; and (3) the prevalence of short metacarpals and cubitus valgus was similar in females with and without spontaneous puberty (Table 2).

### Effect of Gonadal Estrogens

Limb skeletal features are more severe in females than in males, and become overt with puberty in patients with normal karyotype (Figure 1). The so-called idiopathic short-stature phenotype was predominantly exhibited by male patients and prepubertal girls, and LWD was predominantly manifested by pubertal and adult female patients (Table 1). In this context, two matters are noteworthy. First, SHOX appears to function as a repressor of growth plate fusion and skeletal maturation in the distal limb region, so that SHOX haploinsufficiency results in premature growth plate fusion and relatively advanced skeletal maturation in that region.<sup>11,12</sup> Second, skeletal maturation in normal individuals is primarily caused by gonadal estrogens—which increase with puberty—serum estrogen levels being higher in females than in males.<sup>13</sup> Thus, it is likely that gonadal estrogens exert a maturational effect on skeletal tissues that are susceptible to unbalanced premature growth plate fusion, facilitating the development of skeletal lesions in a female-dominant and in a pubertal tempo-influenced fashion.<sup>7,13</sup> Furthermore, the tempo of pubertal development may also play an important role in the development of skeletal features. Severe forms manifested in early maturing girls who are exposed to gonadal estrogens from a relatively early age.<sup>13</sup> This may also account for the sex differences in the severity of skeletal features in SHOX haploinsufficiency, because females enter puberty approximately 2 years earlier than males. Thus, it is inferred that, in SHOX haploinsufficiency, the amount and tempo of gonadal estrogen production in females usually cause LWD, whereas those in males usually lead to the so-called idiopathic short-stature phenotype.

This notion is consistent with the findings in female patients with cytogenetically recognizable Xp deletions. The wrist abnormality is predominantly manifested by females with spontaneous puberty, and the absence of severe Madelung deformity is compatible with a relatively small amount and slow tempo of gonadal estrogen production.<sup>9</sup> Furthermore, this idea is applicable to Turner syndrome as well: (1) the prevalence of Madelung deformity is only approximately 7%,<sup>8</sup> and this relatively low prevalence would be explained by the compromised gonadal estrogen of these patients (Table 3); (2) the

**Table 2. The prevalence of Turner skeletal features in 43 patients with Xp deletions involving SHOX**

|                       | Lymphogenic gene(s)† |         | Spontaneous puberty |          |
|-----------------------|----------------------|---------|---------------------|----------|
|                       | Preserved            | Deleted | Positive            | Negative |
| Distal limb region‡   | 11/19                | 19/24   | *17/20              | *13/23   |
| Short metacarpals     | 7/19                 | 12/24   | 12/20               | 7/23     |
| Cubitus valgus        | 9/19                 | 18/24   | 15/20               | 12/23    |
| Wrist abnormality#    | *8/18                | *2/23   | 19/18               | 11/23    |
| Faciocervical region‡ | 4/19                 | 10/24   | 6/20                | 8/23     |
| High arched palate    | 3/18                 | 8/23    | 4/18                | 7/23     |
| Short neck            | *1/19                | *7/24   | 4/20                | 4/23     |

Denominators indicate the number of patients searched for each feature.

\*  $P < 0.05$  and † $P < 0.01$  by the Fisher's exact probability test.

‡ The ratio of patients with at least one feature of each category.

# Presence of at least one of the following features: decreased carpal angle, metaphyseal lucency and/or epiphyseal hypoplasia at the ulnar side of the distal radius, and angulation of the distal radius and/or ulna.

† Lymphogenic gene indicates the gene involved in lymphatic development located between DMD and MAOA on Xp.

prevalence of spontaneous genital bleeding in Turner syndrome patients is higher than that of Madelung deformity (15%–20% vs 7.5%),<sup>8,14,15</sup> which could be ascribed to relatively small amounts and slow tempo of gonadal estrogen production<sup>14,15</sup>; and (3) estrogen treatment in Turner syndrome does not increase the prevalence of Madelung deformity because this therapy is usually started in late teens with a low dosage and for short periods.<sup>16</sup>

Of the skeletal features in the distal limb region, short metacarpals and cubitus valgus are frequently exhibited in those with Turner syndrome who have gonadal estrogen deficiency. These remain relatively infrequent in patients with SHOX haploinsufficiency who have normal gonadal function (Table 3). This may imply that such skeletal features are also caused by additional factors other than gonadal estrogens. One possibility would be a compressive effect of peripheral lymphedema resulting from haploinsufficiency of the lymphogenic gene (for lymphogenic gene, see below).<sup>17</sup> In support of this, Noonan syndrome patients often have such skeletal features in the presence of peripheral lymphatic malformation.<sup>18,19</sup>

### FACIOCERVICAL SKELETAL FEATURES

Faciocervical skeletal features are occasionally manifested in patients with SHOX haploinsufficiency and normal karyotype (Table 3). In addition, short neck has been described in German subjects.<sup>6,13</sup> This suggests that SHOX haploinsufficiency may also be relevant to the skeletal features in the faciocervical region of Turner syndrome patients. However, the prevalence of these features is apparently lower than that of limb skeletal features in patients with normal karyotype. Furthermore, the prevalence of faciocervical skeletal features is apparently lower in patients with normal

karyotype than in patients with Turner syndrome (Table 3). In particular, micrognathia occurs in approximately 60% of Turner syndrome females, but is rare in patients with normal karyotype.

In contrast, faciocervical skeletal features are frequently exhibited by females with Xp deletions involving SHOX. In this respect, the data are summarized as follows<sup>9</sup>: (1) the prevalence, especially that of short neck, is higher in females with large Xp deletions presumably missing the putative lymphogenic gene (see below) than in those with small Xp deletions presumably preserving that gene; and (2) the prevalence of high-arched palate is similar among females with and without spontaneous puberty, as is that of short neck (Table 2).

### Relevance of the Lymphogenic Gene

Turner syndrome is associated with lymphatic hypoplasia.<sup>20,21</sup> This postulates that a lymphogenic gene escaping X-inactivation may be shared by the X and the Y chromosome, and that haploinsufficiency of the gene results in lymphatic hypoplasia as a dominant phenotype. The lymphogenic gene has been mapped to an approximate 9-Mb region between DMD and MAOA on Xp and to an approximate 4-Mb region between PABY and DYS255 on Yp, by genotype-phenotype correlations.<sup>9,22</sup>

Lymphatic hypoplasia leads to lymph fluid stasis, resulting in distension of the main and tributary lymphatic ducts and in lymphedema. Thus, a mechanical force would be exerted on tissues and organs adjacent to the lymphatic system. It is hypothesized that soft tissue and visceral stigmata are deformational consequences caused by the mechanical force of distended lymphatics and lymphedema.<sup>17</sup> Indeed, it appears reasonable to assume that a distended cervical lymphatic system (cystic hygroma) leads to nuchal region anomalies such as webbed neck and low posterior hairline, and that peripheral lymphedema results in acral region anomalies such as puffy hands and feet and redundant skin.<sup>17,23</sup> It also appears reasonable to postulate that cystic hygroma and distended thoracic and para-aortic lymphatic ducts compress the aortic arch and alter the cardiac hemodynamics, leading to cardiovascular anomalies such as aortic coarctation, and that distended

retroabdominal and iliac lymphatic ducts inhibit normal upward migration and rotation of the kidney, leading to renal malformations such as horseshoe kidney.<sup>17,24,25</sup> It is notable that visceral anomalies in Turner syndrome are limited to the organs in the vicinity of the main lymphatics. Thus, characteristic soft tissue and visceral anomalies can be regarded as the result of a malformation sequence initiated by lymphatic hypoplasia.<sup>17</sup>

By analogy, it is inferred that cystic hygroma and facial edema exert a compressive effect on the developing faciocervical skeletal tissues primarily in the fetal life, facilitating the development of faciocervical skeletal features of SHOX haploinsufficiency. This notion implies that haploinsufficiency of the lymphogenic gene, rather than gonadal estrogens, is relevant to the development of faciocervical skeletal features. This hypothesis explains the difference in the prevalence of faciocervical skeletal features between patients with Turner syndrome and those with SHOX haploinsufficiency (Table 3). Furthermore, since lymphatic distension occurs in the peripheral areas including distal limb regions, this would contribute to the development of cubitus valgus and short metacarpals in Turner syndrome (Table 2).<sup>19</sup>

## GROWTH PATTERNS

### Patients With Normal Karyotype

Patients without overt LWD usually grow along the -2 SD growth curve throughout the growth period (Figure 2a).<sup>13</sup> The magnitude of the height deficit is compatible with the previous estimation that loss of SHOX decreases the adult height by about 12 cm in the absence of overt LWD.<sup>26</sup> This difference in size approximates the magnitude of 2 SD of the adult height in the normal population. This implies that SHOX haploinsufficiency leads to short stature (<2 SD) in roughly half of patients without LWD (approximately 50% of penetrance). Indeed, normal stature has been described in several patients with SHOX haploinsufficiency.<sup>13</sup> In this regard, since normal height has been observed in patients born to tall parents,<sup>13</sup> this implies that statureal growth in patients with SHOX haploinsufficiency is influenced by original height potential as represented by the parental height, as has been reported in Turner syndrome.<sup>27</sup> It

remains to be clarified, however, how SHOX haploinsufficiency causes the idiopathic short-stature phenotype.

**Table 3. The prevalence of skeletal features**

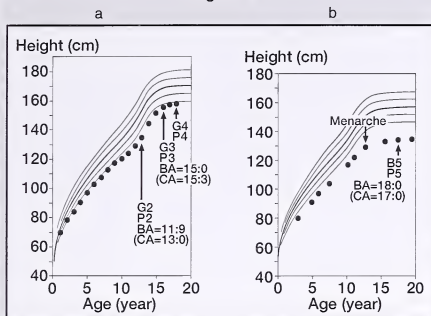
|                                   | SHOX haploinsufficiency<br>(normal karyotype) | Turner syndrome<br>(abnormal karyotype) |
|-----------------------------------|---|---|
| Distal limb region                |   |   |
| Short metacarpals, cubitus valgus | 18%   | 40%                                     |
| Madelung deformity, mesomelia     | 75%   | 7%                                      |
| Faciocervical region              |   |   |
| Short neck                        | 5%  | 40%                                     |
| High arched palate                | 8%  | 35%                                     |
| Micrognathia                      | 0%  | 60%                                     |

Adopted from ref. 7, 8, and unpublished observations.

Patients with overt LWD usually grow along the -2 SD growth curve before puberty, and show definite downward growth shift with puberty (Figure 2b).<sup>13</sup> This type of growth pattern could be



Figure 2



Growth charts in patients with proven SHOX haploinsufficiency and normal gonadal function. The actual heights are plotted on the sex-matched standard longitudinal growth curves (the mean,  $\pm 1$  SD, and  $\pm 2$  SD) for Japanese children. Pubertal stage is indicated according to the classification of Tanner (G: genitalia; B: breast; P: pubic hair). Bone ages (BAs) are given, together with chronological ages (CAs) at the time of BA determination. A boy without recognizable skeletal abnormalities (left), and a girl with short 4th metacarpals, cubitus valgus, and dyschondrosteosis characterized by Madelung deformity (right).

explained by assuming that prepubertal growth is relatively well preserved because of dormant gonadal function, whereas pubertal growth is compromised because of production of gonadal estrogens which facilitate growth plate fusion.<sup>13</sup> The severely affected final height suggests that SHOX haploinsufficiency causes short adult height ( $< -2$  SD) in most patients with overt LWD (probably approximately 70% of penetrance). Furthermore, consistent with the SHOX expression pattern, the longitudinal growth study of patients with SHOX haploinsufficiency and normal gonadal function showed that sitting height was fairly stable throughout the growth period, whereas leg length and arm span were compromised during puberty, thereby worsening mesomelic short stature.<sup>28</sup>

### Patients With Turner Syndrome

SHOX haploinsufficiency alone is unlikely to explain the growth failure and the growth pattern of 45,X Turner syndrome.<sup>2,13</sup> In 45,X Turner syndrome, the mean adult height is about  $-3.2$  SD below the mean of normal females, and the linear growth is associated with a reduced growth rate beginning in early childhood, in the absence of discernible LWD.<sup>2,29</sup> It is noteworthy that 45,X is associated with a gross chromosome imbalance, which has been suggested to result in global developmental defects, including growth failure.<sup>30,31</sup> Although 45,X Turner syndrome females usually have gonadal dysgenesis, gonadal estrogen deficiency is unlikely to influence adult height or childhood growth patterns.<sup>2</sup> Thus, the remaining

growth deficit and the reduced growth rate from early childhood in 45,X Turner syndrome appears to be due to chromosomal imbalance.

One may argue that severe short stature in Turner syndrome is contributed by loss of another growth gene(s) escaping X-inactivation. However, such a growth gene(s) other than SHOX is unlikely to exist on the X chromosome [for details, see Reference 2], although the possibility that a growth gene(s) escaping X-inactivation might exist on Xp has not been excluded.<sup>2,32</sup> The adult height is similar between apparently non-mosaic Caucasian females with 45,X, those with 46,X,del(X)(p11), and those with 46,X,i(Xq);<sup>2</sup> this argues against the presence of a growth gene escaping X-inactivation on Xq.<sup>11</sup> Thus, the shorter mean adult height in patients with larger Xq deletions than in those with small Xq deletions is inexplicable without assuming the growth disadvantage of a chromosomal imbalance.<sup>2</sup> Similarly, the shorter mean adult height in patients with larger Xp deletions than in those with small Xp deletions would also be ascribed to the growth disadvantage of a chromosomal imbalance, rather than to loss of a growth gene on Xp escaping X-inactivation.<sup>2</sup> In addition, short stature in apparently non-mosaic Caucasian females with 46,X,idi(Xp) missing SHOX suggests that a growth gene escaping X-inactivation is absent from most of Xp.<sup>2</sup>

### DIAGNOSTIC IMPLICATIONS

#### Prevalence

The prevalence of SHOX haploinsufficiency has been estimated to be approximately 2% in individuals with normal karyotype with the so-called idiopathic short-stature phenotype ( $< -2$  SD).<sup>4,33,34</sup> However, re-examination of such patients has frequently disclosed mild skeletal abnormalities such as decreased carpal angle, angulation of distal radius, tubular bone alterations, and brachymetacarpia.<sup>35,36</sup> Furthermore, the prevalence should be different between sexes and ages, since normal skeletal features are predominantly exhibited in males of various ages and in prepubertal girls.<sup>7,13</sup> Thus, further studies are necessary to estimate the sex- and age-specific prevalence of these alterations in the so-called idiopathic short-stature phenotype.

The prevalence of SHOX haploinsufficiency is 80% to 90% in patients with normal karyotype and LWD (reviewed in Reference 7), with the lowest value of 60%<sup>37</sup> and the highest value of 100%.<sup>38</sup> Although SHOX haploinsufficiency remains undetected in a small fraction of patients with LWD, it is unknown at this time whether LWD is a genetically heterozygous condition caused by a hitherto unknown autosomal gene(s), or if SHOX mutations reside in the unexamined regions, such as the promoter and enhancer sequences.



In contrast to LWD, SHOX haploinsufficiency is rarely found in normal karyotype patients with short metacarpals and/or cubitus valgus, but without LWD phenotype. To date, only 1 female has been identified with this pattern of phenotype.<sup>13</sup> This would not be surprising, however, because short metacarpals and cubitus valgus appear to be highly heterogeneous conditions and could occur as a normal variant phenotype.

### Clinical Indications

Phenotypic assessment in SHOX haploinsufficiency provides useful clues for the selection of normal karyotype patients to be studied for this condition. First, patients with LWD phenotype should be sought. In this context, of practical importance is to recognize the signs of Madelung deformity on hand and wrist radiographs that are almost invariably obtained for the bone age evaluation in children with short stature. For this purpose, it is recommended to carefully observe the signs of Madelung deformity, such as metaphyseal lucency and epiphyseal hypoplasia at the ulnar border of the distal radius, decreased carpal angle, angulation of the distal radius and ulna, and subluxation of the distal ulna.<sup>7,39,40</sup> In our experience, the first signs of Madelung deformity are often exhibited by metaphyseal lucency and epiphyseal hypoplasia of the medial side of the distal radius in prepubertal patients, as well as by decreased carpal angle in pubertal or adult patients (Figure 3). When such findings are suspected, radiographs of the distal limbs should be obtained in order to search for characteristic features such as radial curvature and shortening. Second, SHOX haploinsufficiency should also be considered for patients with mesomelic short stature, which becomes evident in puberty. Third, familial members of a proband with SHOX haploinsufficiency should be studied irrespective of clinical phenotype. Indeed, familial studies have identified SHOX haploinsufficiency in subjects—especially males—with low-normal height alone.<sup>13</sup>

### Molecular Diagnosis

Molecular studies are necessary to identify SHOX haploinsufficiency, especially in patients with normal karyotype. In this context, it is noteworthy that microdeletions involving SHOX are much more prevalent than intragenic SHOX mutations.<sup>7</sup> The high prevalence of microdeletions would be consistent with repetitive sequences such as subtelomeric interspersed repeats being abundantly present around SHOX,<sup>41</sup> because an unequal crossing over between homologous chromosomes or an intrachromosomal recombination is prone to occur in such a region.

Figure 3



Early and mild signs of Madelung deformity. Metaphyseal lucency and mild epiphyseal hypoplasia of the medial side of the distal radius in a 9½-year-old boy (left), and decreased carpal angle, in addition to angulation of the distal radius and epiphyseal hypoplasia of the medial side of the distal radius, in a 15-year-old girl (right).

Thus, it is recommended to search for a SHOX deletion first, and when SHOX deletion is excluded, an intragenic mutation should be investigated. For SHOX deletion analysis, fluorescence in situ hybridization is recommended because it unequivocally shows the presence or absence of SHOX. Microsatellite analysis for the CA repeat marker at the 3' region of SHOX is also useful because of its high heterozygosity (>90% in our experience), although parental DNA is necessary to confirm SHOX deletion. For SHOX mutational analysis, sequence analysis is essential. In this respect, denaturing high performance liquid chromatography analysis serves as a rapid and reliable screening method.

### THERAPEUTIC IMPLICATIONS

#### Growth Hormone

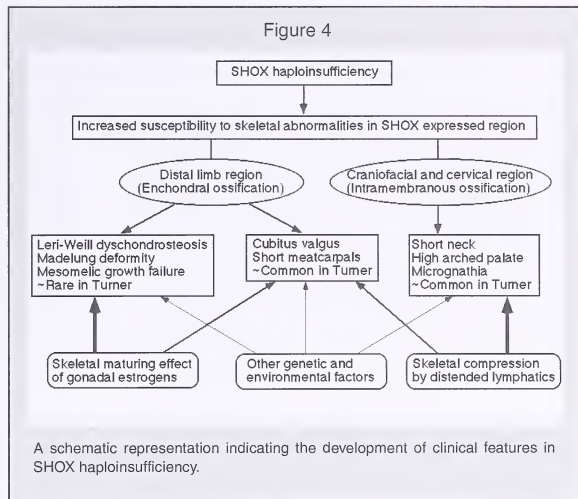
Growth hormone (GH) therapy may be advantageous in SHOX haploinsufficiency because it is effective in Turner syndrome, despite the absence of GH deficiency. Indeed, beneficial short-term effects have been reported in several patients.<sup>33,42,43</sup> However, GH therapy might facilitate the development of skeletal anomalies by accelerating distorted skeletal growth resulting from unbalanced premature fusion, or by stimulating gonadal development and resultant estrogen production.<sup>42</sup> Therefore, careful follow-up is required for GH therapy in SHOX haploinsufficiency.

#### Gonadotropin-Releasing Hormone Analog

Gonadotropin-releasing hormone analog (GnRHa) therapy is expected to serve as prevention or mitigation of the development of skeletal features by suppressing gonadal estrogen production.<sup>42</sup> However, GnRHa therapy has performed poorly in SHOX haploinsufficiency, so that the adequate timing to start and stop the GnRHa therapy is unknown. At present, it may be recommended to attempt GnRHa treatment in an experimental protocol for early maturing girls or in patients with early signs of Madelung deformity, possibly in combination with GH.

## SUMMARY AND SPECULATION

Clinical studies have indicated that SHOX haploinsufficiency is responsible for not only short stature but also Turner syndrome skeletal features and LWD. The expressivity of SHOX haploinsufficiency in the limb and faciocervical regions is primarily influenced by gonadal function status and the presence or absence of the lymphogenic gene, respectively (Figure 4). In this context, although phenotypic spectrum in diseases resulting from haploinsufficiency of transcription factor genes is known to range widely from nearly normal to severely affected phenotypes<sup>44</sup> (a list of haploinsufficiency is given in Reference 45), the underlying factor(s) for clinical diversity remains unknown in nearly all such diseases. Thus, SHOX appears to be the first gene in which modifying factors for haploinsufficient status have been identified.



Finally, two points should be made with respect to SHOX. First, a gene(s) that controls SHOX expression is unknown, as is that controlled by SHOX. Identification of such upstream and downstream genes should serve to facilitate an understanding of the molecular network of human growth. Second, it has been shown that SHOX overdose in association with gonadal dysgenesis constitutes a novel clinical entity leading to tall stature at pubertal age in normal children.<sup>7</sup> Because gonadal estrogen production can be suppressed by GnRHa therapy, this may argue for the possibility of a SHOX gene therapy in patients with growth failure. Further accumulation of clinical and molecular data will provide better clues for the diagnosis and management of SHOX haploinsufficiency.

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## LETTER TO THE EDITOR

## Sexual Outlook for Post-Surgical Ambiguous Genitalia Patients

The December 2003 edition of *Growth, Genetics & Hormones* (Vol. 19, No. 4) abstracted, "The effects of clitoral surgery on sexual outcome in individuals who have intersex conditions with ambiguous genitalia: a cross-sectional survey."<sup>1</sup> This was a postal survey of the sexual function of 39 adults born with ambiguous genitalia, reared as girls. Those who had undergone clitoral surgery reported more sexual difficulties than those without surgery. The authors concluded, "Adult sexual function could be compromised by feminizing genital surgery." An editorial comment emphasized this finding, "The challenge is to devise a corrective procedure that does not do so." The implication is that such a procedure will be surgical in nature.

We wonder whether determination of a satisfying sexual outcome is more complex than this. Both surgical and non-surgical groups reported significantly more problems in several sexual domains than the general population sample. Sexual dysfunctions (particularly sexual aversion disorder and sexual pain disorders) are more common in women who have had genital surgery.<sup>2</sup> Surgical damage to the autonomic pelvic network may lead to iatrogenic sexual dysfunctions.<sup>3</sup> However, Bancroft et al<sup>4</sup> reported that physical aspects of sexual response in women (including arousal and orgasm) were poor statistical predictors of sexual satisfaction: "The best predictors of sexual distress were markers of general emotional well-being," though other variables such as mood, body satisfaction, sexual knowledge, and confidence may also play a role.<sup>1</sup> The authors acknowledge that the poor sexual outlook for intersexed adults may be related to psychological factors as much as to surgical sequelae.

In an accompanying commentary, Slijper<sup>5</sup> comments on some of the psychological factors that modulate sexual functioning, including "sexual shyness" (which could be caused by dissatisfaction with the appearance of the genitals) and "gender behavior" (referring to masculine behavior in XY children assigned female sex). She advocates counseling prior to the onset of puberty to reduce the impact of these factors as a treatment strategy to enhance sexual satisfaction through comfort with one's body and gender assignment. Slijper's authority derives from her membership in a "Gender Team" at a children's hospital in the Netherlands. Unfortunately, there are very few centers such as this, where patients born with disorders of sexual differentiation will receive specialized psychoendocrine treatment. It is possible that such an integrated approach (medical, surgical, and psychological) will result in more positive outcomes for these individuals.

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**First Editor's Comment:** This writer strongly believes that it is inappropriate to rear genotypic and potentially fertile girls with ambiguous or fully masculinized external genitalia as males under most circumstances. The neonate does not exist in isolation but as a member of a family in which the birth of an infant with ambiguous genitalia causes unimaginable stress that can be alleviated only to a modest extent by education, conversation, and reassurance. In most families, corrective genital surgery to conform with the selected sex of rearing is desired as soon as possible. Comparing (admittedly anecdotal and by personal experience) family outcomes in the decades when clitoral surgery in girls with ambiguous genitalia was performed between 2-5 years of age and the current practice of clitoral recession within the neonatal period, far more marital conflicts (spousal abuse, separation, divorce) arose with the delayed rather than early clitoral surgery. Recognizing that corrective genital surgery will be undertaken on the majority of girls with ambiguous genitalia, development of a surgical procedure that will both normalize genital appearance and maintain genital sensation is ideal. Certainly, long-term monitoring and counseling of the family and patient is highly desirable if the facility and personnel to do so are readily available and freely accessible. I agree that we have learned over the past several decades that the most important sex organ is the brain and that normalization of genitalia will not ensure "sexual satisfaction"; even in the normal female population there appears to be a surprising degree of sexual dissatisfaction – a driving force behind the pharmaceutical industry's effort to develop a "female sildenafil".

Allen W. Root, MD

**Second Editor's Comment:** Thanks to Drs. Williams and Sandberg for their comments. The Minto article raises so many important questions and they add two additional issues: (1) should surgery be avoided where possible and (2) how useful are "gender teams"? Clearly, methods and approaches, both surgical and psychological, have changed over the years. Those working in the field will continue to collect experience and share their insights through collaborations and multi-center trials. In an era of "the informed consumer", there does not yet seem to be a perfect approach—but as much information and openness as possible will help parents, families, and affected individuals make decisions that seem right for them.

Judith G. Hall, OC, MD



## ABSTRACTS FROM THE LITERATURE

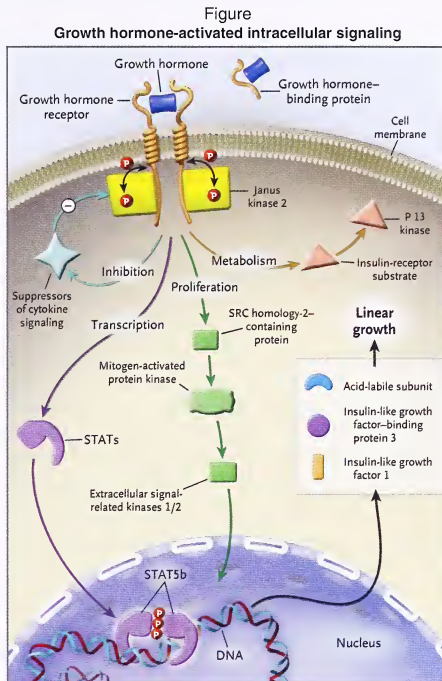
## STATs Role in Growth Hormone Insensitivity

Kofoed and colleagues described a patient with a homozygous mutation in the gene for STAT5b, resulting in growth hormone insensitivity. The girl had abnormal postnatal growth, facial dysmorphism, elevated GH levels after insulin-arginine stimulation, and markedly reduced serum concentrations of IGF-1, IGFBP-3 and acid labile-subunit. Serum concentrations of these proteins remained abnormally low despite 7 days of treatment with GH, and the growth rate failed to increase in response to one year of treatment. Concentrations of GHBP were normal, reflecting the fact that her GHR gene and protein were normal. This patient had no family history of growth retardation, though the parents were first cousins.

Kofoed EM, Hwa V, Little B, et al. Growth Hormone Insensitivity Associated with STAT5b Mutation. *N Engl J Med* 2003;349:1139-1147.

**Editor's Comment:** The authors described a novel mechanism for impaired growth in a patient with severe short stature. This was clearly demonstrated by sophisticated analysis. The finding of elevated serum GH levels after stimulatory tests, in conjunction with low levels of IGF-1 and IGFBP-3, established the presence of GH resistance. Unlike patients with the classic Laron syndrome, this patient had normal levels of GHBP, indicating that the defect was distal to the extra cellular GH receptor domain. A homozygous missense mutation in the STAT5b gene resulted in loss of GH action due to a post-receptor abnormality in the GH-signaling cascade. The GH-activated intracellular signaling involves several steps and, theoretically, each one of these steps could fail and produce GH insensitivity. The role of STATs in stature was reviewed in an accompanying editorial.<sup>1</sup> Eugster and Pescovitz depicted the GH-activated intracellular signaling cascade which is reproduced here. (Figure).

Fima Lifshitz, MD



Phosphorylation of the growth hormone receptor is followed by the activation of the metabolic, proliferative, and transcriptional pathways. STAT5b stimulates the transcription of factors (shown in the box) that are critical for normal linear growth. P denotes phsophorylation, and STAT signal transducer and activator of transcription.

Reprinted with permission from Eugster EA, Pescovitz OH. New revelations about the role of STATs in short stature. *N Engl J Med* 2003;349:1110-1112. Copyright © 2003 Massachusetts Medical Society. All rights reserved

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## IGF-1 Receptor Mutations in Intrauterine Growth Retardation

In a cohort of 41 children with intrauterine growth retardation (IUGR) and sustained postnatal growth retardation, the investigators detected 1 female subject (birth weight  $-3.5$  SDS; adult height  $-4.5$  SDS; exaggerated spontaneous and stimulated growth hormone [GH] secretion; normal to elevated IGF-1 concentrations; adult height apparently unresponsive to therapy with GH) who was a compound heterozygote for loss-of-function missense mutations in the gene encoding

the receptor for insulin-like growth factor 1 (IGF1R). Both parents were heterozygous for different mutations in exon 2 of IGF1R (mother, Lys115Asp; father, Arg108Gln). The birth weights and adult heights of both parents were modestly impaired (mother's birth weight  $-2.0$  SDS; adult height  $-0.6$  SDS; father's birth weight  $-2.0$  SDS; adult height  $-2.8$  SDS). The mutations in exon 2 markedly reduced IGF-1 binding and decreased sensitivity to this growth factor, hence limiting intracellular signal transduction.



A heterozygous nonsense mutation (exon 2: Arg59Stop) resulting in a truncated product was identified in 1 (male) of 9 children studied with short stature (height SDS —3.8 SDS at chronologic age 14 months) and elevated serum concentrations of IGF-1 who also had IUGR (birth weight —3.0 SDS, birth length —4.6 SDS). A similar mutation was present in the mother (birth weight —2.4 SDS; adult height —2.6 SDS) and brother. The mutation led to decreased numbers of *IGF1R* expressed on the plasma membrane of the patient's cultured fibroblasts and presumably to decreased sensitivity to ligand. The authors conclude that inactivating mutations of *IGF1R* are present in a small number of children with both IUGR and postnatal growth retardation, particularly in those with an elevated serum concentration of IGF-1.

Abuzzahab MJ, Schneider A, Goddard A, et al. IGF-1 receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med*. 2003;349:2211-2222.

**First Editor's Comment:** This report documents another abnormality leading to GH non-responsive growth retardation—this in the gene encoding the IGF-1 receptor. There are now documented loss-of-function mutations in the genes encoding the receptor for GH-releasing hormone, GH, the GH receptor, an essential protein (STAT5) in the signal transduction system for GH, IGF-1, and the IGF-1 receptor; abnormalities in the *GHRHR* and *IGF1R* signal transduction systems likely exist as well. An interesting perspective by Rosenfeld<sup>1</sup> discussing factors that control growth accompanies this article.

Allen W. Root, MD

**Second Editor's Comment:** Abuzzahab and colleagues described 2 patients with IUGR and sustained post-natal growth failure due to *IGF1R* gene mutations. In a related paper published the same month, Okubo et al<sup>2</sup> described a girl with IUGR and sustained postnatal growth failure through 10 years of age, despite GH therapy due to a *de novo* terminal deletion of chromosome 15q26.1, which led to a single gene copy of *IGF1R*. *In vitro* studies with cultured fibroblasts from skin biopsy revealed: decreased cell proliferation in response to IGF-1, a reduced IGF-1-stimulated *IGF1R* tyrosine phosphorylation, and decreased [<sub>125</sub>I]IGF-1 binding sites per cell but normal IGF-1 binding affinity. The girl also had facial and musculoskeletal dysmorphisms, a single café-au-lait spot, cardiac anomalies (atrial septal defect and ventricular septal defect), and developmental delays with learning difficulties. Her chromosomal deletion was cytogenetically visible; thus, the girl's phenotype may be due, in part, to contiguous gene deletions beyond the *IGF1R*.

As an elegant counterpoint, Okubo and colleagues also described a boy with 3 copies of the *IGF1R* gene due to chromosomal translocation who had dysmorphic features and was large from birth on.<sup>2</sup> Thus, alterations in the *IGF1R* gene—either mutations or abnormal gene copy numbers—may significantly affect growth, both prenatally and postnatally.

Adda Grimberg, MD

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## Growth, Genetics & Hormones

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### Growth, Genetics & Hormones

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## Overlap Between Schmid Metaphyseal Chondrodysplasia and Cartilage-Hair Hypoplasia

Schmid metaphyseal chondrodysplasia (MCD) is characterized by short stature, bowed legs, coxa vara, metaphyseal changes on skeletal X-rays, and autosomal dominant inheritance. It is usually considered to be easily distinguished from the autosomal recessive cartilage-hair hypoplasia (CHH), in which the skeletal findings are typically more severe and frequently accompanied by hair abnormalities, defective immunity, and hematologic disturbances. However, as Ridanpää and colleagues report, this may not always be the case. Although many patients with Schmid MCD have heterozygous mutations of the *COL10A1* (the gene encoding the type X collagen chain), some do not. In the study of 32 patients with a clinical diagnosis of Schmid MCD reported in this paper, *COL10A1* mutations were identified in 12 patients. Even though they lacked the non-skeletal features of CHH, the 20 patients with no *COL10A1* mutations were screened for mutations of the *RMRP* gene, which encodes the non-translated RNA component of the RNase mitochondrial

RNA processing complex (RNase MRP) and which is mutated in patients with CHH.

Two of the Schmid MCD patients (both 5-year-old boys), one of Canadian descent, the other of French-Canadian descent, were found to be homozygous for a base substitution G for A at nucleotide 70 of *RMRP*. This is considered a worldwide "major" mutation for CHH. In one case, parents were found to be heterozygous for this mutation consistent with the recessive inheritance of CHH. Review of the clinical findings confirmed that both boys had normal hair, no excessive ligamentous laxity, and normal history of infections with normal immunological and hematological findings. One was the product of a consanguineous mating.

The authors also searched for mutations in another gene *H1RNA*, which encodes the RNA component of RNase P, which is structurally and functionally similar to RNase MRP. No mutations were identified. The authors concluded that these patients represent the mild end of the clinical spectrum of CHH, and caution that it should be considered in patients with clinical features of Schmid MCD in whom a *COL10A1* mutation can not be found, especially if there is no family history for bone dysplasia.

**Table**  
Clinical features in Schmid type of metaphyseal chondrodysplasia (MCDS) and cartilage-hair hypoplasia (CHH)

| Feature                        | MCDS               | CHH                 |
|--------------------------------|--------------------|---------------------|
| Neonatal onset                 | —                  | ++                  |
| Progressive                    | ++                 | ++                  |
| Disproportionate (short limbs) | ++                 | ++                  |
| Adult height                   | 130-145 cm         | 110-140 cm          |
| Bow legs                       | ++                 | +                   |
| Increased joint laxity         | —                  | +                   |
| Sparse hair                    | —                  | +                   |
| Immunodeficiency               | —                  | +                   |
| Anaemia and macrocytosis       | —                  | +                   |
| Increased risk of malignancy   | —                  | +                   |
| Hirschsprung disease           | —                  | +                   |
| Mode of inheritance            | Autosomal dominant | Autosomal recessive |
| Responsible gene               | <i>COL10A1</i>     | <i>RMRP</i>         |

++, always present; +, often present; —, absent.

Amended and reproduced with permission from the BMJ Publishing Group. Ridanpää M, et al. *J Med Genet* 2003;40:741-746.

Ridanpää M, Ward LM, Rockas S, et al. Genetic changes in the RNA components of RNase MRP and RNase P in Schmid metaphyseal chondrodysplasia. *J Med Genet*. 2003;40:741-746.

**Editor's Comment:** This paper provides another example of disorders that resemble each other clinically but have different genetic origins. It also underscores why identification of causative mutations is important. As the authors note, it is unknown if mild cases of CHH, such as those reported here, carry the same risk for complications such as skin and lymphoid cancers as more severely affected CHH patients. However, they may well since they carry the same *RMRP* mutation making surveillance for such complications an essential component of their care.

William A. Horton, MD

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## C-type Natriuretic Peptide and Achondroplasia

C-type natriuretic peptide (CNP) is a member of a family of 3 related peptides—atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and CNP. They act by inducing accumulation of intracellular cGMP through 2 subtypes of guanylyl cyclase: guanylyl cyclase A for ANP and BNP, and guanylyl cyclase B for CNP. Although the natriuretic peptides are known mainly for regulating the cardiovascular system, there is growing evidence that CNP is an important positive regulator of endochondral bone growth. For example, genetically engineered mice have short bones when null for CNP and long bones when CNP is overexpressed. In fact, growth plates in these mice are shortened and widened in a manner similar to that detected in mice with loss- and gain-of-function mutations for FGFR3, respectively. These observations led the group headed by Nakao to propose a functional relationship between CNP and FGF signaling in the growth plate, which they have now demonstrated by mouse genetics.

The group first generated transgenic mice in which CNP was overexpressed in the growth plate; expression of the gene encoding CNP, designated *Nppc*, was driven by the type II collagen cartilage-specific promoter (*Col2*). The *Col2-Nppc* transgenic mice displayed excessive skeletal growth that was mainly postnatal. Compared to non-transgenic littermates, the *Col2-Nppc* transgenic mice had longer body length, longer limb bones, a longer cranial base (measured as naso-occipital distance), and wider growth plates by histology.

Next, the *Col2-Nppc* transgenic mice were mated to another transgenic mouse strain in which the achondroplasia-activating mutation of FGFR3 was expressed in cartilage also under the control of the type II collagen promoter (*Col2-FGFR3<sup>ach</sup>*). The latter mouse strain exhibits a dwarf phenotypic with characteristics of human achondroplasia and is considered an animal model for this condition. Offspring of this mating that carried both the *Col2-Nppc* and *Col2-FGFR3<sup>ach</sup>* transgenes had near normal body lengths when measured over 10 weeks. At 3 months, measurements of cranial base length, femurs, and humeri were statistically the same as non-transgenic mice, indicating that over-expression of CNP in the growth plate had rescued the dwarfism caused by the achondroplasia transgene. There was also restoration of the shortened growth plate of the *Col2-FGFR3<sup>ach</sup>* mice toward normal in the mice harboring both transgenes. Of note, the over-expression of CNP did not appear to rescue the reduced proliferation of growth plate chondrocytes detected in the *Col2-FGFR3<sup>ach</sup>* mice.

To confirm the direct effect of CNP on bone growth, the authors treated cultured tibias from *Col2-FGFR3<sup>ach</sup>* mice with different doses of CNP. Bone length showed a dose response to the CNP. The dose that restored bone

length to normal also restored synthesis of 2 markers of cartilage matrix biosynthesis—glycosaminoglycan and collagen—which were reduced in the *Col2-FGFR3<sup>ach</sup>* mice to near normal.

The authors next examined the effect of CNP on FGFR3 signaling pathways in the tibial explants. No differences were observed in FGF-induced STAT1 signaling, which has been implicated in the control of chondrocyte proliferation. However, CNP reduced signaling through the MAP kinase-ERK pathway.

The model that Yasoda et al<sup>1</sup> constructed suggests that FGFR3 signals through STAT1 to down regulate chondrocyte proliferation and differentiation and through the MAP kinase-ERK pathway to negatively control matrix synthesis in the growth plate. They propose that CNP blocks the MAP kinase inhibitory signals of FGFR3 to increase matrix synthesis and thereby counters the restraining consequences of FGFR3 on bone growth. They speculate that these observations could form a basis for a new therapeutic approach to treating achondroplasia.

Yasoda A, Komatsu Y, Chusho H, et al. Overexpression of CNP in chondrocytes rescues achondroplasia through MAPK-dependent pathway. *Nat Med*. 2004;10:80-86.

**Editor's Comment:** This is a very interesting paper that brings to the fore a growth plate regulatory circuit that has not been widely appreciated in the bone growth field. It also suggests that contrary to the popular view that activating FGFR3 mutations acts primarily through inhibition of chondrocyte proliferation and differentiation, they may also act by inhibiting the synthesis of the extracellular matrix that also contributes to bone growth.

The idea that CNP could be used to stimulate growth in achondroplasia is intriguing. Obviously, this work needs to be confirmed and much more investigation done, but in theory, blocking a downstream pathway that propagates growth inhibitory FGFR3 signals has promise.

Of caution is that high levels of CNP likely generated in cartilage of the transgenic mice, which presumably would be needed to counter the effects of mutant FGFR3 in patients, may be very difficult to achieve in a therapeutic setting, especially without having adverse effects on other tissues that respond to CNP such as kidney, adrenal gland, and cardiovascular system or on other regulatory circuits that utilize MAP kinase-ERK pathways. Nevertheless, the unfolding of this story deserves considerable attention.

William A. Horton, MD



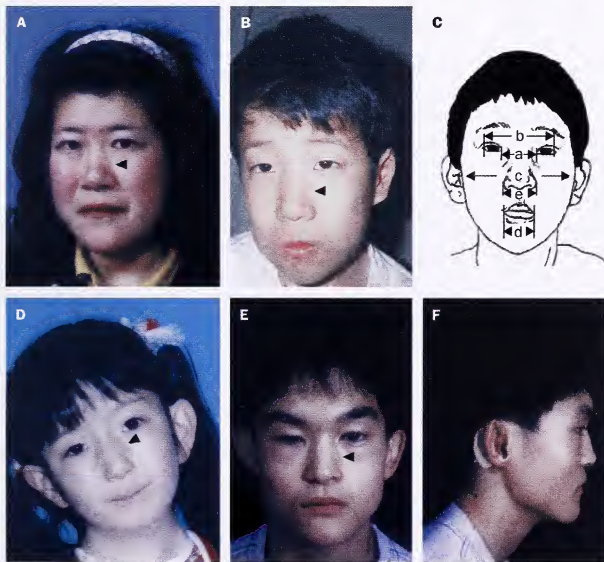
## TBX1 in del 22q11.2 Syndrome (Di George)

In mice, heterozygous and homozygous loss-of-function (LOF) mutations in *Tbx1* (the murine ortholog of a gene present in the DiGeorge critical region [DSCR] on human chromosome 22q11.2) resulted in anomalies of the cardiac outflow tract, thymic and parathyroid gland abnormalities, and craniofacial defects. Nevertheless, previous investigators have been unable to document mutations in *TBX1* in patients with a DiGeorge-like phenotype. The authors demonstrate that heterozygous LOF mutations in *TBX1* can be responsible for the major clinical manifestations of the DiGeorge (DGS-hypoparathyroidism, thymic dysfunction, and cardiovascular anomalies) and conotruncal anomaly face syndromes (CAFS). They present an extremely carefully clinically characterized cohort of 235 Japanese patients with either DGS or CAFS. Fluorescence in-situ hybridization with site specific probes (FISH) analysis revealed microdeletions in the DiGeorge critical region of chromosome 22q11.2 in 225 (96%) patients. In 3 patients

in whom no microdeletion of chromosome 22q11.2 could be detected despite examination with multiple probes, mutations in *TBX1* were identified: exon 4:443T6A leading to Phe148Tyr; exon 8:928G6A leading to Gly310Ser; exon 9: 1223delC leading to a stop signal at codon 459 and truncated product. *TBX1* is a member of a family of T-box transcription factors important for specifying mesodermal differentiation. It is a 10-exon gene that is transcribed into 3 products (*TBX1A*, *TBX1B*, *TBX1C*); the first 2 mutations would affect all products while the third would alter only *TBX1C*. Clinical correlation revealed that those patients with the first 2 mutations had classical CAFS and DGS, respectively; the patient with the third mutation had a less severe form of CAFS. These observations suggest that the clinical manifestations of the DGS/CAFS depend in part upon the extent of *TBX1* loss.

Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet*. 2003;362:1366-1373.

Figure  
Characteristic facial appearance of the patients



(A, B) Typical conotruncal anomaly face of del22q11.2 syndrome in familial cases (mother and son). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides. (C) Items of anthropometric measurement— $a + c$  (wide ocular hypertelorism),  $(b-a) + b$  (short palpebral fissures),  $d + c$ , and  $d + e$  (small mouth).  $a$  = inner canthal distance,  $b$  = outer canthal distance,  $c$  = transverse facial width,  $d$  = oral width,  $e$  = nasal width. (D) Facial appearance of a patient with conotruncal anomaly face syndrome without the 22q11.2 deletion. (E, F) Facial appearance of a patient with DiGeorge's syndrome without the 22q11.2 deletion (E, frontal view; F, side view). Arrowheads show the area of the nose that seems to be divided into two parts (upper and lower) at the joint of the wing and at the sides.

Reproduced from *Lancet*, Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet*. 2003;362:1366-1373.

**Editor's Comment:** This report not only identifies *TBX1* as an important determinant of DGS/CAFS, it also demonstrates the importance of a careful and complete clinical description of a complex and multidimensional clinical disorder. Twenty-five years of experience in treating patients with illnesses associated with microdeletions of chromosome 22q11.2 enabled these investigators to precisely define specific clinical criteria for the diagnoses of DGS/CAFS; they identified several abnormalities of the face that typified these patients (Figure). Thus, they were able to study patients who clearly had either DGS or CAFS. Therefore, LOF mutations in *TBX1* are associated with 5 phenotypes: abnormal face, velopharyngeal insufficiency, cardiac outflow anomalies, and thymic and parathyroid dysfunction. They are not associated with developmental delay often encountered in patients with DGS/CAFS, implying that another gene(s) in the DSCR is likely responsible for this problem. Mutations in *TBX1* were not detected in other subjects with DGS/CAFS, indicating there may be abnormalities in the non-coding region of this gene or that this disorder is polygenic in origin. In this regard, experimental deletion of *Fgf8* leads to a murine phenotype quite similar to that of the DGS/CAFS, suggesting that the products of *TBX1* may regulate the transcription of this gene. An interesting commentary accompanies this report.<sup>1</sup>

Allen W. Root, MD

### Reference

1. Baldini A. *Lancet*. 2003;362:1342-1343.



## Criss-Crossing the Insulin and Insulin-like Growth Factor Pathways

Insulin and the insulin-like growth factor (IGF)-II both bind the A isoform of the insulin receptor (IR-A); how do the 2 hormones achieve specificity in responses? Pandini et al used microarray technology to test the hypothesis that the hormones affect different patterns of gene expression. They studied IGF-I receptor (IGF-1R)-deficient murine fibroblasts (R<sup>-</sup> cells) to isolate the effects on the IR from possible IGF-1R cross-reactivity. Some R<sup>-</sup> cells were transfected with human IR-A cDNA (R<sup>+</sup>/IR-A cells; ~ 5 X 10<sup>5</sup> IR's/cell) for comparison with the R<sup>-</sup> cells (~5 X 10<sup>3</sup> native IRs/cell), and gene expression profiles were compared following exposure to insulin or IGF-II. Two hundred fourteen transcripts were similarly regulated by the 2 hormones, and 45 were differentially transcribed. Expression patterns are summarized in the Table.

To validate the microarray data, changes in 12 genes belonging to different functional categories were confirmed by real-time PCR. Surprisingly, the different gene profiles of the 2 hormones did not fit neatly with the original functional dichotomy. For example, 3 genes selectively up-regulated by insulin were involved in regulating angiogenesis and differentiation, and 3 genes up-regulated longer after IGF-II than insulin were involved in metabolism (cholesterol metabolism, phosphate transport, and selenium supply/oxidative stress prevention). The authors concluded that these studies provided a molecular basis for the biological differences between insulin and IGF-II.

Pandini G, Medico E, Conte E, Sciacca L, Vigneri R, Belliøre A. Differential gene expression induced by insulin and insulin-like growth factor-II through the insulin receptor isoform. *A J Biol Chem*. 2003;278:42178-42189.

**Editors Comments:** The somatomedins were renamed the insulin-like growth factors (IGFs) for their primary structural homology to pro-insulin. Because of their different functions, the IGF and insulin systems were believed to be separate; IGF-I and IGF-II both stimulate cell survival and proliferation through the type 1 IGF receptor (IGF-1R), while insulin affects metabolism through the IR. The IGF-IIIR, which is identical to the mannose 6-phosphate receptor, serves to clear IGF-II from the circulation.

Then things became more complicated. Not only do the ligands share structural homology, but the receptors are also very similar and they overlap in function. Both IGF-1R and IR are transmembrane  $\alpha_2\beta_2$  aggregates with autocatalytic tyrosine kinase activity, and both receptors

activate signaling cascades in common (MAP kinase pathway and PI3 kinase/Akt pathways). The IGFs and insulin can have similar metabolic effects; IGF-II over expression by tumors may cause hypoglycemia, and insulin is recognized as a growth-promoting hormone (best exemplified by the macrosomy of infants of diabetic mothers and babies with congenital hyperinsulinism).

**Table**  
Differential gene expression patterns of IR-A detected by microarray technique

|  | Number of genes up-regulated | Number of genes down-regulated |
|--|------------------------------|--------------------------------|
| By insulin alone   | 3                            | 9                              |
| By IGF-II alone  | 5                            | 1                              |
| Persistently changed by both hormones, more potently after IGF-II than insulin | 5                            | 3                              |
| Transiently changed by both hormones, longer after IGF-II than insulin         | 8                            | 0                              |

In addition to the IR found in metabolically responsive adult tissues—fat, liver, and muscle (the IR-B isoform)—there is also a shorter IR-A isoform (12 amino acids omitted from the  $\alpha$ -subunit by skipping exon 11). IR-A is the predominant isoform in fetal tissues and binds both insulin and IGF-II with high affinity.<sup>1</sup> IR-A is also over-expressed in cancers. Hybrid receptors, composed of an IR hemireceptor combined with an IGF-1R hemireceptor, have also been identified and implicated in neoplasia. These hybrid receptors are not just structural mistakes; they have been shown to bind IGF-I (but not insulin) with high affinity and contribute to IGF-stimulated cell growth.<sup>2</sup>

Thus, the question now is no longer, are the IGF and insulin systems truly related, but rather, how do they achieve specificity in response? One possible mechanism involves differential expression profiles of the various receptors that are cell-type- and ontogeny-dependent. Another involves differences in relative ligand concentrations, ligand binding affinities, and receptor densities. A third possibility evokes different downstream targets.

This paper is an important contribution to the field. Unfortunately, by providing a novel glimpse into the system, it makes it all seem a bit more complicated still.

Adda Grimberg, MD

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## Klinefelter Syndrome: Phenotype and New Research

In August of 2000, the National Institutes of Health sponsored a meeting (co-sponsored by the March of Dimes and Klinefelter Syndrome and Associates) to address gaps in understanding this condition. In an effort to prioritize research initiatives, the participants summarized research data and developed consensus conclusions. Domains covered included: cytogenetic origin and molecular pathogenesis; gonadal and hormonal dysfunction; somatic anomalies; IQ and language development; adult-onset disorders; predicted phenotype and genetic counseling after *in utero* detection of XXY; genetic risks for offspring of the 47, XXY male; and, research priorities.

In contrast to autosomal trisomies in which maternal errors predominate (95% of cases), the origin of 47, XXY is far more variable with regard to source (maternal vs. paternal), as well as form of error (meiotic or mitotic origin). Adverse phenotypic outcomes are assumed to result from the action of excess genes on the X chromosomes that are not inactivated. Researchers are focusing attention on approximately 40 genes on the X short arm that escape inactivation.

Testicular histology in Klinefelter syndrome (KS) is normal or near normal in early infancy, and is followed by a progressive loss of germ cells throughout childhood. Animal models of sex chromosome aneuploidy have been developed to determine whether germ cell and Leydig cell defects are somatic or germ cell in origin.

Although testosterone concentrations fall within the normal range for 50% of late adolescent and young adults with KS, gonadotropins are universally elevated. Does this observation reflect compensated hypergonadotropic hypogonadism, or partial androgen resistance? Should testosterone levels in the normal range serve as the "gold standard" for guiding the timing and dosage of hormone replacement? The suggestion was made (but without supporting evidence) that prepubertal testosterone treatment (even in the early months of life) may normalize aspects of the behavioral phenotype in KS.

The relationship between hypogonadism and reduced libido in men is well documented, as is its effective treatment through testosterone replacement. Less well recognized and understood is the possibility of an increased prevalence in atypical psychosexual development (eg, paraphilias) among men with KS. Because the majority of individuals with KS go undiagnosed, ascertainment bias may be a factor in the association between sex chromosome aneuploidy and sexual disorders.

Neurodevelopmental studies reveal that XXY infants show decreased truncal tone and atypical gross motor skills with delays in walking (mean of 18 months) which can be ameliorated through intervention (mean of 12 months). IQ falls in the low normal range. Intellectual

functioning and career attainment are typically lower in boys with KS as compared with unaffected siblings. Language skills, in particular, are delayed with first words spoken between 18 to 24 months (vs. 12 months normally). These delays persist, and affect multiple aspects of language development. The characteristic passive personality, which is sometimes accompanied by paradoxical behavioral outbursts, may result out of frustration related to deficits in verbal skills under socially challenging circumstances.

Accumulating evidence indicates that XXY is associated with autoimmune disorders. The link in pathogenesis may be chronic estrogen stimulation. Breast cancer appears to be markedly increased in older XXY men, as are extragonadal germ cell cancers and mediastinal teratomas. The authors speculate that constitutional chromosomal abnormalities and extragonadal aneuploidy germ cells predispose to malignant degeneration.

Simpson JL, de la Cruz F, Swerdloff RS, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. *Genet Med*. 2003;5:460-468.

**Editor's Comment:** *Just when you thought you knew all you needed to know about KS, along comes a summary of a conference indicating that research continues unabated. When the diagnosis of KS is made during childhood or adolescence, the pediatric endocrinologist should be the lead healthcare professional involved with the child's care. In light of evidence that neurocognitive deficits associated with KS are apparent at an early age, a developmental assessment should be performed. A referral to a psychologist should be accompanied with background readings (such as this conference proceeding) to orient that clinician to the most current syndrome-specific findings. The objective here is to ameliorate the predictable deficits, thereby improving developmental and quality-of-life outcomes. Such interventions would ideally occur long before issues of testosterone replacement become the focus of clinical management.*

*The suggestion that early testosterone treatment may normalize socialization difficulties in boys with KS is intriguing. Although changes in clinical care await conclusive evidence of such benefits, the discussion forces us to think about testosterone acting in ways beyond induction of pubertal development.*

*Finally, recent findings regarding adult-onset disorders in KS provide the pediatric endocrinologist with the opportunity to emphasize the importance of regular visits with adult specialists.*

David E. Sandberg, PhD

## Soy Formula Complicates the Management of Congenital Hypothyroidism

High fiber soy flour has been reported to cause goiter formation and hypothyroidism in humans and other animals. Soy-based formulas are now made from isolated soy protein to which iodine has been added. Whether or not these contemporary formulas could be associated with neonatal thyroid dysfunction was investigated by Conrad and colleagues in a retrospective analysis of patient charts at Children's Memorial Hospital in Chicago. Seventy-eight children born between 1990 and 1998 who were followed at CMH until at least 1 year of age were eligible for the study. Data included weight, length, total T4, TSH, levothyroxine dose, dietary information, and thyroid scan results. All children diagnosed with congenital hypothyroidism had a thyroid scan at their facility.

Eight children received soy formula, and 70 did not. Treatment was started 2 days earlier (median data) in the soy group. There were no significant differences between initial levothyroxine doses or the 1-year dose between the groups. T4 and TSH levels were comparable before the start of therapy, but TSH levels were significantly higher in the soy group at the first evaluation following initiation of therapy (42.6 mU/l vs 6.6 mU/l,  $p < 0.01$ ). Time to normalization of TSH levels was significantly longer in the soy group (150 days vs. 40 days,  $p = 0.02$ , median data). At 6 months, 62.5% of the soy group had elevated TSH values compared to 17% of the non-soy group ( $p = 0.01$ ). The difference in TSH values persisted throughout the first year of life. There were no significant differences in

height or weight, z-scores, or any other parameter.

The authors discuss possible reasons for their findings, including severity of hypothyroidism, immaturity of the T4-TSH feedback loop, or inadequate dosing, none of which could be demonstrated in this study. The authors speculate that the cause is malabsorption and increased fecal loss of levothyroxine. Free T4 levels were not measured in these children. Neuropsychological data were not available.

Conrad S, Chui H, Silverman B. Soy formula complicates management of congenital hypothyroidism. *Arch Dis Child*. 2004; 89:37-40.

**Editor's Comment:** *This retrospective study provides some provocative information. Although these infants had similar total T4 levels, thyroid dysfunction was present throughout the first year of life in those fed a soy-based formula. Whether or not there are any long-term sequelae related to this disparity remains to be seen. Newer recommendations for treatment of congenital hypothyroidism are based on normalization of TSH levels at a much quicker rate than previously. It would appear that infants fed soy-based formula will require higher doses of levothyroxine than those on other diets. This is important information for pediatricians and pediatric endocrinologists. A dietary history remains an important part of every child's health evaluation.*

William L. Clarke, MD

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### OBESITY OF INFECTIOUS ORIGIN – A REVIEW

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#### INTRODUCTION

Obesity has become the number one public health problem in America.<sup>1</sup> Obesity is a complex, multifactorial disease that involves the interaction of genetic, metabolic, social, behavioral and cultural factors. In the decade from 1980 to 1990, the number of people with obesity increased by 30% in the US; the number of obese adults further increased to 61% between 1991 and 2000.<sup>2</sup> The numerous health risks associated with obesity are well known to the medical community.

The epidemic increase in obesity, its medical consequences, and the rapidly escalating health care costs associated with it have prompted a multidisciplinary approach by health professionals, government, and non-governmental

#### From The Editor's Desk

Obesity has reached epidemic proportions worldwide; the term "globesity" defines the current situation. If the prevalence of obesity remains unabated this will be the first generation of children who die before their parents! The disease is now attracting the attention of pediatric endocrinologists. At the LWPES/APS there were multiple presentations on the subject and a symposium on adiposity. Obesity is now recognized to be at the crossroads of insulin resistance, a condition implicated in the "deadly quartet" of western civilization: diabetes mellitus, hyperlipidemia, hypertension and cardiovascular disease, as well as other common pediatric endocrine conditions, ie, PCOS, acanthosis nigricans, glucose intolerance, etc. The obesity epidemic has its roots in a lifestyle which facilitates consumption of excess calories over and above energy expenditures.

Adipocytes function as an endocrine organ and play an important role in the pathogenesis of obesity and its complications. However the potential role of infectious agents triggering or being associated with obesity and/or its co-morbidities is rarely discussed, nor are the potential endocrine alterations that may be induced by infective processes. In this issue of *GGH* such an omission is addressed by Drs. Dhurandhar, Atkinson and Ahmed. Their review should shed light and attract attention to this poorly understood area and facilitate an understanding of obesity in its entirety. Filling the void of this often neglected aspect may also stimulate research by pediatric endocrinologists wishing to clarify the endocrine interactions with adipocytes and infective agents.

The editors have reviewed a variety of papers addressing subjects of great interest. Noteworthy in the growth field are the papers on the long-term mortality of recipients of pituitary derived growth hormone, the novel dysfunctional growth hormone variant, the growth hormone and IGF-I effects on longitudinal growth, and cancer risk. Also note the papers disproving the risk of type 1 diabetes mellitus with childhood vaccinations as well as those addressing new discoveries of leptin action and a novel treatment of osteogenesis imperfecta. I also want to highlight the 2 papers on intersex, intersexuality and sexual identity which denote the current state of treatment controversies.

I am also pleased to bring to your attention enhancements in the print and web-based journal with the addition of color figures and a more efficient search capability. Please keep us posted with your comments and suggestions so we may continue improving the journal.

Respectfully,  
Fima Lifshitz, MD

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organizations to search for new methods to control it.<sup>3</sup> Such efforts are likely to be facilitated by a better understanding of the etiology of obesity. Sciafani<sup>4</sup> classified the etiology of animal obesity into 9 groups, including obesity of neural, endocrine, pharmacological, nutritional, environmental, seasonal, genetic, idiopathic, and viral origin. Of these factors, the viral etiology of obesity, a relatively recent discovery first noted in 1982,<sup>5</sup> has barely been studied. Over the past 20 years, 8 pathogens have been reported to cause obesity in animal models.<sup>5-11</sup> The relative contribution of these pathogens to human obesity is not yet clear. Considering the emerging reports addressing an infectious etiology of several other chronic diseases,<sup>12</sup> the contribution of certain infections to the etiology and pathogenesis of obesity need not be inconceivable. If shown to be relevant to humans, this relatively novel concept may be potentially important. An adequate understanding of such pathogens is needed for better management of obesity. A new perspective about the infectious etiology of this disease may initiate additional research in the field to assess the contribution of pathogens in human obesity and its co-morbidities and possibly to prevent or treat the obesity of infectious origin.

The known obesity-producing infective agents are listed in Table 1. There are 7 viral pathogens known to cause obesity in animal models, 4 are either known human pathogens or have been shown to be associated with human obesity. In addition *Chlamydia pneumoniae* has been associated with obesity in humans. In this paper we review the present knowledge in the field as this may be of importance to those who deal with patients and/or those who are interested in obesity.

**Table 1. Pathogens responsible for obesity**

| Pathogen (Reference)               | Animal model   | Possible Mechanism(s)  |
|------------------------------------|--|--|
| Human adenovirus-36* (11,15,16)    | Chickens, mice, non-human primates                     | Up-regulation of pre-adipocyte differentiation                       |
| Human adenovirus-37* (33)          | Chickens   | Unknown  |
| SMAM-1 adenovirus* (8,9)           | Chickens   | Unknown  |
| Borna-disease virus* (10,50,51)    | Rats   | Hypothalamic damage  |
| <i>Chlamydia pneumoniae</i> * (68) | No animal model, associated with weight gain in humans | Unknown  |
| Scrapie agent (76-79)              | Mice   | Hypothalamic-pituitary-adrenal axis damage                           |
| Canine Distemper virus (5)         | Mice   | Hypothalamic damage, reduced hypothalamic leptin receptor expression |
| Rous-Associated virus-7 (6,7)      | Chickens   | Reduced thyroid hormone levels                                       |

\* Human pathogens, and/or associated with human obesity.

## ADENOVIRUS AND OBESITY

### Human Adenovirus Type-36

In 2000 we reported that adenovirus type 36 (Ad-36) causes adiposity in animals.<sup>11</sup> Adenoviruses are naked

DNA viruses with icosahedral symmetry and a diameter of 65-80 nm. In humans, adenoviruses are frequently associated with acute upper respiratory tract infections, and may also cause enteritis and conjunctivitis. Adenoviral infections are transmitted via respiratory, fomite, droplet, venereal, and fecal-oral routes; these are easily isolated from nasal swabs or from feces. There are more than 50 types of human adenoviruses listed with the American Type Culture Collection. Ad-36 cross-reacts minimally, or not at all, with other human adenoviruses<sup>13,14</sup> and apparently is antigenically unique. Ad-36 was first isolated in 1978 in Germany in the feces of a 6-year-old girl suffering from diabetes mellitus and enteritis.<sup>14</sup>

In 4 separate experiments, chickens and mice were inoculated with human adenovirus Ad-36.<sup>11</sup> These animals developed a syndrome of increased adipose tissue and paradoxically low levels of serum cholesterol and triglycerides. This syndrome was not present in chickens inoculated with avian adenovirus chick embryo lethal orphan virus (CELO).<sup>11</sup> Sections of the brain and hypothalamus of Ad-36 inoculated animals did not show any overt histopathological changes. Ad-36 DNA was detected in the adipose tissue, but not in skeletal muscles for as long as 16 weeks after Ad-36 inoculation. Subsequently, to ascertain if blood transfusion from Ad-36 infected chickens could produce adiposity in uninfected animals, 4 age- and weight-matched groups of chickens were used: infected donors and recipients (I-D, I-R) and control donors and recipients (C-D, C-R).<sup>15</sup> Blood was taken from the I-D and C-D groups and injected into the recipient groups. The I-D and the I-R groups developed 2.5 and 1.8 times more visceral fat

as compared with the C-D group. Ad-36 DNA was detected in the adipose tissues of I-D and I-R groups, but not in the controls. The 2 infected groups showed significantly decreased serum cholesterol levels and the I-D group had a significant reduction in serum triglycerides. These data confirmed that Ad-36 produces adiposity and paradoxical reductions in serum lipids. In addition, the study fulfilled a Koch's postulate, namely that adiposity was transmitted from infected animals (I-D group) to a new set of animals (I-R group).

Furthermore, two studies were conducted in nonhuman primates to investigate the adiposity—promoting potential of Ad-36.<sup>16</sup> In the first study, spontaneously occurring

Ad-36 antibodies were detected in stored serum samples from adult male rhesus monkeys that were collected over a 7-year period at the Regional Primate Research Center located at the University of Wisconsin, Madison, WI. The monkeys gained approximately 0.1 kg of body

weight during the year preceding seroconversion, and gained 1.8 kg of weight during the following year. Serum cholesterol fell about 35 mg/dL after the appearance of Ad-36 antibodies. In the second experiment, male marmosets inoculated with Ad-36 had a 4-fold weight gain, with a 60% increase in body fat, and a 34mg/dL reduction in serum cholesterol levels as compared with controls over a 6-month period. These data demonstrate that Ad-36 is capable of increasing body fat in non-human primates.

### Mechanism of Action

The exact mechanism of action on adipocytes by Ad-36 is incompletely understood (Figure 1). Ad-36 was recently reported to up-regulate preadipocyte differentiation *in-vitro*.<sup>17,18</sup> Inoculation of 3T3-L1 preadipocytes with Ad-36, but not Ad-2, a non-adipogenic human adenovirus, resulted in increased adipocyte number, cellular lipid accumulation and glycerol 3-phosphate dehydrogenase levels (an adipocyte differentiation specific enzyme marker).<sup>17,18</sup> On the other hand, expression and secretion of leptin (an adipocytokine involved in body weight regulation) by Ad-36 inoculated fat cells was reduced compared to uninfected controls.<sup>19</sup> The phenomenon of increased lipid accumulation and decreased leptin secretion was observed in 3T3-L1 preadipocytes inoculated with Ad-36 or Ad-37, but not in Ad-2 inoculated cells.<sup>20</sup> Extrapolation of these findings to an *in-vivo* situation would suggest increased adipogenesis due to a relative absence of leptin. Thus, the mechanism may involve up-regulation of fat cell differentiation due to a local, direct effect of the virus, as well as a systemic effect of leptin.<sup>21</sup> The interaction of the viral and the cellular genes involved has not yet been elucidated.

### Adipose Tissue-Immune System Interaction

In light of well documented interactions of adipose tissue involvement with modulators and mediators of immune response, an adipogenic effect of certain pathogens should not be surprising. Cousin et al<sup>22</sup> reported that preadipocytes function like macrophages and possess phagocytic and microbicidal activity. Adipocytes too, participate in the immune response. Leptin, an adipocytokine, enhances proliferation and activation of human circulating T lymphocytes and stimulates cytokine production.<sup>23</sup> In addition to leptin-induced modulation of cytokine release, adipocytes themselves secrete various cytokines<sup>24,25</sup> and, in turn, preadipocytes and adipocytes are subject to cytokine directed modulations.<sup>26,27</sup> Certain cytokines, such as tumor necrosis factor alpha (TNF- $\alpha$ ), down-regulate preadipocyte differentiation<sup>27,28</sup> and increase leptin secretion by adipocytes<sup>30</sup> and adenoviral proteins sensitize cells to TNF  $\alpha$ .<sup>31</sup> Although Ad-36 reduces leptin expression and secretion from fat cells,<sup>19</sup> its effect on TNF  $\alpha$  is unknown. It is hypothesized, but not tested that Ad-36 proteins decrease both TNF  $\alpha$  levels and leptin, thereby contributing to up-regulation of preadipocyte differentiation by their relative absence.

Considering the extensive interaction between the immune system and the adipose tissue, expansion of the latter in response to certain infections is conceivable. For instance, Macrophage colony-stimulating factor, which promotes the production of macrophages, is also secreted by adipocytes and, when overexpressed *in vivo*, induces significant adipose tissue hyperplasia.<sup>32</sup> It is unknown if any of the obesity promoting pathogens stimulates macrophage colony-stimulating factor production leading to the growth of adipose tissue.

### Human Adenovirus Type-37

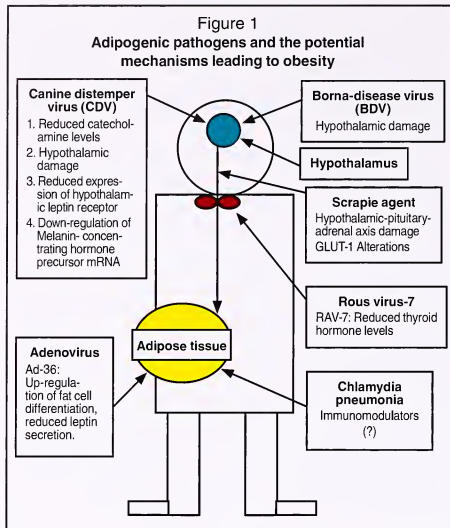
There are other adenoviruses with adipogenic potential properties. In preliminary studies it was demonstrated that Ad-37 increased adiposity in chickens, but that Ad-2 and Ad-31 did not.<sup>33</sup> Currently, minimal additional information is available on Ad-37, but the adipogenic mechanism of this virus may be similar to Ad-36 (Figure 1). However, these results demonstrate that more than one human adenovirus is capable of producing obesity in an animal model, but the adipogenic property is not necessarily shared by all human adenoviruses.

### Adenovirus and Human Obesity

In preliminary studies, human serum samples were obtained from over 500 obese (BMI  $\geq 30$  kg/M<sup>2</sup>) and non-obese volunteers from 3 different sites (Wisconsin, Florida, and New York). The sera were screened for the presence of Ad-36 antibodies using serum neutralization assays. A positive antibody status is suggestive of previous exposure of the individual to the virus. The prevalence of Ad-36 antibodies pooled across the 3 experimental sites was 11% for the non-obese and 30% for the obese subjects.<sup>34,35</sup> Antibody-positive subjects had a significantly higher BMI than antibody-negative individuals. Also, antibody-positive obese subjects had significantly lower serum cholesterol levels compared with the antibody-negative individuals.<sup>34,35</sup> Serum triglyceride measurements were only available at the Wisconsin site, the levels were significantly lower in the antibody-positive subjects versus the antibody-negative counterparts. These data demonstrated that antibody-positive humans were heavier and had lower serum cholesterol and triglycerides levels; these findings were similar to the data of experimentally infected animals with Ad-36. However, extensive research will be needed to establish the contribution of Ad-36 to the etiology of human obesity.

### Adenoviral Infections and Weight Gain in Children: Conjectures

It is well known that respiratory viral infections including adenoviral infections are very common among children.<sup>36,37</sup> Additionally, a very high prevalence of adenovirus is reported in lymphoid tissue obtained by tonsillectomy.<sup>38</sup> Although adipogenic properties of all adenoviruses have not been examined, it is interesting to note that excess weight gain occurs with or without



gain in height in children undergoing tonsillectomy.<sup>39-41</sup> It is not known if tonsillectomy provides the impetus for latent adenoviruses to promote the weight gain. In addition, obese and overweight children have higher levels of markers of inflammation.<sup>42</sup> It is now believed that excess body weight is associated with a state of chronic low-grade inflammation in children as measured by higher levels of C-reactive protein.<sup>43</sup> It is unknown if the inflammatory process is due to infections, or whether it is a causative factor for weight gain in children. Duncan and colleagues<sup>44</sup> showed that fibrinogen and other putative markers of inflammation can predict weight gain in middle-aged adults, which suggests a possible contribution of inflammation to weight gain and/or to co-morbidities associated with obesity. Longitudinal studies that track weight changes in children with and without adenovirus infections are needed to address these issues.

### SMAM-1 Avian Adenovirus

SMAM-1, an avian adenovirus identified in the early 1980s during a poultry epidemic,<sup>45</sup> was found to produce adiposity in chickens.<sup>8,9</sup> We inoculated 3-week-old chickens with SMAM-1 and noted development of excessive visceral fat and paradoxically lower levels of serum lipids compared to the uninfected controls.<sup>8,9</sup> Uninoculated chickens sharing the same room with inoculated chickens (in-contact group) developed the obesity syndrome, presumably due to infection with virus particles carried in the air.<sup>8,9</sup> There was no difference in food intake among the controls, inoculated, and the in-contact group. Visceral fat was greater by 53% and 33% in the inoculated and in-contact groups, respectively. SMAM-1 was reported to be associated with human

obesity. Antibodies to SMAM-1 were found in 11 of 52 subjects.<sup>46</sup> Antibody-positive subjects were heavier ( $95.1 \pm 2.1$  kg vs  $80.1 \pm 0.6$  kg,  $p < 0.02$ ) and had a higher BMI ( $35.3 \pm 1.5$  kg/m<sup>2</sup>, vs  $30.7 \pm 0.6$  kg/m<sup>2</sup>,  $p < 0.001$ ) vs the antibody-negative group. Serum cholesterol was 15% lower and triglycerides were 60% lower in SMAM-1 antibody-positive subjects. Since the prevailing thought was that avian adenoviruses do not infect humans and that human adenoviruses do not cross-react with avian adenoviruses,<sup>47</sup> the findings were surprising. It is possible that a human adenovirus antigenically similar to SMAM-1 produced antibodies that cross-reacted with SMAM-1. Further research is necessary to determine if SMAM-1 is capable of producing obesity and changes in serum lipids in humans. The potential mechanisms whereby infections with this virus lead to obesity remain to be proven (Figure 1).

### Borna Disease Virus

Borna disease virus (BDV) has also been implicated in obesity. This virus was first described in the early 1800s.<sup>48</sup> BDV, has been recently characterized as an enveloped, nonsegmented, negative-stranded RNA virus with a genomic size of approximately 9 kb and nuclear site for replication and transcription.<sup>49-51</sup> The genomic organization is similar to that of members of the Mononegavirales order; therefore, BDV is the prototype of the new family Bornaviridae within this order. BDV infects a broad range of warm-blooded animals from birds to primates. It replicates at lower levels than most known viruses,<sup>52,53</sup> is not lytic, and persists in the nervous system despite a vigorous immune response. Infected animals exhibit movement and behavior disorders.<sup>54,55</sup> BDV-specific antibodies were detected in asymptomatic horses in several countries.<sup>56-60</sup> suggesting that natural infections in animals remain subclinical in most cases.

Gosztonyi and Ludwig<sup>10</sup> reported that BDV infection produces a syndrome of obesity in rats, characterized by lympho-monocytic inflammation of the hypothalamus, hyperplasia of pancreatic islets, and elevated serum glucose and triglyceride levels. The expression of BDV-induced obesity syndrome varies with the age of the animals at the time of inoculation, the genetic background of the host and the viral strain used.<sup>10</sup> Rats infected as newborns with BDV show progressive neurological disease. On the other hand, weanling or adult rats similarly inoculated with BDV develop acute encephalitis and die within 1 to 4 months. Some of these rats survive the infection and develop marked obesity.<sup>61</sup> The obese phenotype has a characteristic distribution of inflammatory lesions and BDV-antigen in the rat brain. Infiltration with mononuclear immune cells and viral antigen expression are restricted to the septum, hippocampus, amygdala and ventromedian tuberal hypothalamus. Therefore, infection with obesity-inducing BDV most likely results in neuroendocrine dysregulations leading to development of obesity.<sup>62</sup> This might be due to the restriction of viral



antigen expression and inflammatory lesions to brain areas that are involved in the regulation of body weight and food intake (Figure 1).<sup>62</sup>

BDV may also be a human pathogen.<sup>49</sup> BDV-specific antigen and BDV-RNA were detected in 4 autopsied human brains with hippocampal sclerosis and astrocytosis. BDV-seropositive neurologic patients have been observed to become ill with lymphocytic meningoencephalitis.<sup>63</sup> In humans BDV is also associated with schizophrenia and mental depression<sup>64,65</sup> that are responsive to treatment by amantadine, an antiviral agent.<sup>66,67</sup> However, the contribution of BVD infections and the relationship to obesity in humans is unknown. Although it would be interesting to know if those with such infections gain more weight; such a relationship has not been reported.

### **Chlamydia pneumoniae**

The relationship between *Chlamydia (C) pneumoniae* infection and coronary heart disease (CHD) is of interest. There are studies that showed that *C. pneumoniae* was related to the development of CHD.<sup>68</sup> While others have found negative results,<sup>72-74</sup> in Australia newly identified cases of CHD compared with matched controls were tested for the presence of serum IgG and IgM against *C. pneumoniae*, *C. trachomatis* and *C. psittaci*. None of the subjects had IgM against chlamydia and only few were positive for *C. trachomatis* and/or *C. psittaci*.<sup>73</sup> The prevalence of seropositivity for *C. pneumoniae* was not significantly different for subjects with or without CHD. Similarly, a number of known CHD risk factors such as hypertension, serum lipids, and glucose levels lacked a significant difference between the antibody-positive and antibody-negative groups. However the antibody-positive group had significantly greater BMI and smaller LDL particle size. Antibody prevalence was significantly greater for subjects with insulin levels above the median and for those with LDL particle size below the median. However, after multivariate analysis, only BMI continued to be associated with seropositivity.

Although the association of *C. pneumoniae* antibodies with CHD may be questioned, the increased BMI with seropositivity to this infection is very intriguing. Approximately 10% of the subjects were obese. The greater prevalence of antibodies in patients in the highest BMI quartile as well as the relationship of BMI with the presence of positive *C. pneumoniae* antibodies may be the result of impaired immunity. Unlike *C. pneumoniae*, antibodies to *C. trachomatis* and *C. psittaci* did not show such a selective or high prevalence among those with higher BMI. A possible explanation offered by Dart et al,<sup>68</sup> which has neither been proved nor disproved, is that *C. pneumoniae* infection may be causally related to increased BMI, though the mechanism involved in this process is not completely known.

### **Scrapie Agent**

Scrapie is a neurodegenerative disease of prion proteins, with a long incubation period, known to occur in sheep and goats. Scrapie affects the brain and is transmissible from animal to animal. The key features of such infections include abnormal behavior and deficits in motor function. Certain scrapie strains induce obesity in experimental animals.<sup>70,71</sup> The obesity-promoting characteristic is a function of the scrapie strain, but not the mouse type. Regardless of the mouse strain tested, scrapie strain ME7 induced obesity. The effect was not observed with scrapie strains 139A or 22L in mice.<sup>78</sup> Vacuolation in the forebrain of the mouse was caused by ME7, whereas 22L and 139A caused vacuolation in the cerebellum and white matter, respectively.<sup>77</sup> The difference in the obesity-promoting potential of the agents may be linked to the differences in the brain lesions. Kim et al<sup>79</sup> demonstrated that ME7-induced weight gain in mice was associated with increased adrenal gland weight and adrenalectomy prevented ME7-induced obesity. Based on these findings, they suggested that scrapie-induced obesity depends on an effect of scrapie on the hypothalamic-pituitary-adrenal axis (Figure 1). Recently, Vorbrodt et al<sup>80</sup> demonstrated differences in the distribution of glucose transporter (GLUT-1) in the microvascular endothelium of scrapie-infected SJL/L hyperglycemic mice. These animals showed clinical signs of scrapie, obesity, and reduced glucose tolerance. GLUT-1 receptor density was significantly lower in microvasculature supplying the thalamus, cerebellum and, to a lesser degree, the hippocampus, but was unaffected in microvessels supplying the cerebral cortex and olfactory bulb.<sup>80</sup> Glucose, the major energy source for the brain, is passed across the blood-brain barrier by facilitative diffusion catalyzed by GLUT-1. Reduced GLUT-1 density in the scrapie-infected mice impairs transvascular glucose transport in the above-mentioned brain regions and presumably disturbs their function, which may lead to obesity.<sup>79</sup>

### **Canine Distemper Virus**

Canine distemper virus (CDV) was reported to cause obesity in mice in 1982.<sup>5</sup> CDV is a member of the genus Morbillivirus of the family Paramyxoviridae that causes severe health problems including respiratory, gastrointestinal, and central nervous system disease in dogs and other wild mammals.<sup>81</sup> CDV-induced encephalomyelitis in dogs is the most common cause of death.<sup>82</sup> CDV invades the nervous system and replicates in neurons and glial cells of the white matter during a period of severe viral-induced immunosuppression.<sup>83</sup> An increase in body weight and fat cell size and number was reported in Swiss albino mice experimentally infected with canine distemper virus.<sup>5</sup> Six to 20 weeks after CDV infection obesity was observed in approximately 26% of the mice with intracerebral infection compared to 16% of mice with intraperitoneal infection. Catecholamine levels were reduced significantly in the infected obese mice. The phenomenon of CDV-induced obesity in mice is believed to be due to virus-induced



hypothalamic damage.<sup>84-86</sup> Bernard et al<sup>87</sup> reported down-regulation of expression of the leptin receptor in the hypothalamus of CDV infected obese mice, and suggested this as the cause of the weight gain. Recently Verlaeten et al<sup>88</sup> demonstrated that melanin-concentrating hormone precursor mRNA, an anorexigenic neuromodulator was down-regulated in the late stage of acute phase of CDV infection in mice. Bernard et al<sup>87</sup> speculated that the data demonstrated a "hit and run" type of relationship between CDV and the expression of obesity, ie, the initial viral impact in the hypothalamus may initiate changes that would continue to promote obesity in animals even after the acute infection subsided. CDV is not considered a human pathogen, and its contribution to human obesity is unknown. However, measles virus is a human virus closely related to the CDV, and both belong to the paramyxovirus family, though its relationship to human obesity is not known. Animal experiments showing the effect of measles virus on adiposity are also unavailable.

### Rous Associated virus 7

Carter et al<sup>6</sup> reported that Rous-associated virus 7 (RAV-7) induced obesity in chicken characterized by stunting, hyperlipidemia, and hypercholesterolemia. Inoculation of 10-day-old chick embryos with RAV-7 produced fat deposition around crop and abdominal fat pads in the adult birds.<sup>6</sup> Intravenous inoculation of 1-day-old chickens with RAV-7 did not produce stunting and obesity.

Chicken embryos infected with RAV-7 developed fatty, yellow colored livers, hepatomegaly, anemia, and immune suppression.<sup>6</sup> Livers of infected animals constituted 6.2% of the body weight vs 2.4% of the body weight in the uninfected controls. These signs and symptoms manifested within 3 to 4 weeks after hatching. Obesity, stunting of growth and hyperlipidemia were the most striking features observed in the RAV-7 infected chickens. The mean body weight of the 50-day-old RAV-7 infected chickens was 515 g compared to 194 g of the same age controls. Both the RAV-7 infected and control groups were offered the same amount of food. Although the usual triglycerides levels for chickens are around 100 mg/dL, chickens from the RAV-7 group had serum triglycerides levels over 2000 mg/dL. The authors suggested that the reduced thyroid hormone level in the RAV-7 infected chickens was the cause of the observed obesity and hyperlipidemia.<sup>6</sup> Although lymphoblastoid infiltration of the thyroid gland was noted in the RAV-7 infected chickens, antibodies to thyroglobulin indicative of autoimmune thyroiditis, were absent. Administration of exogenous thyroxine prevented the syndrome.

### CONCLUSIONS AND SPECULATION

Although obesity has multiple causes, an overlooked possibility is that in some instances obesity could be due to an infection. Seven viral pathogens are reported to

cause obesity in animals. Of which, at least 4 are human pathogens and are associated with human obesity. In addition *Chlamydia pneumoniae* has also been associated to human obesity; however more research is needed to further define the mechanisms and the role of these pathogens in its etiology and/or co-morbidities.

It is possible that viral infections exacerbate and facilitate the development of obesity, or its complications, by working in conjunction with other adipogenic factors. For example obese children have been shown to have a cluster of conditions that put them at a high risk for developing diabetes and heart disease.<sup>89</sup> Over one-third of obese children studied presented with dysmetabolic syndrome, defined as hypertension, low HDL cholesterol, high insulin levels, elevated blood glucose and triglyceride levels. In addition, they presented elevated levels of C-reactive protein (CRP); which reflect an inflammatory reaction associated with an increased risk of heart disease. Furthermore, there were decreased levels of adiponectine with increased adiposity. Adiponectine is an anti-inflammatory hormone produced in fat cells that helps regulate glucose and cholesterol metabolism and may help protect blood vessels.

The insidious onset of human obesity makes it difficult to retrospectively link obesity or any of its co-morbidities to a particular episode of infection. Thus, a causative role for infectious pathogens in human obesity is difficult to establish. Due to ethical considerations, humans cannot be experimentally infected with these pathogens; linking the infection to long term weight gain is often impossible. In order to determine the role for viral pathogens in human obesity it is necessary to collect overwhelming indirect evidence in the area, and that remains to be done.

Elucidating the role of obesity of infectious origin could have two goals, prevention and treatment. The prevention of obesity of infectious origin could be achieved by vaccination against individual adipogenic pathogens; whereas the treatment may be more difficult and will depend on the adipogenic mechanism of individual pathogens. Antiviral agents may be of help only if the body continues to harbor the pathogen. Antivirals may be useless if the virus operates in a "hit and run" fashion. In such cases, the offending pathogen will have been cleared from the body long before its resulting impact on weight gain is noticed. Such cases will have to be treated by responding to the metabolic consequences of the infection in a genetically susceptible individual.

Understanding the causes and the mechanisms of obesity of infectious origin will be of immense help in individualizing the management of obesity by permitting cause-specific treatments. Recognizing the role of the above-stated pathogens and identifying more such candidates contributing to human obesity is the first step.

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## Growth, Genetics &amp; Hormones

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## ABSTRACTS FROM THE LITERATURE

### Dysfunctional Growth Hormone Variant

In order to expand the known mutational spectrum of the growth hormone (GH) gene, 74 patients with familial short stature were screened for mutations in the pituitary-expressed *GH1* gene. Two novel mutations were identified: a missense mutation Ile179Met substitution and a — 360A→G promoter variant; and two other previously known heterozygous lesions were also detected: a Val110Ile variant polymorphism and a Thr-24Ala neutral polymorphism. The Ile179Met variant exhibited a similar degree of resistance to proteolysis and secretion as the wild type GH. Molecular binding studies suggested that the Ile179Met substitution perturbed the interactions between the GH and the GH receptor affecting signaling transduction. This resulted in 50% reduced extracellular related kinase (ERK) activation as compared with that induced by the wild type GH. The authors concluded that these mutations reduced the ERK pathways activation and may thus play a role in mediating GH action of patients with familial short stature (SS).

Lewis MD, Horan M, Millar DS, et al. A novel dysfunctional growth hormone variant (Ile179 Met) exhibits a decreased ability to activate extracellular signal-regulate kinase pathway. *J Clin Endo Metab* 2004;89:1068-1075

**Editor's Comment:** This paper provides the molecular evidence that accounts for the growth failure of some patients with familial SS. It further expands the knowledge of the growth alterations in the presence of normal GH secretion, defined as peak GH levels higher than 10ng/ml after provocative stimulation. There may be other alterations of the GH secretion and/or signaling pathway that lead to SS without altering circulating GH levels. These can be detected in as many as 25% of children with idiopathic SS if appropriately investigated with sophisticated techniques not readily available to the clinician.

The accompanying editorial succinctly reviewed the known molecular alterations associated with SS.<sup>1</sup> If we look we will continue to find other mechanisms accounting for growth alterations and/or SS. The etiology of some SS patients with short parents has been clearly elucidated by these investigators.

Fima Lifshitz, MD

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### Disproving Another Vaccination Scare

The Danish Civil Registration System, implemented since 1968, enabled Hviid and colleagues to perform a very powerful longitudinal study examining the proposed association between vaccination and incidence of type 1 diabetes mellitus (T1DM). Because each Dane is assigned a unique identification number, the population can be followed longitudinally and individual data on different variables can be independently compiled from multiple registry sources, thereby eliminating selection and recall biases. Using this rationale, Hviid et al. followed through December, 2001 all children born in Denmark January 1, 1990 to December 31, 2000 (n = 739,694), and identified 681 cases of diabetes. Using Poisson regression models, the rate ratios for diabetes among children who had received at least one dose of the different vaccines versus unvaccinated children ranged from 0.91 to 1.14 (95% confidence intervals ranged 0.71-1.57). [hemophilus influenza B 1.02; DPT 0.94; DPTp 1.06; MMR 1.08; and oral polio 0.74 (p>0.05)] The rate ratio for maximal number of vaccinations (13) versus no vaccinations was 1.32 [0.42-4.10]. Likewise, the rate ratios did not increase in the 2-4 years after vaccination, the proposed time of disease clustering. Of the 681 cases of diabetes, 26 had siblings with diabetes. Even in this genetically predisposed subgroup (rate ratio for diabetes

in siblings versus no siblings was 40.1 [26.9-59.6]), the rate ratios for diabetes did not significantly increase with vaccination status.

Hviid A, Stellfeld M, Wohlfahrt J, Melbye M. Childhood vaccination and type 1 diabetes. *New Engl J Med* 2004;350:1398-1404.

**Editor's Comment:** The increasing incidence of T1DM has been associated temporally with the widespread introduction of general childhood immunizations. Further, T1DM has been described as clustering 3-4 years after vaccination. This has led some to conclude that vaccination plays a role in the development of T1DM. Associations are NEVER sufficient to prove causation. Thankfully, these authors performed such a terrific study which clarified that childhood vaccinations did not increase the risk of developing T1DM. For recent reviews of the pathogenesis of T1DM, see references 1-3, and reference 4 for immunologic effects of vaccination.

Adda Grimberg, MD

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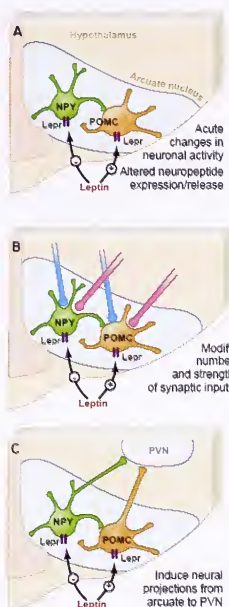
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## Leptin Actions on Hypothalamic Neurons & Arcuate Nucleus

Leptin decreases feeding behavior and encourages weight loss.<sup>1</sup> It stimulates hypothalamic neurons within the arcuate nucleus that synthesize anorexigenic or appetite-suppressing neuropeptides [proopiomelanocortin (POMC) and its products  $\alpha$ -melanocyte stimulating hormone and cocaine- and amphetamine-regulated transcript (CART)]. It also suppresses neurons that synthesize orexigenic or appetite-stimulating neurotransmitters [neuropeptide Y (NPY) and agouti-related protein (AgRP)]. These neurons then project to the paraventricular and dorsomedial hypothalamic nuclei, and lateral hypothalamic area (PVH, DMH, LHA, respectively). There, other neuropeptides propagate the feelings of hunger or satiety.<sup>2</sup> Leptin acts directly upon these neurons through the leptin receptor. Pinto et al identified direct anatomical functional effects of leptin upon these arcuate neurons. They transgenetically programmed wild-type (WT) and *ob/ob* mice to co-express fluorescent proteins with POMC (topaz) and NPY (sapphire). As expected each neuropeptide was expressed in a different arcuate neuron. They then examined, by patch clamp recordings in arcuate nuclear slices *in vitro*, the numbers of excitatory and inhibitory afferent inputs into these discrete neurons and quantitated by electron microscopy their anatomically distinct synapses. In *ob/ob* animals, there were far more excitatory than inhibitory impulses into (and excitatory synapses on) NPY neurons than in WT mice. There were many more inhibitory impulses into (and inhibitory synapses on) POMC neurons in *ob/ob* than WT mice. Administration of leptin to *ob/ob* mice reversed these patterns. In WT mice, administration of ghrelin, a gastric appetite-stimulating peptide,<sup>3,4</sup> increased inhibitory and decreased excitatory synapses into/on POMC neurons but did not appear to affect NPY-containing neurons. The authors concluded that there is "neural plasticity" in the arcuate cells containing POMC and NPY and that the effects of leptin and ghrelin are at least partially mediated by such changes.

Bouret et al examined the effect of leptin deprivation and leptin administration upon the density of the neural projections between the arcuate nucleus and the paraventricular nucleus (PVH), dorsomedial hypothalamic nucleus (DMH), and lateral hypothalamic area (LHA) in intact and leptin deficient (*ob/ob*) mice. They placed a "fluorescent ... tracer that labels axonal projections in fixed tissues" into sections of the arcuate hypothalamic nucleus then examined the pattern of fluorescent projections to the target area(s). In WT animals, the density of these projections increased with age. Relative to WT animals at all ages, leptin deficiency was associated with a greatly decreased number of projections from the arcuate nucleus to all target regions, but not to non-target areas. Administration of leptin to *ob/ob* adult mice did not alter this pattern. However, leptin given at very high doses intraperitoneally (1 mg/100 mg body weight IP) between



### Leptin and neurodevelopment.

Leptin acts on the neurocircuitry of the hypothalamus in three ways. (A) Leptin acts directly on the neurons of the arcuate nucleus by binding to the leptin receptors (LepR) that they express. The altered activity of these neurons in response to leptin results in changes in their production and release of the neuropeptide NPY and the POMC product,  $\alpha$ -melanocyte stimulating hormone. (B) By acting on an unknown site, leptin produces rapid changes in the strength and number of excitatory and inhibitory synapses that have inputs on NPY and POMC arcuate neurons. (C) Leptin induces neurite outgrowth of arcuate neurons, stimulating projections from the arcuate to the paraventricular nucleus (PVN) of the hypothalamus during a critical postnatal period.

Reprinted with permission from: Elmquist JK, Flier JS. *Science* 2004;304:63-66. Copyright © 2004 AAAS. All rights reserved.

Illustrated by Katharine Sutiliff

postpartum days 4 through 12 restored the density of projections to normal by the 80<sup>th</sup> day of life. The authors concluded that leptin is essential for the development of hypothalamic neural pathways that convey leptin downstream signals and that this property was expressed in the neonatal period and perhaps promoted by the neonatal surge in leptin secretion.

Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 2004;304:108-110.

Pinto S, Roseberry AG, Liu H, et al. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 2004;304:110-115.

**Editor's Comment:** In an accompanying commentary Elmquist and Flier<sup>5</sup> discussed the significance of the neuroexcitatory and anatomical effects of leptin. They suggested that through the influence of leptin on the excitatory and inhibitory inputs into the arcuate neurons and by stimulation of their neural connectivity, an as yet hypothetical body weight set point might be a functional reality. In mice, there is a surge in leptin secretion in the first week after birth that is not accompanied by a decrease in food intake. The possibility that a body weight set point may be related to and perhaps programmed by the secretion of leptin in the immediate post-partum period (in mice<sup>6</sup>) is intriguing. In human neonates, serum levels of leptin decline over the first 6 days of life and then do not change



appreciably over the first 17 days after birth.<sup>5</sup> Serum leptin concentrations are higher in female than male infants and related to BMI through 12 months of age, but low relative to values in older children and adolescents.<sup>6,7</sup> If there is a set point for body weight as there is for height in man, it is unfortunately easily abridged.

Allen W. Root, MD

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## Attitudes Toward Clinical Management of Intersexuality: The Voices of 46,XY Adult Patients

Controversies regarding the care of individuals born with intersexuality prompted a stream of adult followup studies of psychosocial and psychosexual functioning. Far less attention has been directed at the attitudes held by former patients toward treatment policies. The paper by Meyer-Bahlburg et al represents a marked exception. Specifically, participants were asked about their satisfaction with assigned gender as well as their opinions regarding the desirability of a 'third gender,' and the optimal age for genital surgery.

Attitude data were collected on 46,XY adults who had presented to a pediatric endocrinology clinic with varying degrees of genital ambiguity. The study was a postal survey followed by a physical examination. A total of 72 completed the questionnaire (32 men and 40 women; 18-60 years old). Based upon appearance of the genitalia at time of referral, participants were classified with ambiguous genitalia (AMBI; 21 men, 18 women), micropenis (MICRO; 11 men, 5 women), or female external genitalia (FEG; 17 women). The AMBI group consisted of individuals born with microphallus associated with perineoscrotal hypospadias secondary to various intersex syndromes. MICRO syndromes were attributed to hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, and idiopathic types. The FEG group was made up mostly of patients with complete androgen insensitivity.

Most participants were "mainly satisfied" with assigned gender (85%). In male AMBI and MICRO 68% replied their genitalia appeared unusual, and 76% complained that their penis was too small. Whereas, in female AMBI and MICRO, 39% thought their genitals looked unusual. The majority of participants (73%) were either mainly or somewhat satisfied with sexual functioning.

Only 15% endorsed an assignment of a third gender as a strategy to avoid genital surgery. However, there was a statistical trend for those not satisfied with their own gender to endorse this. When asked about surgical correction of a hypothetical child born with ambiguous genitalia, 67% did not endorse the option of postponing genital surgery until adulthood. When asked to employ hindsight regarding their own genital surgery, 47% thought the procedure should be performed during infancy, 24% recommended postponing surgery until adolescence, and 22% thought

the procedure should have been postponed until their adult years. FEG women almost uniformly endorsed waiting for surgery until adulthood.

Meyer-Bahlburg HFL, Migeon CJ, Berkovitz GD, Gearhart JP, Dolezal C, Wisniewski AB. Attitudes of adult 46,XY intersex persons to clinical management policies. *J Urology* 2004;171:1615-1619.

**Editors' Comment:** Several findings of this study are noteworthy. First, the majority of the 46,XY adult patients with intersexuality expressed satisfaction with assigned gender. This finding has been corroborated in independent studies.<sup>1</sup> Second, 45% were mainly satisfied with their current sexual functioning (while 28% were somewhat satisfied and 27% mainly dissatisfied). Readers should be cautioned against assuming that dissatisfaction with sexual functioning is necessarily related to the quality of the surgical reconstruction. Sexual problems in the general population of men and women are reported to be high.<sup>2</sup> Without a healthy comparison group, the rates of satisfaction/dissatisfaction reported in this study are difficult to evaluate. In addition, the best predictors of sexual distress in women are markers of general emotional well-being and emotional relationship with the partner during sexual activity. In contrast, physical aspects of the sexual response in women, including arousal, vaginal lubrication, and orgasm, are poor predictors.<sup>3</sup> Because survey respondents may assign different interpretations to single questionnaire items, the precise meaning of responses await more detailed assessments. Consistent with patient advocacy groups (eg, the Intersex Society of North America), the majority of survey participants opposed a third gender option. It is reassuring that the message obtained from former patients and patient advocacy groups coalesce in this critical aspect of clinical decision-making.

David E. Sandberg, PhD  
Sherri Berenbaum, PhD

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## Ethics Guidelines for Intersex Conditions

The Hastings Center, explored ethical and social issues raised by surgery aimed at making children appear more typical. A multidisciplinary group considered medical, psychosocial, and ethical issues associated with surgical interventions in children born with atypical genitalia, commonly grouped as intersex.

Parents of newborns with intersex may believe that medical evaluation will reveal their infants "true sex" and that genital surgery should proceed as soon as possible to avoid negative psychological sequelae. However, gender identity is not perfectly predicted by sex chromosomes or other physical parameters. Empirical evidence challenging predictions of positive outcomes from "cosmetic" procedures were reviewed as was diminished sexual responsiveness associated with surgical procedures. The practice of shielding patients from details of their diagnosis and surgical treatment is purported to precipitate disruption of relationships with parents and health care professionals. The authors point out that guidance documents put forth by professional societies are not based on valid clinical investigations and that there exists substantial variability among different specialties. The following conclusions were reached.

- 1) A comprehensive assessment of actual clinical practice should be undertaken.
  - 2) Current surgical procedures that normalize genital appearance, are not alone justified. Surgery does not assure that the individual will avoid being discriminated against.
  - 3) Appearance-altering surgeries do not need to be performed urgently. Surgical expediency does not necessarily outweigh the psychosocial and ethical considerations of waiting until the patient can participate in decision-making.
  - 4) Immediately following diagnosis families require comprehensive services, including access to mental health professionals with intersex expertise. Psychological support is essential.
  - 5) To reduce the feelings of humiliation and shame, children should be informed of their differences in an age-appropriate manner.
  - 6) Ethical practice demands rigorous follow-up studies focusing on well-being and quality of life. Retrospective studies should include those who have not had surgery and prospective studies should compare the outcomes of non-surgical alternatives. Careful study design is crucial.
  - 7) Clinicians need more intersex education including diagnosis, the development of gender, and sexual health.
- In an accompanying commentary, Dr. Erica Eugster juxtaposes panel recommendations against the "real life challenges of providing compassionate and responsible care to infants with intersex conditions and their families." Eugster draws attention to the potential psychological risks of postponing genital surgery until the patient is mature enough to provide informed consent. She ponders the implications for parenting of denying the option of early surgery when the family demands surgery. She concludes the ultimate decision should rest with the parents. To safeguard

physical well-being of the child, Eugster reinforces a recent recommendation that genital surgery be undertaken in centers of excellence with intersex expertise.

Eugster welcomes the development of multidisciplinary teams, but recognizes its rare application. Her experience demonstrates that some families passively and/or actively reject counseling. The full integration of counseling services with a multidisciplinary team may temper such resistance. Regarding disclosure of medical information to the child, Eugster welcomes the guidelines for psychoeducational counseling but acknowledges that conflicts may be encountered when family members wish to shield the child from diagnostic details. Eugster enthusiastically endorses increased human sexuality education for clinicians but recommends a targeted strategy of focused training workshops that would serve the purpose of filling staffing gaps in multidisciplinary teams with intersex expertise.

Frader J, Alderson P, Asch A, et al. Health care professionals and intersex conditions. *Arch Pediatr Adolesc Med* 2004;158:426-428.

Eugster EA. Reality vs recommendations in the care of infants with intersex conditions. *Arch Pediatr Adolesc Med* 2004;158:428-429.

**First Editor's Comment:** *These papers provide an excellent discussion on the ethical care of patients with intersex conditions. Both pieces underscore the value of outcome studies in guiding clinical practice, and yet the vision of multicenter, multidisciplinary research is largely unrealized. There are inherent limitations on research (eg randomized clinical trial is not an option in assessing the benefits of early versus later genital surgery). Studies examining the relative benefits of multidisciplinary teams versus the current standard of care would be compelling and feasible. The systemic constraints in 'real life' medicine represents even greater challenges. The intense effort required in creating and maintaining multidisciplinary teams serves as a disincentive. Creative problem solving is needed; we will not obtain answers to the most important questions if we restrict our inquiry to those issues that are the easiest to study.*

David E. Sandberg, PhD

**Second Editor's Comment:** *The reader is encouraged to review the article "Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth",<sup>1</sup> as well as the accompanying commentaries by many experts regarding sex determination, differentiation, and identity.<sup>2</sup> Altogether these articles should be carefully considered when caring for these patients. As Eugster stated, "The most important determinant of outcome may be an individual family's ability to accept and unconditionally love their child."<sup>3</sup>*

Fima Lifshitz, MD

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## IGF-1 and Cancer Risk

The authors undertook a meta-analysis of 26 published (and closely scrutinized) data-sets examining the relationship between circulating concentrations of IGF-I and IGFBP-3 and the risk of developing prostatic, breast, colon, and lung cancer in adult men and women employing extremely strict, unified, and sensitive analytical methods. After stratification of the analyte levels, they compared the 75<sup>th</sup> percentile of circulating protein concentration with the 25<sup>th</sup> percentile and then calculated odds ratios for the development of cancer. There were significant associations between the concentration of IGF-I and development of premenopausal breast, prostatic, and colon cancer (odds ratios: 1.93, 1.83, and 1.58, respectively). There was no association between IGF-I values and risk of breast cancer in postmenopausal women or of lung cancer. The IGFBP-3 level was associated with an increased risk of development of breast cancer in premenopausal women (odds ratio 1.96) and possibly with a protective effect on development of lung cancer. The investigators concluded that because of the proliferative and anti-apoptotic effects of various IGFs, higher circulating concentrations of IGF-I are a risk factor for development of 3 non-smoking related common malignancies.

Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004; 363:1346-1353.

**First Editor's Comment:** The association of hyper-somatotropism with increased risk for development of cancer of the colon is well known.<sup>1</sup> The present report concluded that higher IGF-I concentrations contributed to the development of several malignancies. The mechanism(s) by which IGF-I enhanced or facilitated neoplasia and the roles that IGFBPs and IGFBP proteases (such as prostate specific antigen) played in this process are not known with certainty.<sup>2</sup> The precise point at which a malignancy began and the corresponding IGF-I value is unknown. However, the association between IGF-I concentrations and malignancy should give one pause when recommending the use of growth hormone (GH) in short subjects without GH deficiency, particularly in the absence of data indicating that an increment in adult height of 2 or more inches results in greater academic, social, and economic well-being.

Allen W. Root, M.D

**Second Editor's Comment:** The major lesson from the IGF/cancer association is to beware of over generalizations; this is a story of complicated nuances. Over 200,000 patient-years' experience globally with recombinant human growth hormone (rhGH) therapy revealed a very strong safety profile. This safety profile may not hold as our use moves from purely physiologic replacement to increasingly pharmacologic use, in terms of both higher doses and newer indications. For example, increased mortality ensued when rhGH was administered to critically ill adults in an attempt to foster anabolism.<sup>3</sup> Conversely, we cannot jump to the conclusion that IGF-1 (and GH) are necessarily harmful. While high IGF-1 levels have been associated with higher cancer risk, low IGF-1 levels have been associated with increased risk for age-related memory loss, Alzheimer's dementia and diabetes-associated dementia.

The underlying science is also filled with nuances that likely contribute to the contradictory results found in the literature. The relative contributions of endocrine (circulating) versus autocrine or paracrine GH/IGF axis components to cancer progression remain unclear and technical issues, such as IGFBP interference with some IGF assays<sup>4</sup> and interassay variations,<sup>5</sup> can cloud the results. Due to its dynamic nature, measuring components of the IGF system may yield different results, ie a shift in the amount of free versus total IGF, the IGFBP profile, or the amount of intact versus proteolyzed IGFBP.

More research is needed, as in long-term surveillance of the rhGH recipients into late adulthood when the natural incidence of cancer increases. As a safety marker, IGF-1 levels should be closely monitored in all rhGH recipients to avoid supraphysiologic concentrations. Clinical nuances may also be explored regarding potential risk modulation by altering rhGH dose or duration of treatment.<sup>4</sup> Ultimately, treatment should be individualized and tailored to the risk/benefit analysis for each patient. That includes appreciating the true benefit (or not) of height augmentation.

Adda Grimberg, MD

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## Novel Treatment for Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a relatively common genetic disorder characterized by bone fragility, skeletal deformities, ligamentous laxity, thin skin, blue sclerae, and other "connective tissue" features. It results from heterozygous mutations of the *COL1A1* and *COL1A2* genes that encode the  $\alpha 1$  and  $\alpha 2$  chains of type I collagen; its manifestations reflect the distribution of type I collagen. The most severe forms of OI, eg, OI type II, result from mutations in which the mutant procollagen chains are synthesized, participate in and disrupt the assembly of triple helical type I collagen molecules. Such mutant genes or alleles might be considered dysfunctional alleles. In contrast, milder forms, such as OI type I, result from mutations that inactivate one of the two *COL1A1* or *COL1A2* alleles, leaving the patient with only one functional *COL1A1* or *COL1A2* allele. Accordingly, one strategy to treat severe OI is to convert the dysfunctional *COL1* allele to a nonfunctional or null allele. A group headed by David Russell (Chamberlain et al) has demonstrated the feasibility of inactivating dysfunctional *COL1A1* alleles in adult, or often termed mesenchymal, stem cells (MSC) from patients with severe OI.

MSC available from the marrow of bone samples discarded at surgery, have the potential to repopulate bone marrow and locally generate new bone. In principle, MSC could be harvested from a patient with severe OI, genetically manipulated to inactivate a dysfunctional type I collagen gene, and be transplanted back into the patient where they would home to bone marrow and locally produce bone tissue with properties of mild OI.

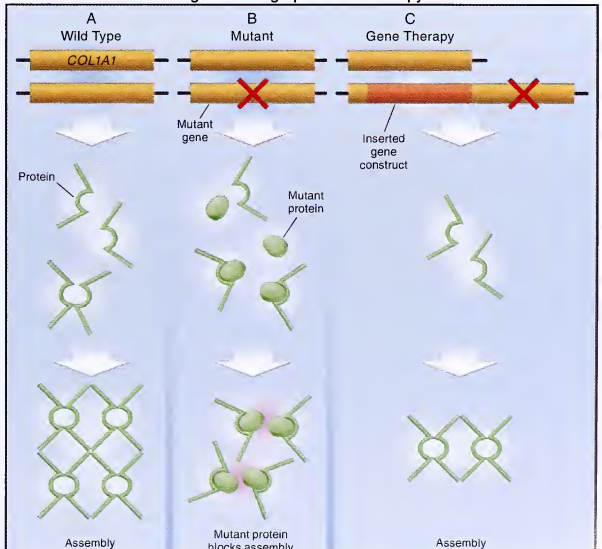
In this investigation, MSC were isolated from 2 patients with severe OI whose specific mutations were identified and shown to be of the dysfunctional type. The cells were infected with an adenovirus-associated virus-based gene targeting vector designed to promote homologous recombination between the vector and the *COL1A1* gene. Successful targeting inserted foreign DNA, including the gene for neomycin resistance, into exon 1 of the *COL1A1* gene, thereby preventing its expression and converting it to a null allele. The targeting vector did not distinguish between the mutant and normal *COL1A1* allele. The MSC were then grown in an antibiotic to select recombinant cells and then analyzed to confirm that targeting was successful. Analysis of several cell clones, as well as pools of cells, revealed that a high percentage of the resistant MSC had undergone targeting at one *COL1A1* allele. Significant improvements

were observed in measures of collagen processing, stability, and fibril ultrastructure for targeted cells, and accumulation of suspected mutant collagen present in the original cells disappeared in the targeted MSC. To determine if the targeted MSC retained their ability to become osteoblasts, the targeted MSC were implanted subcutaneously into immunodeficient mice, removed after 8 weeks, and analyzed. Although amount varied, bone formation was detected in all of the implants and the osteocytes were shown to be of human origin.

The authors note potential problems with their approach, such as inability to specifically target the mutant versus the normal *COL1A1* allele and possible immunogenicity of the foreign neomycin-resistance gene. But they argue that these can eventually be overcome and that their approach has certain advantages over allogeneic bone marrow and MSC transplantation, which has been reported in a clinical trial for severe OI.

Chamberlain JR, Schwarze U, Wang P-R, et al. Gene targeting in stem cells from individuals with osteogenesis imperfecta. *Science*. 2004;303:1198-1201.

Figure. Boning Up on Gene Therapy.



Type I collagen, a structural constituent of bone, is made up of two subunits, one of which is encoded by the *COL1A1* gene (Panel A). Mutations in the genes encoding these subunits cause osteogenesis imperfecta. Dominant negative mutations result in mutant protein that interferes with the activity of wild-type protein encoded by the unaffected gene (Panel B). Diminishing the levels of mutant protein may be a way to treat the disease, and a recent study by Chamberlain et al provides support for this approach and suggests that it could be achieved through gene therapy. Using a molecular construct engineered to insert itself into the *COL1A1* gene (Panel C), the investigators showed that the processing and stability of collagen could be improved by the targeting of mutant mesenchymal stem cells and that the targeted cells become bone cells. Although the construct probably targets mutated and wild-type alleles equally, the approach works because the cells in which the wild-type allele is inactivated do not produce collagen and the cells in which the mutated allele is targeted produce adequate amounts of the normal protein.

Reprinted with permission from Prockop DJ. *N Engl J Med* 2004;350:2302-2304. Copyright © 2004 Massachusetts Medical Society. All rights reserved.



**First Editor's Comment:** There are 2 major challenges in order for this form of genetic treatment to be successful. The first is being able to alter the mutant gene so that the mutation is corrected or nullified, as in this case. This paper demonstrates that conversion of a dominant-negatively acting allele to a null allele works, at least in cell culture and in mice, and can be carried out in a time frame that is realistic for clinical use. Although there are still many problems to resolve, the gene-targeting strategy has considerable promise. The second challenge is to achieve sufficient engraftment of genetically modified cells to repair excessively fragile bones. Fortunately, therapists can exploit the high vascularity of bone and the natural behavior of MSC to home to marrow and differentiate as functional osteoblasts. However, previous attempts at allogeneic MSC transplantation and similar experiments in mice have resulted in modest engraftment, at best. Figuring out how to safely and effectively impart therapeutic cells with a competitive advantage over their

dysfunctional endogenous counterparts in bone may prove to be the greater of the 2 challenges. Nevertheless, given the absence of other successful treatments for severe OI, it remains a viable potential option.

William A. Horton, MD

**Second Editor's Comment:** The exciting field of gene therapy has been given a shot in the arm by these studies. The recent comment by Prockop<sup>1</sup> on targeting gene therapy for OI is worth reading in order to facilitate the appreciation of this novel concept (figure).

Fima Lifshitz, MD

## Reference

1. Prockop DJ. *N Engl J Med* 2004;350:2302-2304.

## Teasing Apart GH from IGF-I Effects on Longitudinal Bone Growth

Wang and colleagues examined tibial growth in mice with targeted deletions of the insulin-like growth factor-I gene (*Igf1*) or growth hormone (GH) receptor gene (*Ghr*) to elucidate the direct versus indirect (ie IGF-I-mediated) effects of GH on longitudinal bone growth. The study design was based on the fact that *Igf1*<sup>-/-</sup> mice do not produce IGF-I in either the circulation or local tissues, but have high levels of GH due to the loss of IGF-I negative feedback. They would therefore be expected to retain any IGF-I-independent effects of GH action. In contrast, *Ghr*<sup>-/-</sup> mice lose all GH effects. The authors focused on tibial growth from postnatal days 20 to 40, a period of maximal GH action in normal murine growth which precedes sexual maturity. Further, because the two genetic mutants were created in different background mouse strains, all results were analyzed as a percent of the wild-type littermates. This controls for both genetic variations between the two strains and for any uterine or environmental factors that may affect growth.

Body weights of both mutants were about 60% less than wild-type littermates. Tibial morphology remained grossly normal in both, but the tibial growth rate was about 37% less in *Igf1*<sup>-/-</sup> mice and 65% less in *Ghr*<sup>-/-</sup> mice. The germinal zone, the upper growth plate region that produces chondrocyte precursors, was enlarged in *Igf1*<sup>-/-</sup> mice but smaller in *Ghr*<sup>-/-</sup> mice, suggesting IGF-I-independent effect of GH. IGF-II mRNA levels, as assessed by *in situ* hybridization, were increased in the former and decreased in the latter mutants. Similarly, the proliferative zone was unaffected in *Igf1*<sup>-/-</sup> mice but diminished in *Ghr*<sup>-/-</sup> mice; here, too, IGF-II mRNA was increased in the former but decreased in the latter. In contrast, the hypertrophic zone was markedly

reduced in both mutants. It remains unresolved whether prechondrocyte proliferation is directly enhanced by GH or by GH-induced local IGF-II production.

Wang J, Zhou J, Cheng CM, Kopchick JJ, Bondy CA. Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. *J Endocrinol* 2004;180:247-255.

**Editor's Comment:** Confirming similar results in femoral studies of different genetic mouse strains, this paper nicely demonstrated IGF-I-independent effects of GH on chondrocyte production and proliferation, and IGF-I-dependent effects on chondrocyte hypertrophy in murine tibial growth plates. It also opens the possibility that the IGF-I-independent effects may be mediated by GH-induced local production of IGF-II. Similar analyses in *Igf1*<sup>-/-</sup> mice will be needed to answer this question, as are additional experimental models to determine the contribution of IGF-II in a physiologic context as opposed to a possibly compensatory role when IGF-I is deleted. A study comparing *Igf1*<sup>-/-</sup>, *Igf1*<sup>-/-</sup> and GH deficient *lit/lit* mice found a greater contribution of IGF-I than IGF-II to bone mineral accretion and pubertal bone growth.<sup>1</sup> Thus, more than 10 years after the first description of the *Igf1*<sup>-/-</sup> and *Igf1*<sup>-/-</sup> mice continue to teach us.<sup>2</sup> For an excellent review of the GH/IGF system in controlling somatic growth, see reference 3.

Adda Grimberg, MD

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1. Mohan S, Richman C, Guo R. *Endocrinology*. 2003;144:929-936.
2. Baker J, Liu JP, Robertson EJ, Elstradiadis A. *Cell*. 1993;75:73-82.
3. Butler AA, Le Roith D. *Annu Rev Physiol* 2001;63:141-164.

## Transient Adrenocortical Insufficiency of Prematurity

There have been several reports of very low birthweight (VLBW) infants who experience systemic hypotension that is unresponsive to volume expansion and inotropic agents, but very responsive to corticosteroids. Ng et al have performed a prospective study of the pituitary adrenal axis in 137 VLBW infants, of whom 78 had refractive hypotension (group 2) and 59 remained normotensive (group 1). Human corticotropin releasing hormone (hCRH) (1mcg/kg IV bolus) was administered between 08:00 h and 10:00 h on days 7 and 14 of life. Serial samples for ACTH and cortisol were obtained at baseline, 15, 30, and 60 minutes after injection. Inclusion criteria were gestational age <32 weeks, birthweight <1500 grams, no postnatal systemic or inhaled corticosteroids, and an indwelling arterial line. Exclusion criteria were persistent hypoglycemia, systemic infection, necrotizing enterocolitis, or major surgery.

Results from groups 1 and 2 combined, showed that basal and peak cortisol and change in cortisol over the first 30 minutes after hCRH injection correlated significantly with the lowest recorded BP during the first 14 days of life and the BP measured at the initiation of the study on day 7. In contrast, ACTH levels on days 7 and 14 and cortisol on day 14 did not correlate with the lowest BP. Serum cortisol levels (after hCRH) on day 7 correlated negatively with the total dose of inotropic agents, while plasma ACTH levels were positively correlated.

The ACTH response to hCRH was significantly greater on both days 7 and 14 in group 2 infants, but cortisol responses were greater in group 1 than group 2 on day 7. Day 14 cortisol responses were similar in both groups. The authors state that this study demonstrates adrenal hyposponsiveness in group 2 infants at day 7. Those

were the infants with significant hypotension requiring inotropic agents. By day 14, the transient nature of this endocrine dysfunction was evident as there were no significant differences between the two groups of infants. The authors term this dysfunction, transient adrenocortical insufficiency of prematurity or TAP.

Ng PC, Lee CH, Lam CWK, et al. Transient adrenocortical insufficiency of prematurity and systemic hypotension in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F119-F126.

**Editor's Comment:** *This is an interesting, well-conducted prospective study of a problem that is relatively common in many NICUs. Neonatologists have been using small doses of hydrocortisone in premature babies with hypotension refractory to inotropic agents for some time. However, the definition of the defect responding to this non-replacement, non-stress level of hydrocortisone administration has not been clarified. Ng et al have provided a clear demonstration that these infants have a transient adrenal, not pituitary immaturity, which requires hydrocortisone administration. They note that a previously reported trial of hydrocortisone versus dopamine for the routine treatment of hypotension failed to confirm its benefit. This is not surprising, given the distinct differences in the 2 groups of infants studied. A short course of hydrocortisone in premature infants with hypotension refractory to inotropic agents seems a reasonable therapeutic maneuver. Data now show that these corticosteroids do not need to be given for prolonged periods.*

William L. Clarke, MD

## Long-term Mortality in the U.S. of Pituitary-derived Growth Hormone Recipients

Mills and colleagues from the NIH, FDA, and CDC presented long-term mortality data on patients who received pituitary-derived growth hormone (pGH) from the National Hormone and Pituitary Program (NHPP) during the years 1963–1985. Data through December 1996 were obtained for 6107 of the 6272 children who received pGH. Information regarding the reason for pGH treatment and the specific cause of death was obtained. Death certificates were reviewed in all but 3 instances. Causes of GH deficiency were categorized as idiopathic, organic (including tumor-related or non-tumor related—eg septo-optic dysplasia, histiocytosis, trauma, etc.), or other (including unknown causes, neurosecretory defect, Turner syndrome, etc.). Subjects were classified as having isolated GH deficiency, multiple hormone deficiencies, unspecified deficiency (insufficient information to classify), or not applicable (non-GH deficient). Subjects with adrenal insufficiency and/or a history of hypoglycemia were identified.

Observed mortality was compared to that expected in a similar US cohort. Relative risks were calculated and a proportional hazards model constructed.

There were 433 deaths from 1963–1996 compared to an expected number of 114. Thus the overall risk of death was nearly 4 times that of the general population (RR, 3.8; 95% CI, 3.4–4.2;  $p < .0001$ ). Only subjects with idiopathic isolated GH deficiency had a death rate similar to that expected for the population at large. The highest risk categories included patients with either benign or malignant tumors, adrenal insufficiency, and hypoglycemia. Tumors, hypoglycemia, adrenal insufficiency, and multiple hormone deficiencies were demonstrated to be significant, independent risk factors by proportional hazards analysis. There were 26 deaths from Creutzfeldt-Jakob disease (CJD). Two deaths were from colorectal cancer; one of whom had familial polyposis and the other had received radiation for a CNS tumor. One subject died from Hodgkin's disease. Thus, the reported associations between GH therapy and colorectal

cancer or Hodgkin's disease were not observed.

Of interest is that 24.5% of the deaths were sudden and unexpected. Of those, multiple hormone deficiencies were present in at least 74%, a history of hypoglycemia was present in 31% and seizures had occurred in 52%. Deaths followed a clinical course suggestive of adrenal insufficiency in 56% of deaths. Sudden unexpected death was also associated with the presence of a medical problem other than isolated GH deficiency—craniopharyngioma (24%) or other intracranial tumors (14%). Hypoglycemia in children was associated with a 9 fold increase in risk. The death rate in those with adrenal insufficiency remained stable as children aged.

The authors emphasized 3 findings. First, hypoglycemia was an important risk factor for death, which decreased as the children aged and presumably could identify and treat their own symptoms. Second, tumors were an important cause of death, even though the risk of colorectal cancer, Hodgkin's disease and overall cancer deaths were not increased. Third, adrenal insufficiency was an unexpected high-risk factor leading to death even in adulthood. They stated that increased steroid doses for even supposedly trivial infections was important as 30 of these 35 subjects were found dead or comatose and most likely died of unrecognized or inadequately treated adrenal insufficiency.

Mills JL, Schonberger LB, Wysowski DK, et al. Long-term mortality in the United States cohort of pituitary-derived growth hormone recipients. *J Pediatr* 2004;144:430-436.

**First Editor's Comment:** *This report presents some alarming and some re-assuring information. That patients who had received pGH have a markedly increased relative risk of dying is alarming. That the cause of their deaths, in many instances could be prevented by appropriate glucocorticoid administration for rather trivial infections suggests that endocrinologists are not teaching*

*or reminding patients of the importance of increasing their medications or seeking medical assistance at the first sign of infection. The reassuring news is that there does not appear to be an increased risk of colorectal cancer, Hodgkin's disease or other cancers in this cohort. Furthermore, there have been no new cases of CJD in subjects who began pGH treatment after 1977.*

*These data are interesting and compelling and deserve to be read by those who care for these children. At quick glance, it might appear that pGH administration was a dangerous treatment. On closer inspection, the facts are much friendlier. One can anticipate discussing these findings with parents of these patients.*

William L. Clarke, MD

**Second Editor's Comment:** *This is an important paper which clearly documents higher mortality risks of hypopituitary patients and the surprisingly high number of unexpected sudden deaths. The concern with CJD is justified and requires continuous surveillance, but there isn't much we can do to prevent it in those who harbor the prion. However there is a lot we must do when treating hypopituitary patients to prevent unexpected fatalities. Adrenal insufficiency must be aggressively treated particularly in patients who vomit when ill. It is not clear why the patients in this report failed to do so, but it is clear that more emphasis is needed so patients receive appropriate steroid replacement during periods of stress. Familiarity breeds complacency—or so it seems. However, there were 20 sudden deaths in patients without adrenal insufficiency. The possibility that uncontrolled diabetes insipidus played a role should also be kept in mind, particularly when oral DDAVP therapy may not be effective, such as when a patient vomits.*

Fima Lifshitz, MD

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### PREGNANCY IN ADOLESCENTS WITH TYPE 1 DIABETES

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#### INTRODUCTION

Adolescence is a time of many changes. For an adolescent with type 1 diabetes mellitus (T1DM), change means becoming more self-reliant in dealing with chronic illness. Rebellion, acting out, and the desire to be "normal" may drive the adolescent with chronic disease to make poor choices.<sup>1,2</sup> Such choices may have detrimental effects on dietary intake, medication usage, and social behavior.<sup>1,3</sup> For diabetic adolescents, low self-esteem and depression may contribute to behavior resulting in an unplanned pregnancy. This is of high risk to both the woman and fetus and is associated with high rates of congenital malformations, spontaneous abortions, and stillbirths.<sup>4,5</sup> Additionally,

#### From The Editor's Desk

Volume 20, number 4, of *GGH* marks the first year of the journal under my direction. I am proud of what we accomplished and I thank the editorial board for their support. The scope of pediatric endocrinology keeps on diversifying and with it, the journal's contents. The lead articles of the 2004 volume reviewed important subjects, some beyond the immediate concerns of our colleagues. In this issue the topic of pregnancy in adolescents with type 1 diabetes mellitus by Brindley & Jovanovic addresses the risks and consequences of adolescent pregnancy; these may be costly to both the mother and the fetus, thus underlining the importance of dealing with contraception as part of the treatment of our patients. Also included are the abstracts and editorial comments of exciting papers selected by our editorial board, these deal with pertinent clinical concerns and basic discoveries in the etiopathology of patients encountered in pediatric endocrine practice. Please note the **new feature** introduced to the journal in this issue, namely the electronic abstracts which are only displayed on the website. This new feature allowed the publication of important abstracts with erudite comments; these could not be included in the printed version of the journal because of space limitations. These e-abstracts are concurrently listed in the table of highlights with those published in both the printed and the electronic version of the journal.

During the last year, we accomplished a tremendous growth in the number of subscribers that enjoy *GGH* through the Web and we welcome over 1700 new readers. The reach of the journal also increased, over 37% of our online readers are now from countries beyond the United States, almost a 45% increase in worldwide distribution, with an excess of 30,000 visitors to date. However, we were often challenged with wrong email addresses and returned notifications. The protective filters pose obstacles to the exchange of legitimate scientific information through the internet. We no longer include a Table of Contents in the email announcement as this may trigger filters (ie, intersex). Thus, I want to remind our subscribers to please inform us of email address changes and to notify their I.T. staff to allow [www.GGHjournal.com](http://www.GGHjournal.com) through the institutional filter systems.

I am pleased to inform you that we **will not** discontinue the printed version of the journal as was planned. It will be published and distributed by surface mail within the United States. Finally, a word of thanks to our sponsor, Genentech Inc., for their continuous support through an unrestricted educational grant award for the publication of *GGH*.

Respectfully,  
Fima Lifshitz, MD

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the pregnancy can complicate diabetes. Retinopathy and nephropathy may worsen,<sup>6,7</sup> and preeclampsia and hypertension of pregnancy occur more frequently.<sup>8</sup> However routine physician visits usually focus on the state of the disease without addressing the sexual habits and/or contraceptive options for adolescents. Planned pregnancies are not relevant for most teenagers, thus pregnancy is usually unintended.<sup>9,10</sup> Medical intervention usually begins after embryogenesis and organogenesis,<sup>11</sup> and the level of glycemic control, is often sub optimal at the time of conception and early development.<sup>9,12</sup> This review aims to bring to the attention of pediatric endocrinologists the importance of this issue.

## MENARCHE AND MENSTRUAL DISTURBANCES

The hypothalamic-pituitary-ovarian axis is often incompletely mature in adolescents with T1DM<sup>13</sup> resulting in delay of menarche, irregular menses, and secondary hypogonadotropic amenorrhea, oligomenorrhea or polymenorrhea. Those with poorer control were those with oligomenorrhea/amenorrhea. Strotmeyer, et al<sup>14</sup> also reported a highly significant difference between age of menarche in patients with debut of diabetes before age 10 years compared to healthy controls and sisters (Table 1).

Moreover, poor metabolic control of T1DM is associated with worsening menstrual disturbances.<sup>15</sup> Diabetic adolescents with irregular menses, primary amenorrhea, secondary amenorrhea, or oligomenorrhea had a significantly higher glycosylated hemoglobin (A1C) level (11.4% vs 9.7%), than diabetic adolescents with regular menses.<sup>15</sup> As the A1C value increased above 10%, the prevalence of menstrual disturbances also increased; when the glycemic control improved, menstrual regulation ensued.<sup>16</sup> Diabetic adolescents with irregular cycles had a mean A1C of 12.8%, compared to a mean of 10.5% in those with regular cycles. Poor glycemic control is unfortunately a common problem

in adolescents with T1DM.<sup>3,17,18</sup> The mean A1C in children was 8.6%<sup>17</sup> and in adolescents peaked at 9% to 9.5%. In the United Kingdom, the mean A1C was 9.1%, with less than 15% of pediatric and adolescent patients having an A1C level <8.0%.<sup>18</sup> Poor glycemic control among adolescent diabetic patients is also associated with other issues that compound the control of the disease.<sup>1,3,19</sup> The prevalence of eating disorders, anorexia nervosa and bulimia nervosa in adolescent females with T1DM is increased compared to age-matched controls. Those patients with an eating disorder (DSM-IV criteria) had a higher A1C level than those who did not (9.4 vs 8.6%).<sup>1,19</sup> Additionally, psychiatric disorders such as anxiety and depression are more common in female adolescents with chronic disease than in their healthy counterparts.<sup>20</sup> Although not specific to diabetes, the Adolescent Health Survey of Barcelona,<sup>20</sup> reported significantly elevated rates of low self esteem, personal problems, and feeling sad in chronically ill adolescents. A similar increase in psychological disorders occurred more often in adolescents with T1DM and was associated with poorer glycemic control.<sup>21,22</sup>

The changing insulin needs of adolescents as they mature through puberty may also contribute to the tendency for poor glycemic control. The peak insulin requirement (up to 2 units/kg/day) occurs at Tanner Stage 3. As the diabetic young woman progresses through Tanner Stage 5, there is a gradual reduction in insulin requirements. Insulin misuse or insulin omission may also be used as a weight-control method.<sup>1,3,19</sup> As a result, glycosuria increases and the sense of being able to eat "anything" may be reinforced. On the other hand, when the daily insulin dose is high, there is a tendency for weight gain.

## UNPLANNED PREGNANCY

Adolescents with T1DM are as likely as non-diabetic adolescents to engage in unprotected sexual activity. However, at their medical appointments, physician visits are more likely to focus on the state of T1DM, compliance with medical regimens, and laboratory data, and not deal with birth control and/or contraceptive usage. Chronically ill adolescents are less likely to receive contraceptive counseling and sexual education than healthy counterparts.<sup>23,24</sup> Young women with T1DM are less likely to receive the most effective hormonal contraceptive, a combined estrogen-progesterone pill, than those without the disease.<sup>24-26</sup> Other often less effective methods of contraception such as condoms, IUDs, and surgical sterilization are more often recommended for diabetic women than for non-diabetic women.

Furthermore, T1DM adolescents may, in an attempt at independence, act in ways that are counterproductive.<sup>1</sup> This may include changing medications or dosing schedules, eating forbidden foods, experimenting with drugs or alcohol or engaging in other behaviors that are risky to their health. For some adolescents with diabetes,

**Table 1**  
Descriptive characteristics of women with and without T1DM<sup>14</sup>

|                                 | T1DM       | Without Diabetes Sisters | Controls   | p      |
|---------------------------------|------------|--------------------------|------------|--------|
| n                               | 143        | 186                      | 158        |        |
| Age at menarche (years)         | 13.5 ± 1.9 | 12.5 ± 1.4               | 12.6 ± 1.4 | <0.001 |
| Ever oral contraceptive use (%) | 44.0       | 79.0                     | 79.8       | <0.001 |
| Mean number of pregnancies*     | 2.3 ± 1.6  | 2.9 ± 1.4                | 2.6 ± 1.4  | <0.001 |
| Miscarriages (%)*               | 31.2       | 32.1                     | 27.9       | 0.76   |
| Stillbirths (%)*                | 10.1       | 0.6                      | 0.9        | <0.001 |
| Ever smoked (%)                 | 41.8       | 48.4                     | 50.0       | 0.33   |
| College attendance (%)          | 64.6       | 65.6                     | 75.9       | 0.06   |
| Income >\$40,000 (%)            | 40.8       | 59.1                     | 52.7       | 0.006  |
| Mean BMI (kg/m <sup>2</sup> )   | 24.6 ± 4.5 | 25.2 ± 5.3               | 27.4 ± 7.3 | 0.003  |

Data are mean ± SD.

\* excluding women who had never been pregnant

Adapted from Strotmeyer ES, Steenkiste AR, Foley TP Jr, Berga SL, Dorman JS. *Diabetes Care* 2003; 26:1016-1021.

pregnancy may be the only way to prove that one is a "normal" adolescent female.

The United States and the United Kingdom have the highest rates of teenage pregnancy in the world; in the U.S., 52 of 1000 adolescents between the age of 15 and 19 gave birth in the year 2000.<sup>27</sup> Within the first month of initial intercourse, 20% of adolescent young women become pregnant and nearly 50% have a second pregnancy while in their teenage years.<sup>9</sup> Young women with diabetes are more likely to become pregnant than their age-matched controls (ages 16–24), or age-matched young women with phenylketonuria, another chronic metabolic disorder with strict dietary control issues. They are more likely to have been pregnant before, but not more likely to have given birth.<sup>10</sup>

Adolescent pregnancy is associated with higher than expected rates of intrauterine growth retardation (IUGR) and premature births.<sup>28,29</sup> Low birth weight, preterm delivery, small for gestational age, and other malformations were associated with maternal age <18 years.<sup>29</sup> Poorly controlled diabetic women have higher rates of perinatal mortality and fetal malformations than nondiabetic women.<sup>5,30</sup> Although the data on pregnant women with diabetes is obtained from groups involving various age groups, the adolescent with T1DM may be at a greater risk.<sup>31</sup> Lack of dietary folate supplementation prior to conception, as well as lack of proper nutrition, inadequate weight gain, and poor metabolic control may contribute to poor pregnancy outcome in T1DM.<sup>31,32</sup> Inadequate weight gain during pregnancy also increases risks of neural tube defects.<sup>33,34</sup> Other negative factors include lack of pregnancy planning and delayed access to prenatal care or poorly attended prenatal classes; the majority of such pregnancies occur in unwed and poorly educated young women.<sup>28,29</sup>

### EFFECT OF DIABETES ON PREGNANCY

Unplanned pregnancies are often complicated in healthy teenagers, but pregnant adolescents with chronic diseases are at greater risk. Pregnancy in T1DM is considered a high risk to both the woman and the fetus. In these pregnancies, the rates of pregnancy-induced hypertension, preeclampsia, premature delivery, and cesarean section were more than 4-fold the rates observed in the non-diabetic population. Also, the prevalence of infants born large for gestational age was much higher (20% vs 3.5%) and the gestational age was significantly less. Elevated maternal A1C level early in pregnancy was an independent risk factor for pregnancy-induced hypertension and preeclampsia.<sup>8</sup> Moreover, the presence of diabetic nephropathy (defined as persistent proteinuria or albuminuria >300 mg/day) in the first 20 weeks of pregnancy, was associated with an increased risk of IUGR, fetal distress, and preeclampsia. Preterm deliveries and/or cesarean section births were increased as well. The presence of microalbuminuria (30–300

mg/day) can also complicate the pregnancy of T1DM patients; they present increased rates of preeclampsia, preterm births, and infants with IUGR.<sup>35</sup> The pregnancy of a diabetic young woman may also be complicated by the use of multiple medications. Treatment of chronic hypertension or pregnancy-induced hypertension may be required.<sup>36</sup> Unfortunately compliance with medical regimens is low in T1DM adolescents,<sup>1,3</sup> making the treatment of hypertension challenging.

Maternal hyperglycemia has been shown to complicate pregnancy more than any other factor. A1C levels at the time of fertilization and embryogenesis have been linked to a higher rate of spontaneous abortions and congenital malformations.<sup>5</sup> Those with A1C levels >7.5% had a 4-fold increase in spontaneous abortions and a 9-fold increase in congenital malformations. The risks of diabetic ketoacidosis (DKA) during pregnancy include life threatening metabolic derangements for the woman and intrauterine demise for the fetus.<sup>37</sup> DKA in adolescents usually results from insulin omission and infection.<sup>38</sup> Furthermore, the tightly controlled blood sugars recommended in pregnancy<sup>39,40</sup> increase the risk for maternal hypoglycemia and fetal injury. Of interest is that pregnant T1DM women have a lower risk of miscarriage and of delivering infants with birth defects and congenital malformations than women with T2DM.<sup>41</sup>

### EFFECT OF PREGNANCY ON DIABETES

While diabetes can complicate pregnancy, the pregnancy itself may complicate the woman's diabetes. The ever-changing insulin needs of a pregnant diabetic can be very difficult to meet, even for the most dedicated patient. The diabetic adolescent is challenged to strictly follow the dietary demands of pregnancy and the rigorous insulin regimens. There are also medical complications of diabetes that may develop and/or worsen during pregnancy. Young adults with a mean duration of diabetes of 12.7 years were shown to have retinopathy (70% background and 10% proliferative) at baseline.<sup>42</sup> Bouhanick et al reported a retinopathy rate of 50% 15 years after the onset of disease.<sup>43</sup> The Diabetes in Early Pregnancy Study<sup>44</sup> assessed the progression of retinopathy with fundus photography early after conception and followed through 1 month postpartum. There was progression to retinopathy in 10% of those who had none to start with, and in 50% of those who had baseline moderate-to-severe non-proliferative retinopathy. The Diabetes Control and Complications Trial (DCCT)<sup>8</sup> also reported an increased risk of progression of retinopathy in both the conventionally treated and in the intensively treated group of pregnant women. The proposed mechanism of the progression of this complication is either suboptimal control or a rapid change in the control of the illness that occurs early in pregnancy. Elevated A1C at baseline and degree of improvement of glucose control through week 14 were found to correlate with greater progression of retinopathy.<sup>38</sup> The progression slows or regresses after pregnancy; 6½-year follow-up studies indicated that

retinopathy in previously pregnant patients was similar to that observed in never pregnant controls.<sup>6</sup>

In contrast, pregestational diabetic nephropathy may not be adversely affected by pregnancy.<sup>5,36</sup> Although the albumin excretion rate in T1DM pregnant women increased in the intensive treatment group of the DCCT, it was not different from that in non-pregnant controls at 6.5 years of follow-up. Although microalbuminuria may worsen in pregnancy, it generally returns to baseline within a few months postpartum.<sup>45</sup> Maintaining glycemic levels and blood pressure close to normal are the best strategies to prevent progression of renal disease.<sup>46</sup> Patients with moderate to severe nephropathy early in pregnancy may progress and continue to do poorly postpartum. Purdy et al<sup>7</sup> reported that postpartum renal function in diabetic women with creatinine >1.4 mg/dL at the onset of pregnancy, declined permanently in 45%, transiently worsened in 28%, and remained stable in 27% of the women.

## FETAL OUTCOMES

Congenital malformations are 4 to 10 times more likely in offspring of diabetic women than in non-diabetic women. These anomalies account for the majority of the increased perinatal mortality associated with pregnancies complicated with diabetes.<sup>4,5,30</sup> The major congenital malformations include cardiovascular, neural tube and skeletal abnormalities (Table 2).<sup>46,47</sup> Renal abnormalities and hypospadias also occur at increased rates.<sup>30</sup> Spontaneous abortions are more frequent,<sup>8</sup> but the degree of increase is somewhat controversial. Hanson et al<sup>46</sup> reported a highly significant increase when the mother's glycemic control was poor (A1C >10.1%). Danish women with diabetes self-reported rates of spontaneous abortions at 17.5% compared to 10% to 12% in nondiabetic controls.<sup>48</sup> In the review of Strotmeyer et al,<sup>14</sup> 10% of the pregnancies in T1DM ended in stillbirth, compared to 0.6% of the sisters and 0.9% of the controls ( $p<0.001$ , Table 1). Similarly, Casson et al<sup>30</sup> reported a 5-fold increase in stillbirths in such pregnancies. In an audit of stillbirths in T1DM, Lauenborg et al<sup>49</sup> identified causes for stillbirths as DKA, chorioamnionitis, placental abruption, placental infarctions, severe IUGR, and thrombosis of the umbilical cord. The women with stillbirths had sub optimal glycemic control (A1C >7.5%) early in pregnancy more often than the women without stillbirths, 64% vs 33% ( $p<0.004$ ), and continued to have poor control during pregnancy. Maternal DKA was associated with a very high fetal mortality rate.<sup>37,38</sup> Other adverse outcomes of pregnancies complicated by diabetes include a higher rate of macrosomia, IUGR, neonatal respiratory distress syndrome, and shoulder dystocia.<sup>30,39</sup> Folate deficiency and inadequate weight gain are well established causes of neural tube defects, especially in poorly nourished adolescents.<sup>31-34</sup>

**Table 2. Congenital malformations in infants of diabetic mothers**

| System         | Malformation  | Age of gestation* |
|----------------|---|-------------------|
| Neurologic     | anencephaly, holoprosencephaly, microcephaly  | 4                 |
| Skeletal       | sacral agenesis, caudal agenesis  | 3                 |
| Cardiovascular | ventricular septal defect, transposition of the great vessels, patent ductus arteriosus, pulmonary stenosis | 5-6               |
| Renal          | duplication of ureter, renal agenesis,  | 5                 |
| Other          | hypospadias   | 4                 |

\* weeks

Medications taken prior to conception, especially during fertilization and organogenesis, may have detrimental effects on the fetus. For example, angiotensin-converting enzyme (ACE) inhibitors may cause fetal oliguria, severe fetal hypotension, and osseous cranial anomalies.<sup>36</sup> Likewise, the use of the acne medication isotretinoin during pregnancy has been associated with very severe fetal abnormalities of the central nervous system, cardiovascular system, craniofacial formation, as well as parathyroid hormone deficiency.<sup>50</sup> Moreover, cigarette smoking, drugs, and alcohol use may cause untoward effects on the fetus. Severe hypoglycemia may lead to maternal seizures and loss of consciousness which may cause automobile accidents and may result in neuropsychological problems, with electrophysiological impairment in the child.<sup>2,39,40</sup> Rebound hyperglycemia after hypoglycemic events is thought to be a cause of fetal macrosomia.<sup>40</sup>

## PRECONCEPTION CARE AND MANAGEMENT DURING PREGNANCY

A diabetic woman who wishes to become pregnant needs preconception advice and counseling. Before pregnancy, glycemic control should be maximized and the underlying disease should be assessed thoroughly. Preconception counseling in T1DM decreases the risk of congenital malformations, spontaneous abortions, and stillbirths (Table 3).<sup>11,12,47,51,52</sup> Although the data reported were in adult pregnant women, the information is applicable to adolescents. Malformation rates and mortality rates dropped from 14% to 2.2% and 7% to 2%, respectively during a 15-year period.<sup>51</sup> The rates began to rise when the program was discontinued. The T1DM women who received preconception counseling for a mean duration of 17 weeks prior to becoming pregnant had a reduced rate of congenital malformations compared with controls (1.2% vs 10.9%), though the level of glycemia in both groups was similar.<sup>11</sup> Thus, preconception intervention is most beneficial in positively impacting the critical periods of embryogenesis and organogenesis. Preconception care in diabetic adolescents, coupled with ongoing prenatal intervention, reduces the high rate of spontaneous abortions and improves infant outcome.<sup>12,52</sup> Glycohemoglobin levels at first diagnosis of pregnancy are lower in women who attend such programs and correlate with better glycemic control during conception and embryogenesis.<sup>11,12,52</sup>



The majority of teenage pregnancies are not intended and out-of-wedlock adolescent pregnancies are not well received in the United States.<sup>27,29</sup> Thus, preconception counseling is usually not applicable. The necessary intense care of a pregnant adolescent with T1DM is cumbersome and difficult, though when applied through the entire pregnancy it leads to better outcomes. A team approach to care for T1DM is most effective and should be instituted as soon as possible.<sup>11,12,47,51,52</sup> Prenatal care should include nutritional counseling and weight gain guidance based on the preconception body weight and adequacy of the nutritional intake to avoid hypoglycemia and ketosis.<sup>53</sup> Dietary habits and preferences need to be considered to facilitate compliance and to meet nutrient requirements. Although controversial, most agree in striving for euglycemia, while ensuring appropriate weight gain of the woman and fetus. Jovanovic allows for an intake of 40% calories as carbohydrate, 20% as protein, and 40% as fat, with the caveat that breakfast is small (less than 10% of total calories). These percentages should be adjusted for glycemic control, insulin usage, and level of activity. Others favor a more liberal intake of carbohydrates (45%-55%) as long as the premeal insulin dose is adjusted appropriately.<sup>53</sup> Much of the available data on dietary intake are from gestational diabetes studies without the specific concerns of T1DM adolescent patients who more easily experience hypoglycemia or ketosis and are not very compliant.

Prenatal vitamins including folate and calcium should be initiated as soon as pregnancy is diagnosed. Folate doses are often increased up to 5 milligrams in order to prevent the neural tube abnormalities frequently found in infants of T1DM mothers.<sup>54</sup> If there is any indication of drug, alcohol, or cigarette use, these should be discouraged and discontinued. Any pre-pregnancy medications such as diuretics, ACE inhibitors, or acne treatments should be stopped immediately. Antihypertensive medications that are safe in pregnancy should be started and adjusted to maintain tight blood pressure control (ie, calcium channel blockers).<sup>55</sup> Alpha-methyldopa and hydralazine are two antihypertensive medications that have been used more extensively in pregnant women.

Early photography of the fundi will serve as a baseline to assess the degree of retinopathy and may infer the degree of microangiopathy present. Close follow-up with an ophthalmologist is necessary at least every trimester, if baseline photographs are normal, and more frequently

if baseline photographs show any degree of abnormality. A detailed antenatal evaluation for diabetic women with nephropathy, including evaluation of serum creatinine, uric acid, urea nitrogen, creatinine clearance, and urine culture is important. Creatinine clearance and protein excretion should be assessed at least every trimester, and more frequently if abnormal. Monitoring for anemia (due to renal loss of erythropoietin or iron deficits) and of thyroid function (due to the high rate of coexistent autoimmune thyroid disease in patients with T1DM<sup>56</sup>), are recommended and appropriate treatment instituted at once to avoid possible poor outcome for the infant.<sup>57</sup>

Many authors have reviewed the targets for optimum glycemic control; however, a consensus has not been reached. Jovanovic recommends 1-hour postprandial whole blood glucose <120 mg/dL (6.7 mmol/L), and fasting blood glucose <90 mg/dL (5.0 mmol/L). The American Diabetes Association recommends <140 mg/dL (7.8 mmol/L), and <100 mg/dL (5.6 mmol/L), respectively.<sup>58</sup> The A1C levels should be checked regularly to ensure compliance with the program and to guide insulin doses and dietary advice. The rapid-acting insulin analogs have been shown to be safe in gestational diabetes, and preliminary data indicate that they are safe in T1DM pregnancies. However, there are no data on long-acting insulin analogs use in pregnancy. Furthermore, obstetrical care and diabetes care should be jointly agreed upon to maximize patient participation and outcome of the pregnancy. Regular follow-up visits, ongoing dietary counseling, and emotional and psychosocial support are needed. Plans for the newborn, including child rearing, adoption, or alternative care (eg, grandparents) should be initiated as early as possible. A plan for the adolescent to complete her education is another major issue best approached early. Counseling for the mother-to-be, the father of the baby (if available), and the future grandparents is recommended.

## PREGNANCY PLANNING AND PREVENTION

Due to the high risk nature of pregnancy in adolescents with T1DM, pregnancy planning and/or prevention should play a major role in their care. The focus should be on prevention of pregnancy and improving the sexual education of the adolescent population.<sup>10,28</sup> The American Diabetes Association recommends that all women with diabetes of child-bearing potential use appropriate contraception and receive counseling about the risk of malformations associated with unplanned pregnancies

and poor glycemic control.<sup>55</sup> Unfortunately, contraception for teenagers has been politicized, thus without parental involvement and/or consent it may be difficult to obtain in the United States.

**Table 3. Outcome of Pregnancies Complicated by Diabetes**

| Authors                          | Congenital Malformations |                | Spontaneous Abortions |               | p value   |
|----------------------------------|--------------------------|----------------|-----------------------|---------------|-----------|
|                                  | Preconception care       | Routine care   | Preconception care    | Routine care  |           |
| Dicker, et al <sup>52</sup>      |                          |                | 5/59 (8.5%)           | 10/35 (28.6%) | <0.001    |
| Steel, et al <sup>47</sup>       | 2/143 (1.4%)             | 10/96 (10.4%)  |                       |               | <0.005    |
| Kitzmillier, et al <sup>11</sup> | 1/84 (1.2%)              | 12/110 (10.9%) |                       |               | <0.01     |
| Rosenn, et al <sup>46</sup>      | 0/28                     | 1/71 (1.4%)    | 2/28 (7%)             | 17/71 (24%)   | NS, <0.04 |



and the United Kingdom—the countries with the highest teen pregnancy rates.<sup>27</sup> Contraception in diabetic young women can be accomplished with cooperation between the patient, primary care physicians, gynecologists, and endocrinologists or diabetologists.<sup>23</sup> The factors associated with consistent birth control use in diabetic women and women with phenylketonuria were social support and positive attitudes toward birth control.<sup>10</sup> Low dose combination hormonal contraceptive pills are recommended; these can be safely used in adolescent T1DM patients in whom vascular disease is a low risk.<sup>25</sup> Barrier methods and spermicidal agents may be less acceptable to teenagers, resulting in poor compliance.

Emergency contraception, the so-called morning after pill, is another consideration. Use of such preparations has been limited due to prescription requirements, fear of hormones, possible adverse effects, and misinformation on availability and use.<sup>59</sup> The proposal to switch levonorgestrel emergency contraception (approved for prescription use in 1999, sold under the brand name Plan B®, Barr Pharmaceuticals, Pamona, NY) to over-the-counter status was not approved in May 2004 by the US Food and Drug Administration. Plan B consists of 2 (0.75 mg) pills of levonorgestrel to be taken as soon as possible within 72 hours after unprotected sexual intercourse. The rate of pregnancy is 0.4% if treatment is initiated within 24 hours and 2.7% if given within 72 hours. There are extensive data on the safety of this medication, though specific data on adolescents with diabetes are not available. The most frequent side effects are nausea and menstrual disruption. During a 29 month period, between 2001 and 2003, 40% of 7774 callers to a telephone prescription service in North Carolina (designed to increase access to emergency contraceptive pills) were teenagers.<sup>57</sup> Adolescents with diabetes frequently depend on pediatric endocrinologists for their care, thus a prescription for Plan B emergency contraception should be considered in advance of the crisis which may follow unprotected sexual intercourse. Additionally, the option for termination of pregnancy should be presented in a factual manner to the young woman, regardless of her religious background, so it may be performed as early as possible.

There are other considerations that need to be addressed in the course of the treatment of the adolescent with T1DM, particularly the encouragement of daily use of folic acid supplementation, even in those who are not sexually active or when pregnancy is not a consideration.

## CONCLUSION AND SPECULATION

The adolescent diabetic woman struggles with daily reminders of her disease—multiple fingerstick glucose checks, insulin injections, and an uncertain future of possible complications. Although preconception care is preferable, most adolescents do not intend to become pregnant. Unplanned pregnancy can be avoided with education, support, and contraceptives, offered to the

adolescent by diabetic educators, parents, and physicians. If pregnancy does occur, timely institution of excellent diabetes and obstetrical care promises at least a brighter future for the young woman and the infant.

Future challenges for the physician caring for pregnant young women with T1DM may include use of the rapid-acting insulins, long-acting insulins, and insulin pumps. Furthermore, if islet cell transplantation continues to show promise, consideration for unplanned pregnancies in young women on long-term immunosuppressant medications will need to be addressed. Interesting new data on gestational diabetes using fetal growth ultrasound to manage a patient, rather than strict dietary control and stringent glycemic guidelines<sup>58</sup> may offer a useful approach in pregnant T1DM adolescents. Outcomes including caesarean rate, small and large neonates, hypoglycemia, and neonatal intensive care admissions, were equivalent. Perhaps the lighter the burden we place on the teenager to conform to medical guidelines, the better chance we have of dealing with rebellion. However, adolescents will always be adolescents, for generations to come.

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## ABSTRACTS FROM THE LITERATURE

### Celiac Autoimmunity, Celiac Disease and Growth

The objective of this study was to evaluate growth and clinical features of children who tested positive for antibodies associated with celiac disease (CD). A cohort of HLA-DRB1\*03-characterized newborns from 1234 families in Denver, Colorado were prospectively followed since birth for the development of IgA autoimmune transglutaminase antibodies (TG). Clinical evaluation, growth, anthropometry and biochemical assessments, as well as small bowel biopsies were performed. There were 33 children who tested positive to TG; 18 of them completed the studies, underwent repeated testing and were compared with 100 pair-matched controls. The TG-positive children had antibodies detected at a mean age of 4.4 ( $\pm 1.2$ ) years and the mean age at clinical evaluation was 5.3 ( $\pm 1.5$ ) years. They had significantly lower z-scores for height, weight and BMI ( $-0.3 \pm 0.7$ ), but not for weight- or height-for-age. They also had decreased mid-arm circumference and mid-arm muscle mass area. TG-positive children experienced more symptoms which increased over time, including abdominal pain, constipation and irritability/lethargy and these were independently associated with decreased weight gain. Thirteen (72%) of the 18 TG children had small intestinal mucosa evidence

of CD (Marsh 2-3), 2 showed increased intraepithelial lymphocytes (Marsh 1), and 3 had normal biopsies. No relationship was found between copies of HLA-DRB1\*03 and biopsy scores. The authors concluded that screening for CD identified TG-positive children who demonstrated mild alterations in weight and body composition and reported more symptoms than control subjects. They also had intestinal mucosa evidence of CD.

Hoffenberg EJ, Emery LM, Barriga KJ, et al. Clinical features of children with screening-identified evidence of celiac disease. *Pediatrics* 2004;113:1254-1259.

**Editor's Comment:** This prospective study provided important data of the natural history of CD autoimmunity in a genetically susceptible population. It also discerned the clinical findings of TG-positive children and the small intestinal mucosa alterations. However, the response to a gluten-free diet was not reported; expert consensus panels require the assessment of the response to dietary therapy as important evidential data for the diagnosis of CD. Additionally, the nutrient intake or fecal-fat excretion was not reported. It is possible that children decreased food ingestion to minimize the discomfort of malabsorption, thus

contributing to decreased weight and body composition. The prevalence of CD in children ranges between 0.4% to 1.0%,<sup>1,2</sup> whereas the prevalence of CD autoimmunity was close to 3% in this genetically susceptible population. TG-positive children presented few if any symptoms, and the autoimmune markers had a lower predictive value (75%) of detecting small bowel evidence of CD.<sup>3</sup> Symptomatic CD patients are at risk of long-term consequences, including osteoporosis, lymphoma and other autoimmune processes,<sup>4,5</sup> though no data are available on the risks of patients with CD autoimmunity and silent disease. However, CD screening of at-risk patients is increasingly being done by pediatric endocrinologists in patients with T2DM, short stature, Turner or Down syndromes who have shown a prevalence of CD autoimmunity of up to 15%. Screening for CD is best accomplished by measurements of TG and endomysial antibody immunofluorescence IgA. Both have a high sensibility and sensitivity, whereas antigliadin antibodies do not. However, the case finding efforts need

to be tempered with the cost of labeling children with CD, with the realization that this disease is difficult to prove and that only half of the patients follow a strict gluten-free diet.<sup>6</sup> The benefits from early diagnosis and treatment of silent patients have not been demonstrated. Thus more research is warranted along with careful monitoring of height and weight progression of children with CD autoimmunity.

Fima Lifshitz, MD

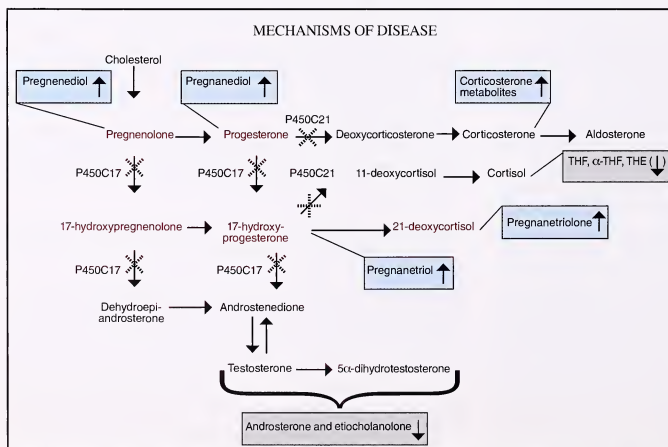
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## Congenital Adrenal Hyperplasia, Antley-Bixler Syndrome and Mutant P450 Oxidoreductase

Patients with biochemical evidence of apparent combined deficiencies of 17 $\alpha$ - and 21-hydroxylase have been recognized for almost 2 decades. The clinical features include infant females born with mildly to moderately virilized external genitalia in whom virilization does not progress post partum, to adult women with menstrual irregularities (amenorrhea). Affected males have normal external genitalia, cryptorchidism, or hypospadias. Serum and urine glucocorticoid and androgen values are

normal or low, while levels of precursors (progesterone, 17-hydroxyprogesterone, pregnenolone, pregnenolone, pregnenolone) are elevated. Recent analysis of the genes encoding the enzyme proteins (*CYP17A1*, OMIM 202110, chromosome 10q24.3; *CYP21B*, OMIM 201910, chromosome 6p21.3) did not disclose any mutations. The Antley-Bixler syndrome (ABS, OMIM 207410) is characterized by facial (midface hypoplasia with proptosis, choanal atresia, frontal bossing, dysplastic ears) and skeletal abnormalities (cranial, humero-radial and radio-ulnar synostoses, femoral and ulnar bowing, camptodactyly, joint contractures), and in some patients by genitourinary abnormalities (renal agenesis, vaginal atresia, virilization of female external genitalia, undermasculinization of male genitalia). Heretofore, ABS has been primarily attributed to mutations in *FGFR2*.



**Steroidogenesis and its impairment in patients affected by apparent combined P450C17 and P450C21 deficiency**  
Serum steroids reported in increased amounts are shown in red, and pathologically altered urinary steroid metabolites are shown in blue boxes if raised and gray boxes if reduced, linked to the steroid from which they are derived. Crosses indicate impairment of enzymatic activity.

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Fluck et al and Arlt et al reasoned that perhaps the primary problem in patients with apparent combined deficiencies of 17 $\alpha$ - and 21-hydroxylase might be due to decreased levels of a common co-factor (Figure). Both enzymes require transfer of electrons to achieve the activated state. The electron donor is cytochrome P450



oxidoreductase (*POR*, OMIM 124015, chromosome 7q11.2), a flavoprotein that contributes electrons to all microsomal P450 enzymes. It does so by binding to NADPH through its flavin adenine dinucleotide (FAD) domain to which NADPH contributes 2 electrons; these electrons are then transferred to the flavin mononucleotide (FMN) domain of *POR* that, in turn, donates them to the target P450 enzyme. Mutations in *CYP17A1* that involve its binding to *POR* lead to decreased 17 $\alpha$ -hydroxylase activity. Accordingly, these investigators analyzed *POR* in 7 patients with combined deficiencies of 17 $\alpha$ - and 21-hydroxylase deficiency, some of whom had clinical and skeletal anomalies consistent with ABS. They found compound heterozygous or homozygous loss-of-function mutations in all patients including: 531T $\Rightarrow$ G: Tyr178Asp; 731+1G $\Rightarrow$ A: donor splice site intron 6; 859G $\Rightarrow$ C: Ala287Pro; 1370G $\Rightarrow$ A: Arg457His; 1475T $\Rightarrow$ A: Val492Glu; 1706G $\Rightarrow$ A: Cys569Tyr; 1822G $\Rightarrow$ T: Val608Phe. The Ala287, Arg457, and Val492 mutations were in the FAD domain that binds NADPH and, predictably, changed steric conformation or charge leading to greatly reduced 17 $\alpha$ -hydroxylase and 17-20-lyase activities. The Cys569 and Val608 mutations were in the region that binds NADP $^{+}$  and resulted in less loss of enzyme activity. Patients with ABS tended to have the more severe mutations in *POR*, while those with the less severe defects only had disordered steroidogenesis. In no patient studied was a mutation in *FGFR2* identified.

Fluck CE, Tajima T, Pandey AV, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet.* 2004;36:228-30.

Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet.* 2004;363:2128-35.

**Editor's Comment:** Pregnant women who are heterozygous carriers of a loss-of-function mutation in *POR* may manifest gestational hyperandrogenism (acne, hirsutism) possibly due to the effects of both fetal hyperandrogenemia and impaired endogenous steroidogenesis. This resembles the hyperandrogenism seen in patients with a luteoma of pregnancy or placental aromatase deficiency. Arlt et al suggest that in the fetus with loss of *POR* activity, an alternate pathway of androgen synthesis is pursued: 17 $\alpha$ -hydroxyprogesterone is converted to 5 $\alpha$ -pregnane-3, 17 $\alpha$ -diol-20-one and the latter to androsterone by sequential actions of 5 $\alpha$ -reductase type I, 3 $\alpha$ -hydroxysteroid dehydrogenase, and low levels of 17 $\alpha$ -hydroxylase. Since this pathway disappears in early infancy, virilization does not progress. These data suggest that ABS is genetically heterogeneous; one type is due to loss of *FGFR2*, and is not associated with genital malformation; the second type is due to loss of *POR*. *POR* is required for activity of both adrenal and hepatic microsomal P450 enzymes. Indeed, in infants of mothers treated with the antifungal agent fluconazole, that inhibits ergosterol synthesis by interfering with lanosterol 14 $\alpha$ -demethylase activity, skeletal deformities resembling those in neonates with ABS have been observed. The skeletal deformities observed in children with deficiency of *POR* may reflect an error in this pathway that affects skeletal embryogenesis.

Allen W. Root, MD

## Prevention of Progression from Pubarche to Polycystic Ovarian Syndrome

There is evidence that girls with low birth weight (LBW) and precocious pubarche (prior to 8 years of age) are at high risk of polycystic ovarian syndrome (PCOS) even if not obese. Ibáñez and colleagues performed a randomized early prevention study in 24 such girls 6 to 12 months post-menarche. In each, precocious pubarche was diagnosed by high serum androstenedione and/or DHEAS levels. To be included in the study, girls had to have a birth weight for gestational age  $<-1.5$  SD, BMI  $<26\%$ , hyperinsulinemia on a 2-hour OGTT (peak serum insulin  $>150$   $\mu$ U/mL or mean serum insulin  $>84$  mU/L), and subclinical ovarian hyperandrogenism (17-HO progesterone response  $>160$  ng/dL to GnRH agonist). They were randomized to receive either metformin 850 mg once daily or no treatment for 12 months. Serial clinical and biochemical measurements were made throughout the study.

There were no differences in any parameter between the treated and untreated groups at baseline. All subjects had increased androgen levels, abnormal lipid profiles, increased total body fat and reduced lean body mass. By 12 months, the treated group showed significant decreases in androgen levels, LDL cholesterol, and total body and truncal fat mass, and increases in HDL

cholesterol and lean body mass. In addition, insulin resistance was normalized. Most of these effects were seen between 3 and 6 months of treatment. The untreated group had significant worsening of each of these parameters. The authors conclude that the early post-menarchal years are an important period in the evolution of PCOS in girls with the predisposing clinical criteria. The authors also noted that the intervention was effective although limited to a once-daily medication without any other lifestyle change.

Ibáñez L, Ferrer A, Ong K, Amin R, Dunger D, de Zegher F. Insulin sensitization early after menarche prevents progression from precocious pubarche to polycystic ovary syndrome. *J Pediatr.* 2004;144:23-29.

**Editor's Comment:** This is an important and well-designed study performed by a group of investigators with significant research experience in this area. Their suggested pathophysiologic schema for the development of PCOS consists of girls with LBW but normal catch-up growth who maintain reduced muscle mass and become insulin resistant. This predisposes them to central obesity and excessive fat mass despite appearing lean, as well as to PCOS. Ibáñez and colleagues also suggest that their



data provided evidence that the endocrine-metabolic state is primary rather than secondary in this process. These are provocative conclusions and, if applicable to other patient populations, suggest an important role for insulin sensitizers, such as metformin, in the prevention of PCOS. Most pediatric endocrinologists are encountering more patients with PCOS. Therapy often includes metformin, an androgen-receptor blocker, and/or oral contraceptives,

but the results are rarely satisfactory. Clearly, there is a need to prevent the development of this syndrome. The etiology may not be the same in all cases, but close follow-up is merited in all girls born with LBW, as well as all girls presenting with premature pubarche. It is not unreasonable to suggest preventive therapy in some of these children.

William L. Clarke, MD

## Improving Accuracy of Linear Growth Measurements

A survey study of pediatric and family primary care practices in 8 areas of the United States found that 70% employed inaccurate techniques for measuring children.<sup>1</sup> As follow-up, Lipman et al analyzed the effectiveness of an intervention aimed at improving the accuracy of linear growth measurements. From the 259 prior practice responders, 8 per geographic area were randomly recruited and divided into intervention and control arms of the trial of 55 practices (44 pediatric and 11 family practice). Practices cared for an average of 4000 children, and employed an average of 3.6 staff responsible for the measurements (21% RNs, 23% LPNs, 56% nurses' aides/medical assistants) with an average of 8.2 years experience. At baseline, correct overall measurement technique was demonstrated on 30% of measurements. Proper equipment was used in 58% of standing measured children and in 18% of recumbently measured children. The measurements differed by an average of 1.2 cm within the same child by study staff (differences ranged up to 12.1 cm). The intervention group received: a written pre-test of knowledge of growth measurement, a slide presentation and handouts on both proper measuring techniques and the physiology/pathophysiology of growth disorders, the installation of accurate measuring equipment and demonstration (plus return demonstration) on the correct measurement of height

and length, and a written post-test assessment. The control group received no intervention. Measurement techniques were re-evaluated after 3 and 6 months in both groups. Accurate measurement in the control group remained at 37% at 3 months and 34% at 6 months. The intervention group increased the accuracy of the measurements to 55% at 3 months and 70% at 6 months. At conclusion, the intervention group's mean difference in measurement from study staff decreased to 0.5 cm.

Lipman TH, Hench KD, Benyi T, et al. A multicentre randomised controlled trial of an intervention to improve the accuracy of linear growth measurement. *Arch Dis Child*. 2004; 89:342-346.

**Editor's Comment:** Growth is the single most important indication of a child's health.<sup>2</sup> Growth monitoring is an integral part of pediatric care. The American Academy of Pediatrics has recommended that height and weight be measured at least at birth; age 2-4 days; 1, 2, 4, 6, 9, 12, 15, 18 and 24 months; and yearly through age 21.<sup>3</sup> It is disheartening that Lipman et al found high prevalence of incorrect techniques among pediatric and family practices. Even more disheartening is that 10% of pediatric practices and 40% of family practices did not measure children at every well-child visit.<sup>1</sup> This is a worldwide problem with a lack of equipment or trained personnel, inaccurate plotting

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and misunderstanding of the reference curves.<sup>4</sup> Likewise, a study of an academic pediatric clinic found that 35% of well-child encounters failed to plot growth measurements and/or document a growth abnormality.<sup>5</sup> This study demonstrated that an intervention program can effectively improve the accuracy of growth measurement in clinical practices, and that the improvement increased with time. Thus, rather than forgetting the lessons learned, continued use of proper technique and proper equipment reinforced and improved performance. The most common reason for practices refusing to participate in this interventional study was "provider unwillingness due to low importance assigned to linear measurements." The importance of proper technique and equipment cannot be overemphasized as lack of these may lead to missed or delayed recognition of growth failure or can lead to unnecessary investigation and

specialist referrals. As the authors pointed out, the current average inaccuracy exceeds the difference between the defined cut-offs for normal and abnormal growth velocities. (See Online Resources at [www.GGHjournal.com](http://www.GGHjournal.com) for links to growth charts.)

Adda Grimberg, MD

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## Stature and Psychosocial Adjustment in Adulthood

The Wessex Growth Study in the United Kingdom is a prospective, longitudinal school-based study that followed the physical and psychosocial development of short healthy students, and their average stature classmates from school until 18 to 20 years of age. This report represents the third in a series; the prior studies occurred when subjects were 7 to 9 and 11 to 13 years of age. The objective was to ascertain whether any psychosocial sequelae of short stature (during childhood or at the time of follow-up) could be detected in young adulthood. The short stature group (<-2 SDs score for height) was compared to classmates of average height (10–90<sup>th</sup> percentiles). There were 48 short normal (SN) and 66 control (C) subjects; these were statistically indistinguishable on multiple sociodemographic variables. Ulph and colleagues used the Adolescent to Adult Personality Functioning Assessment (ADAPFA) to measure social and interpersonal role performance in 6 domains: education and employment; love relationships; friendships; coping; social contacts; and negotiations. Critical behaviors related to education received beyond school, employment status, relationships with a partner, parenthood, drug taking, drinking, and involvement with violence—referred to as activities of daily living—were also assessed. The data were analyzed with respect to both height at recruitment (ages 7–9) and as adults (ages 18–20). The participants were classified into 3 height groups: <2<sup>nd</sup> percentile (n=19); 2<sup>nd</sup>–50<sup>th</sup> percentile (n=61); and >50<sup>th</sup> percentile (n=34). The middle group consisted of both initial SN and C participants.

Height at recruitment was not associated with ADAPFA scores. The mean ADAPFA scores on 3 domains were (nonsignificantly) higher in the SN group, indicating poorer adaptation. (Gender and SES were significant predictors of several domain scores validating ADAPFA sensitivity.) There was no effect of adult height on outcome measures, nor was there a significant difference in the proportion of 3 adult height groups that received scores falling within

the clinical range. ADAPFA score was highest in the shortest group and for 2 specific domains. The measure of activities of daily living did not differentiate participants by recruitment or adult height. The authors conclude that healthy short stature adults did not have compromised psychological, social, or educational adaptation when sociodemographic variables were taken into account.

Ulph F, Betts P, Mulligan J, Stratford RJ. Personality functioning: the influence of stature. *Arch Dis Child*. 2004;89:17–21.

**Editor's Comment:** The Wessex Growth Study is the first and only prospective longitudinal study of social, educational and psychological adaptation of physically healthy short children from a community sample that employs a methodologically sophisticated approach. Because study participants were selected from schools, the referral bias that stems from recruitment through pediatric endocrinology clinics was obviated. Previous findings demonstrated that stature was not a statistically significant predictor of self-concept, behavioral or emotional functioning, or academic performance, although those with short stature were less satisfied with their height.<sup>1,2</sup> These earlier observations were reinforced by the current findings that adult stature was not a predictor of psychosocial adaptation. Importantly, statistical analyses in all waves of the study controlled for the influence of sociodemographic variables that are well-recognized predictors of quality-of-life outcomes, and which can be confounded with stature.

The authors conspicuously failed to mention that this cohort had also been examined during adolescence.<sup>3</sup> At that time, short boys reported being more than twice as likely as average stature boys to be the object of teasing, and much more likely to say that this upset them and that they spent break time alone. Short stature may thus place the individual at increased risk for psychosocial stress. However, the association between negative experiences

(teasing or juvenilization) and validated measures of behavioral and emotional functioning is relatively weak. Another study has shown the overall level of psychosocial adaptation of short youths derived from a clinic sample was comparable to that of the general population.<sup>4</sup> It can be inferred that, on average, short youths exposed to negative experiences adaptively cope so that signs of impairment do not emerge.

David E. Sandberg, PhD

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## The Marfan Syndrome –TGF- $\beta$ Connection

The Marfan syndrome (MFS) (OMIM 154700) is one of the original connective tissue disorders described by McKusick and colleagues. Its cardinal manifestations are aortic dilatation and dissection, dislocation of the lenses and overgrowth of long bones caused by over 600 mutations in the gene encoding fibrillin-1 (*FBN1*). Most evidence has suggested that the presence of abnormal fibrillin-1 interferes with the functions of tissues in which it resides. For example, the disturbance might weaken the ability of the aortic wall to resist hemodynamic forces, of the suspensory ligaments in the eye to hold the lens in place, or of the perichondrium to restrain the linear expansion of growing bones. However, controversy has persisted over the existence of a second MFS gene locus. A large French family with features of MFS was reported for which linkage was established not to *FBN1* on chromosome 15, but to markers located on chromosome 3p24.2-p25; the locus was designated *MFS2* (OMIM 154705).

Mizuguchi et al evaluated a child with MFS and a complex *de novo* chromosome rearrangement that included a breakpoint at the *MFS2* locus at 3p24.2-p25. The gene encoding the TGF- $\beta$  receptor 2 (*TGFBR2*) was disrupted and a point mutation disturbed its splicing in affected members of the French family. They identified 3 additional missense *TGFBR2* mutations from analysis

of 3 French families and 10 unrelated Japanese patients with MFS who had no mutation or linkage to *FBN1*, and demonstrated that several of the MFS mutations resulted in loss of receptor function.

TGF- $\beta$  receptors belong to the serine-threonine kinase family of cell surface receptors. When activated, they recruit and phosphorylate serine and threonine residues on cytoplasmic proteins that propagate TGF- $\beta$  signals to downstream pathways in cells. TGF- $\beta$ s regulate many cellular processes including proliferation, cell cycle arrest, apoptosis, differentiation and formation of extracellular matrix. Type II receptors are thought to mediate growth inhibitory signals, and *TGFBR2* is considered to be a tumor suppressor gene.

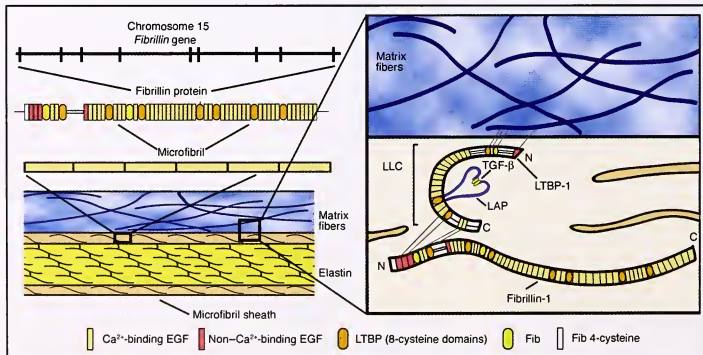
Byers described fibrillin-1 binding to so-called latent TGF- $\beta$  binding proteins (LBP). These bind to inactive, latent TGF- $\beta$ s. In this way, fibrillin-1 can influence the extracellular availability of active TGF- $\beta$ . In MFS, the model suggests that mutations reduce the amount of fibrillin-1 in the matrix. The consequence is diminished sequestration of LBP-bound TGF- $\beta$  and a relative increase in the abundance of active TGF- $\beta$  in the matrix. In TGF- $\beta$ -responsive tissues, this results in exaggeration of processes that are regulated by TGF- $\beta$ , such as growth (Figure). Support for this idea comes from work recently published by Neptune et al, in which it is demonstrated that

emphysematous changes in the lungs of fibrillin-1 null mice could be partially ameliorated by antibodies that block TGF- $\beta$  function.

Mizuguchi T, Colod-Beroud G, Akiyama T, et al. Heterozygous *TGFBR2* mutations in Marfan syndrome. *Nat Genet*. 2004;36:855-60.

Byers PH. Determination of the molecular basis of Marfan syndrome: a growth industry. *J Clin Invest*. 2004;114:161-63.

Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet*. 2003;33:407-11.



Fibrillin-1 is translated from mRNA encoded by *FBN1* gene on chromosome 15. (Reprinted with permission from: Byers PH. *J Clin Invest*. 2004; 114:161-63 Copyright ©2004 Am Soc Clinical Investigation.)



**Editor's Comment:** Taken together, these 3 papers strongly implicate TGF- $\beta$  playing a role in the pathogenesis of MFS. As suggested by these groups, the findings open up many new possibilities for treatment that focuses on excessive TGF- $\beta$  signaling as the target. These papers also highlight somewhat

of a renaissance in thinking about extracellular matrix and its components; they are not just static structural proteins, but dynamic elements that play important functional roles in development and disease.

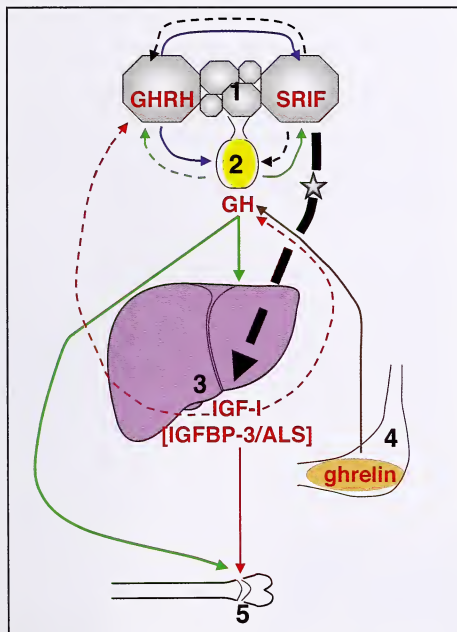
William A. Horton, MD

## Somatostatin: New Effects on the GH-IGF Axis

Somatostatin (SRIF) exerts multiple, mostly inhibitory, effects on endocrine and exocrine secretions, gastrointestinal function and cell proliferation. SRIF produced by the hypothalamic parvocellular neurons (paraventricular nucleus) inhibits pituitary growth hormone (GH) release and, subsequently, GH-induced insulin-like growth factor (IGF)-I production.<sup>1</sup> Murray and colleagues performed experiments that elegantly demonstrated peripheral inhibition of hepatic IGF-I production by SRIF and its analog, octreotide. RT-PCR of cDNA from isolated rat hepatocytes revealed expression

of both somatostatin receptor subtypes (SSTR) 2 and 3. IGF-I mRNA expression and protein secretion by isolated rat hepatocytes increased in a dose-dependent fashion after incubation with GH. This effect was inhibited by pretreatment with SRIF or octreotide, neither of which affected IGF-I levels without GH, nor affected other GH signaling pathways like phosphorylation of extracellular signal-related kinases (ERK) or induction of c-myc. SRIF or octreotide pretreatment decreased binding of radiolabeled GH to hepatocytes, and also decreased phosphorylation and nuclear localization of STAT5b, the main pathway by which GH induces IGF-I. SRIF inhibition of GH-induced IGF-I required inhibitory G proteins (G<sub>i</sub>s; frequently transduce signals from SSTRs) and involved protein tyrosine phosphorylation (PTP) activation but not increased suppressors of cytokine signaling (SOCS) 2 or 3. Furthermore, inhibition of GH-induced IGF-I production was confirmed in perfused whole rat livers *ex vivo*. Both models clearly excluded any central actions of SRIF on the GH/IGF axis.

Murray RD, Kim K, Ren S-G, Chelly M, Umehara Y, Melmed S. Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. *J Clin Invest*. 2004;114:349–356.



**The GH-IGF axis.** Pituitary (2) GH secretion is controlled by hypothalamic (1) GHRH (from the arcuate nucleus) and SRIF (from the paraventricular nucleus), by negative feedback from IGF-I (3) and by ghrelin stimulation (4). GHRH actions are shown by blue arrows, SRIF by black, GH by green, IGF-I by red and ghrelin by brown. All solid arrows signify stimulatory actions; dashed arrows are inhibitory. Both GH and IGF-I act on target organs such as the growth plates of long bones (5) to stimulate cell survival, differentiation and proliferation. The paper by Murray et al adds a new mechanism of action for SRIF: hepatic inhibition of GH-induced IGF-I production, indicated by the starred, thick-dashed arrow.

**Editor's Comment:** This paper described a new mechanism of action of SRIF and its analogs: the peripheral inhibition of GH-induced hepatic IGF-I production (Figure). SRIF analogs are the primary medical treatment for acromegaly, a state of GH excess usually caused by GH over-expression by a benign pituitary adenoma.<sup>2</sup> IGF-I elevation may become discordant with GH suppression in some treated patients, and biochemical control may not correlate with clinical improvement. These findings oppose the dogma of SRIF analog action at the hypothalamic-pituitary level. The new data support additional, peripheral action of SRIF and its analogs in suppressing GH-induced IGF-I production. This not only has important implications for the treatment of acromegaly, but also for the potential use of SRIF analogs in the treatment of cancer.<sup>3,4</sup>

Adda Grimberg, MD

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## ADHD Treatment & Growth

The multimodal treatment study of 540 attention deficit hyperactivity disorder (ADHD) patients reported the intent-to-treat analyses of 7 to 9 year old subjects who were treated for up to 24 months. Four naturalistic subgroups were formed in accordance with their patterns of medication intake over 2 periods: the first 14 months and during the 14-24 month period (Med/Med, Med/NoMed, NoMed/Med and NoMed/NoMed). Exploratory mediator analysis was performed to assess the effects of changes of medication intake, changes in scores of medication effectiveness (symptom ratings of 5 conceptually distinct domains of function) and growth (height and weight measures). The behavioral effectiveness of the medication use was greatest among children who ingested medications throughout the 24-month observation period. Those who stopped taking their medication and/or those who never received it showed increasing behavioral problems. However, there was significant growth deterioration among those who took medication for the longest periods. Between the Med/Med group and the NoMed/NoMed group the mean difference at the end of 24 months was  $-1.94$  cm in height growth suppression, being similar in the 2 periods of observation. The weight gain changes were larger during the initial phase ( $-2.5$  kg) than during the second period ( $-1.22$  kg). Similar growth deterioration was observed in the 2 other groups while they received medication, with improvement during the NoMed periods. The authors concluded that consistent treatment with stimulant medication was associated with maintenance of behavioral effectiveness but continued growth suppression.

MTA Cooperative Group. National Institutes of Mental Health multimodal treatment study of ADHD follow-ups: changes in effectiveness and growth after the end of treatment. *Pediatrics* 2004;113:762-769.

**Editor's Comment:** This paper is difficult to read; however, it provides important data obtained with sophisticated methodologies and statistical analyses. The cooperative group clearly documented behavioral benefits of ADHD treatment, but there were consequences of the stimulant medication on growth as well as substantial difficulties in compliance. The high rate of patients who did not adhere to the drug regimen allowed the formation and assessment of 4 naturalistic groups. It has long been debated whether these medications alter growth progression; this study clearly demonstrated that they do. The growth-suppression effect persisted as long as the medications were ingested. This study also provided evidence that treatment interruption limits growth-suppression effects. The somewhat larger body weight deterioration that was observed might be due to the anorexic effects of these medications. Suboptimal nutrition appears to be an underlying cause of reduced growth, an aspect that should be thoroughly investigated. For a particular ADHD patient with growth concerns, when the stimulant cannot be interrupted, the physician should attempt to overcome the decreased dietary intake and correct nutrient deficits to foster appropriate growth. The pediatric endocrinologist is increasingly seeing more of these patients and should be aware of this important paper.

Fima Lifshitz, MD

## Cornelia de Lange Syndrome – Gene Mutations

The Cornelia de Lange syndrome (CdLS) (OMIM 122470) is characterized by a typical face (synophrys, upturned triangular nose, thin upper lip, long philtrum, downturned corner of the mouth), impaired growth, developmental delay, limb reduction defects, and anomalous development of the heart, eyes and genitourinary tract. It occurs *de novo* or may be transmitted as a dominantly inherited trait with variable expressivity. By studying families in which there were 2 or more affected members with a chromosome

translocation or deletion, both groups of investigators localized the disorder to chromosome 5p13.1 and identified mutations in a gene termed "Nipped-B-like" or *NIPBL*. There were heterozygous missense, nonsense, deletion and insertion mutations of *NIPBL*, all of which would have resulted in a truncated or untranslated protein product. The normal product of this 47 exon gene has 2804 amino acids (termed by the Tonkin group "delangin") that likely act upon chromosomes as an adherin, linking

the interactions of promoters and enhancers of homeobox genes. Further studies revealed that the human gene and mouse homolog of *NIPBL* were expressed during gestation in the anlagen of the limbs, cranium and branchial arches, placenta, kidneys, liver, heart, skeletal muscle and thymus. Homologs of *NIPBL* were identified in flies, mosquitoes, worms, plants and fungi.



Characteristic features of CdLS (Reprinted with permission from: Krantz ID, McCaullum J, DeScipio C, et al. *Nat Genet.* 2004;36:631-635. Copyright © 2004. Nature.)

Krantz ID, McCallum J, DeScipio C, et al. Cornelia de Lange syndrome is caused by mutations in *NIPBL*, the human homolog of *Drosophila* melanogaster *Nipped-B*. *Nat Genet.* 2004;36:631–635.

Tonkin ET, Wang T-J, Lisgo S, et al. *NIPBL*, encoding a homolog of fungal *Scc2*-type sister chromatid cohesion proteins and fly *Nipped-B* is mutated in Cornelia de Lange syndrome. *Nat Genet.* 2004;36:636–641.

**First Editor's Comment:** Mutations in *NIPBL* were found in approximately 20% of patients with the clinical manifestations of the CdLS who were examined, implying that this disorder is likely to be genetically heterogeneous. Other sites that have been linked to the CdLS are located on chromosomes 2q37, 10p13, and 14q24, but an abnormality in one or more of the genes in these regions has not been detected to date. It is likely that as mutations in other genes that lead to the CdLS are identified, our understanding of the genetic regulation of somatic differentiation will be greatly enlarged.

Allen W. Root, MD

William A. Horton, MD

## Metabolic Syndrome in Obese Children

The metabolic syndrome (MS) described as a link between insulin resistance, hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM), with an increased risk of atherosclerotic cardiovascular disease, has been reported to have a prevalence of 6.8% among overweight adolescents and 28.7% among obese adolescents (NHANES III). In order to determine the effect of the degree of obesity on the prevalence of the MS and its relationship to insulin resistance, Weiss and colleagues studied 439 obese (BMI >97% for age and sex), 31 overweight (BMI 85%–97% for age and sex), and 20 non-obese children and adolescents (4–20 years of age) with baseline measurements of BMI, blood pressure (BP), plasma lipids, C-reactive protein, interleukin-6, and adiponectin. Oral glucose tolerance tests were performed as well. Degree of obesity was defined by BMI z-scores (moderately obese = 2.0–2.5 and severely obese >2.5). The overall prevalence of MS was 38.7% in moderately obese subjects and 49.7% in severely obese subjects. Glucose, insulin, insulin resistance, triglycerides, C-reactive protein, interleukin-6, systolic BP, and prevalence of glucose intolerance (defined as 2hr glucose of 140–200 mg/dL) increased with increasing obesity. HDL cholesterol and adiponectin decreased with increasing adiposity. Three factors explained 58% of the variance observed: obesity and glucose metabolism, dyslipidemia and BP. Through multiple regression analysis of risk factors associated with the syndrome, a significant risk included age, sex, BMI z-score, race or ethnic group, and insulin resistance. Each half-unit increase in the BMI z-score was associated with a significant increase in the risk of the MS. White children had a higher risk than black children, and girls had a lower risk than boys.

**Second Editor's Comment:** The multiplicity of clinical features of CdLS, combined with reports of chromosomal rearrangements in some patients, had long suggested that CdLS might be a contiguous gene syndrome. These papers demonstrate that the clinical manifestations reflect the diverse functions of a single gene product—delangin—during development. Most of the reported cases have severe clinical phenotypes and mutations that predict full loss of function, such as frameshift mutations leading to premature stop of translation. However, in a few cases mutations predicted to alter some, but not all functions, ie, 3 bp deletion that would remove a single amino acid appears to produce milder features, suggesting that the manifestations reflect loss or alteration of specific functions related to different regions of delangin. Correlation of clinical findings with specific mutations in more patients, combined with experimental modeling of specific CdLS mutations in mice, should help to sort out the relationship between genotype and phenotype.

A 2-year follow-up study was performed in 77 children; 34 with and 43 without MS. At follow-up 24 of 34 children continued to have MS. The 10 who improved had a lower BMI initially, gained less weight over the 2 years, and had decreased insulin resistance. The MS developed in 16 of 43 children who did not meet the criteria at baseline; they had gained more weight than the others. Eight subjects developed T2DM, and all had impaired glucose tolerance at baseline. The authors conclude that MS is much more common than previously reported and that each element of the syndrome is adversely affected by increasing weight. Of particular concern were the markers of inflammation, interleukin-6 and C-reactive protein, which escalated with increasing obesity and presumably put these children at high risk for cardiovascular disease.

Weiss R, Dziura J, Burger TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med.* 2004;350:2362–2374.

**Editor's Comment:** This manuscript presents some frightening data. The prevalence of MS, once a diagnosis reserved almost exclusively for adults, is very common among obese adolescents. Weiss et al showed that not only adolescents, but children meet the criteria for this diagnosis, and that markers of cardiovascular inflammation are present at a very young age. The progression to develop MS over 2 years as weight continues to increase is remarkable.

Despite the importance of the documentation that this manuscript presents, the findings and conclusions are not surprising to most physicians who provide care to America's young. Indeed, the epidemic of childhood obesity is evident to any observer of children. It is heartening that some federal research funds are now being made available to study this problem and that Medicare is beginning to recognize

*obesity as a medical condition. Regardless of the data documenting the prevalence of obesity and the morbidities and co-morbidities associated with it, the behavioral and societal interventions required to stop its progression have not been adequately addressed. Reduction in the incidence and severity of obesity will require more than medical*

*research—it will require significant input of pediatricians and family physicians, schools, media, and commercial enterprises. Being apprised of the seriousness of the problem is just another wake-up call.*

William L. Clarke, MD

## Index for Volume 20 (2004)

### **Volume 20, Number 1**

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## GROWTH HORMONE THERAPY IN CHRONIC KIDNEY DISEASE

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### INTRODUCTION

In the last few years, there has been a shift in emphasis on the medical management of children with chronic kidney disease (CKD) from strategic attempts to preserve renal survival to optimizing global biological potential, and thereby maximizing quality of life. Early diagnosis and prompt treatment have become the cornerstones of modern care. Thus, in addition to measures like anemia

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#### From The Editor's Desk

This issue contains 9 printed and 8 e-abstracts of important papers in the field with editorial comments. Thus, we have doubled the content of *GGH* with the introduction of e-abstracts. This feature also allowed the publication of longer abstracts, data, and comments. Please take advantage of this added feature. I welcome your comments regarding the on-line and print aspects of the journal. Your feedback is important as we continue to grow and strive to serve your needs. The lead article by Drs. Bamgbola and Kaskel on Growth Hormone Therapy in Chronic Kidney Disease is a very comprehensive review of the pathophysiology of the disease as it pertains to growth hormone. It includes provocative ideas about possible future direction for treatment with growth hormone and insulin-like growth factor. I am sure you will enjoy it and save it as a reference source.

The expanded journal and all of its content is carefully prepared by the editorial board and all the lead articles are reviewed to comply with the high scientific standards of a peer review journal. *GGH* is also in compliance with the code of conduct for medical publishers on the internet (Health on the Net Foundation [www.hon.ch](http://www.hon.ch)).

Respectfully,  
Fima Lifshitz, MD

control and improved nutritional intake, there is increasing use of recombinant human growth hormone (rhGH).

Although the FDA-approved indication for use of rhGH in CKD is growth failure, there are other clinically significant metabolic effects of the hormone. In this review, we shall highlight the potential benefits of rhGH therapy in CKD, including its positive impact on cellular growth and metabolism, immune regulation, and energy homeostasis. The roles of rhGH in modulation of psychosocial function, sleep physiology, and bone metabolism in children with CKD will also be discussed.

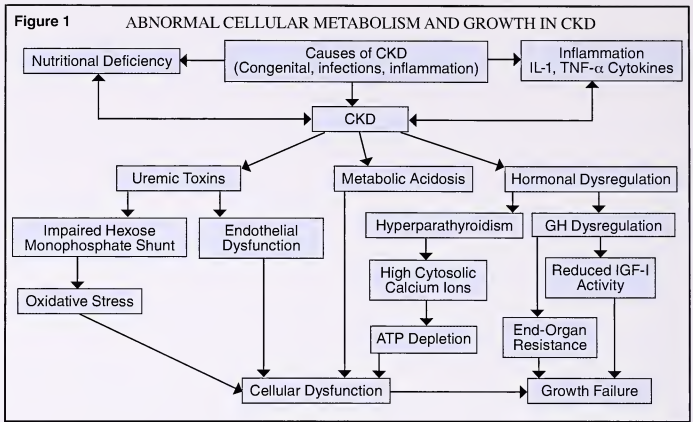
### GROWTH FAILURE

More than 50% of adults with childhood-onset CKD attain final heights that are below the third percentile.<sup>1</sup> The burden of growth retardation in patients with renal disease is enormous, resulting not only in physical handicaps but also the potential for psychological and social distress.

CKD, whether caused by congenital anomalies, chronic infection, immune disorders, or connective tissue diseases, may be associated with nutritional deficiency and



growth retardation (Figure 1). Conversely, consequences of renal disease such as metabolic acidosis, endocrinopathy, chronic anemia, persistent micro-inflammation, recurrent infection, and cardiac dysfunction may also result in growth failure. Inadequate dietary intake (often less than 80% of RDA) and defective protein metabolism are common features of CKD. However, increased food intake does not necessarily translate into a healthy nutritional outcome, and it often leads to greater adiposity rather than musculo-skeletal growth.



Furthermore, metabolic acidosis, which is a common outcome of CKD, accelerates protein degradation by activation of the ubiquitin-proteasome pathway, stimulation of branched-chain keto-acid-dehydrogenase, and promotion of end-organ resistance to anabolic effects of GH.<sup>2</sup> In addition, steroid therapy, often used as an anti-inflammatory agent in some kidney diseases, or for immune suppression following renal transplantation, may not only impair GH release but also increase end-organ resistance. In this regard, there is a positive correlation between the cumulative dose of steroids and adult-height deficit in pediatric allograft recipients. Treatment with steroids may inhibit GH synthesis by stimulation of (hypothalamic) somatostatin production. Consequently, by acting on multiple receptor-sites of the pituitary gland, GH-releasing peptide-2 (a GH secretagogue) has the therapeutic potential of bypassing the inhibitory effect of somatostatin.<sup>3</sup> Similarly, the use of rhGH alone or in combination with insulin-like growth factor (IGF)-I promotes musculo-skeletal growth, essentially by attenuating the inhibitory effect of steroids on protein synthesis.<sup>4</sup>

Whereas somatic growth at an early age is predominantly determined by factors such as birth size and adequate nutritional status, functional availability of GH is essential during childhood, and gonadotropin is a necessary adjunct for post-pubertal maturation.<sup>1</sup> Consequently, provisions of an optimal metabolic and nutritional milieu are often sufficient for growth in children with CKD who are less than 2 years of age, while use of rhGH is commonly required in older children.

## GH/IGF AXIS

Although the pulsatile release of GH is blunted in uremia, the total amount of GH secretion from the pituitary gland is often increased.<sup>5</sup> IGF-I and -II are derived from both hepatic cells and local tissues (of target organs) in response to a

primary activation of the GH receptor (GHR).<sup>6,7</sup> Despite the higher plasma level of circulating GH,<sup>8</sup> there is less synthesis of IGF-I due to end-organ resistance.<sup>9</sup>

Factors that contribute to GH tissue resistance in CKD include hyperparathyroidism, metabolic acidosis, and pro-inflammatory cytokines.<sup>9-12</sup> The mechanism of the end-organ resistance is inhibition of calcium-mediated intracellular signaling and impaired transcription of GHR-mRNA. Thus, GH activation of growth plates in uremic animals results in reduced local synthesis of IGF-I, impaired chondrocyte replication, and therefore retarded skeletal growth.<sup>13</sup>

The physiologic functions of GH are mediated by 2 different but complementary mechanisms: GH directly activates target organs while its indirect effects are mediated through IGF-I.<sup>7</sup> While GH increases the hepatic production rate of glucose and glycerol (an index of lipolysis), IGF-I acts in concert with insulin to increase peripheral glucose uptake and to reduce protein breakdown.<sup>14</sup>

IGF-I is a small, single-chain peptide belonging to the same family of genes as IGF-II and pro-insulin,<sup>15</sup> and its free bioactive form accounts for 1% of total plasma concentration.<sup>7,16</sup> IGF-I has a very short half-life (20 minutes), rapidly losing its metabolic function in the absence of a carrier binding-protein (IGFBP).<sup>6,7</sup> The most abundant of the 6 IGF-binding proteins (IGFBP-1 to -6) is IGFBP-3; it binds to circulating IGF-I and acid labile-sub-unit (ALS) as a 150 kDa ternary complex, thereby protecting it from premature degradation.<sup>7,16</sup>

IGF-I receptors are heterotetramers comprised of 2 alpha and 2 beta sub-units attached by disulfide bridges. IGF-I ligand binds to the extracellular alpha sub-unit which in turn induces the transmembrane beta unit,

resulting in an autoactivation of tyrosine kinase and phosphorylation of an intracellular tyrosine residue.<sup>15</sup> Interaction between insulin receptor substrates (IRS-1 and -2) and the receptor-tyrosine residue evokes a signal transduction thereby activating the downstream MAP-3 kinase (and protein kinase-B) pathways.<sup>15</sup> The 2 pathways mediate protein synthesis, cellular growth, cell motility, and inhibition of apoptosis.

IGFBP-3, by sharing a similar molecular structure, competitively inhibits IGF-I receptors.<sup>15</sup> However, the receptor molecules have stronger affinity for the IGF-I ligand. Consequently, there is a regulated but slow release of the plasma IGF-I from its carrier proteins at the designated target tissue. In uremic plasma, IGFBP-3 peptides are more rapidly degraded into smaller fragments. The smaller molecules of IGFBP-3 have less avidity for IGF-I and are often poorly excreted by the diseased kidneys. The reduced renal clearance of the relatively inefficient IGFBP-3 fragments and retention of inhibitory binding proteins, including IGFBP-1, -2, -4, and -6, substantially reduce the bioavailability of IGF-I.<sup>16,17</sup>

#### Future Directions for GH/IGF-I Treatment

Despite end-organ resistance to GH in uremia, exogenous administration of rhGH accelerates skeletal growth by increasing the molar ratio of IGF-I to IGFBP-3. However, CKD patients often require dose levels of rhGH 2 to 3 times higher than doses administered to GH-deficient subjects.<sup>7</sup> In addition, combined therapy with rhGH and rhIGF-I results in a greater than additive effect, or synergistic interaction, in CKD patients.<sup>6</sup>

Given the prevalent organ resistance to GH in CKD, therapeutic approaches that increase functional availability of IGF-I may be more effective than the simple administration of rhGH as is currently practiced.<sup>6,7</sup> These measures may include the use of exogenous IGFBP-3 to replace the inhibitory smaller fragments and IGF-I analogs to displace endogenous IGF-I from its binding proteins.<sup>6,7</sup> While the binding protein may prolong the half-life of IGF-I, IGF-I analogs may increase the effective concentration of the bioactive free IGF-I. Therapeutic administration of combined IGF-I and IGFBP-3 complexes have been successfully used to enhance positive nitrogen balance in burn patients.<sup>6</sup>

Furthermore, synthetic GH-releasing peptide (GHRP) and its endogenous equivalent, ghrelin, may be available for oral administration in the near future.<sup>7</sup> These GH secretagogues are more potent than the conventional GH releasing hormone (GHRH) in stimulating a pulsatile release of GH. They act on specific receptors of the anterior pituitary gland, thereby restoring its normal physiologic characteristics. These include capacity for feedback regulation and a greater than 6-fold increase in IGF-I synthesis.<sup>6</sup> This therapeutic approach has been introduced into clinical practice with the combined use

of GHRP and thyroid-releasing hormone to reactivate pulsatile pituitary secretion of GH and thyroid-stimulating hormone, thereby preventing protein catabolism and muscle wasting in protracted critical illness.<sup>18</sup>

#### Delayed Puberty, Hypogonadism, and rhGH

There is a complex interaction among GH, IGF-I, and sex steroids in maximizing growth potential and body composition and in promoting sexual and reproductive capacities in human subjects.<sup>19</sup> Although the mechanism is unknown, the increase in pituitary GH synthesis during mid-puberty in boys is preceded by an increase in plasma testosterone. Similarly, the GH/IGF-I axis is activated by small increases in plasma estrogen in girls at the onset of puberty. GH and IGF-I influence reproductive function directly by modulation of gametogenesis and indirectly by enhancing steroidogenesis. Achievement of critical body weight is associated with pubertal onset, suggesting that somatic effects of rhGH treatment may play a role in the attainment of spontaneous puberty.<sup>20,21</sup>

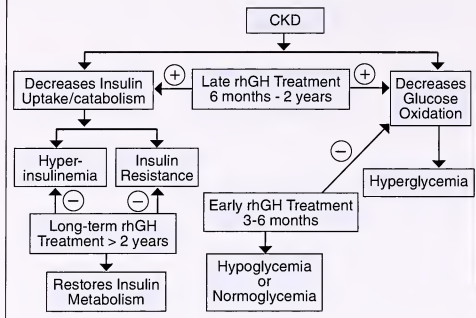
The common findings of hypogonadism and delayed puberty in CKD are characterized by a loss of the normal pulsatile hypothalamic release of gonadotropin-releasing hormone (GnRH).<sup>22</sup> Puberty may be delayed for up to 2 years, while peak height velocity is often less than 50% of normal in CKD patients. There is a low expression of GHR in a GHR gene knockout-mouse model, similar to the findings in human CKD subjects. These mice have delayed maturation of seminal vesicles, spermatids, and testes, with a poor testicular response to leutinizing hormone, supporting a role for rhGH in induction of pubertal maturation.<sup>23</sup> The use of rhGH/IGF-I administered with GnRH analog (experimental hypogonadism) in men has been shown to preserve protein synthesis and lipid oxidation compared with controls, indicating an independent effect of the combined regimen in the maintenance of fat-free mass.<sup>24</sup> Similarly, combined therapy with rhGH and testosterone synergistically promotes muscle IGF-I gene expression, whole body protein anabolism, bone turnover, physical performance, and sexual function.<sup>25,26</sup>

#### METABOLIC CHANGES AND rhGH THERAPY

##### Insulin and Glucose Metabolism

Insulin and glucose metabolism in CKD (Figure 2) is characterized by reduced activity of glycolytic enzymes with a consequent decrease in glycolysis, glycogen synthesis, and storage. In uremic rats, there is 25% to 45% reduction in hepatic gluconeogenesis and glucose formation rate from fructose and pyruvates.<sup>9</sup> Similarly, due to a defective intracellular (post-receptor) signaling there is impairment of hepatic insulin metabolism in uremic rats. In addition, although pancreatic insulin secretion is reduced, its renal degradation is substantially compromised in CKD. The resultant hyperinsulinemia stimulates plasminogen activator inhibitor,

**Figure 2** INSULIN & GLUCOSE METABOLISM IN CKD: Effects of rhGH Therapy



reduces fibrinolysis and, therefore, promotes vascular thrombus formation.

### rhGH Therapy and Glucose Metabolism

In the early phase of rhGH therapy, insulin-like effects (including hypoglycemia and protein synthesis) predominate and serve to overcome the uremic-induced insulin resistance (Figure 2). This effect is due to a cross-affinity of IGF-I with insulin receptors leading to an increased glucose uptake and cellular oxidation.<sup>27</sup> On the other hand, with long-term rhGH administration, there is impairment of insulin-mediated glucose uptake, increased lipid oxidation, and formation of insulin-resistant (glycolytic type II) muscle fibers.<sup>28</sup> Consequently, hyperglycemia ensues with an increase in glycosylated hemoglobin. In general, restoration of normal glucose tolerance has been shown to occur within 2 years of starting rhGH therapy.<sup>29,30</sup> These paradoxical effects of rhGH may result from functional and structural diversities of its fragments. For example, GH fragment 1-15 is endowed with insulin-like effects, whereas GH fragment 177-191 possesses anti-insulin properties, and the 20K-GH variant promotes cellular growth.<sup>31</sup>

### Protein Metabolism in CKD

Although hepatic synthesis of total serum protein is often preserved in CKD subjects, production of specific proteins such as IGF-I and apolipoprotein A1 are commonly reduced.<sup>9</sup> Similarly, there is a 30% to 40% reduction in enzymatic activity of the urea cycle, with a down-regulation of ureagenesis and accumulation of nitrogenous substances, including middle molecule toxins (poorly dialyzed, larger-sized uremic molecules) such as advanced glycation end products, and  $\beta$ 2-microglobulin.<sup>9</sup>

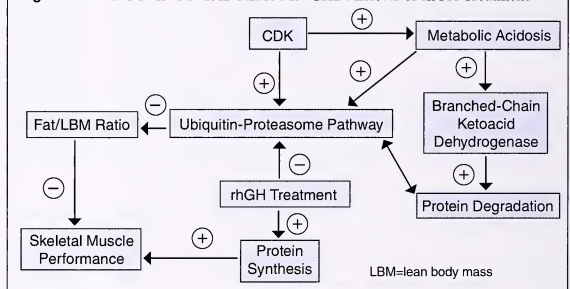
As previously stated, metabolic acidosis and uremic-induced inflammation cause protein degradation by activation of ubiquitin-proteasome pathway, induction of

branched-chain ketoacid dehydrogenase, and promotion of end-organ resistance to insulin and GH/IGF-I (Figure 3). The physiologic impact of activated uncoupling proteins (UCP polymorphism) on mitochondrial oxidative phosphorylation is substantial and may account for up to 20% of basal energy expenditure.<sup>32</sup> Tumor-necrosis factor (TNF)- $\alpha$  cytokine, often elevated in CKD, promotes negative nitrogen balance by up-regulating UCP-2 and -3 genes in skeletal muscles of experimental rats.<sup>33</sup>

### rhGH Therapy on Protein Metabolism

Treatment with rhGH increases protein synthesis, not only by stimulating uptake of amino acid, but also by promoting intracellular peptide assembly.<sup>34</sup> Protein degradation is prevented by inhibition of lysosomal and ATP-ubiquitin-proteasome pathways. Thus, the net effect of rhGH therapy in CKD is an efficient use of dietary branched-chain amino acids with improved skeletal muscle performance.<sup>35,36</sup> Consequently, administration of rhGH therapy after long-term mechanical ventilation has been shown to result in improved respiratory muscular strength, reduction in ventilator settings, and successful extubation in post-surgical patients.<sup>37</sup> Similarly, combined use of GH/IGF-I as an adjunct to total parenteral nutrition results in a net positive protein balance in critically ill patients.<sup>38</sup> On the other hand, in a multi-institutional, randomized, controlled trial of critically ill adults, the use of high dose rhGH resulted in longer length of hospitalization and a higher mortality rate.<sup>39</sup>

**Figure 3** PROTEIN METABOLISM IN CKD: Effects of rhGH Treatment

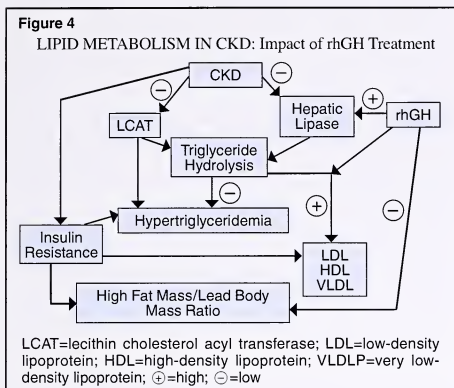


### Lipid Metabolism in CKD

CKD subjects exhibit a reduction of lecithin-cholesterol acyl transferase (LCAT) enzyme, down-regulation of apo-A1 genes, and inhibition of hepatic lipase activity.<sup>9</sup> (Figure 4) Consequently, there is impaired hydrolysis of triglycerides (TG) in high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL), resulting in hypertriglyceridemia. Plasma low-density lipoprotein (LDL) has been shown to be elevated due to a down-regulation of its receptor function.<sup>9</sup> In addition, insulin resistance may promote dyslipidemia and pro-coagulant activity in CKD.<sup>40</sup> The pattern of lipid profiles in CKD patients are strikingly similar to findings



in metabolic syndrome. Both clinical syndromes share other characteristics such as hypertension, altered body composition, low-grade persistent inflammation, and hyperinsulinemia with a common outcome of premature cardiovascular (CV) disease.<sup>41</sup>



### rhGH Therapy and Lipid Metabolism

In general, rhGH therapy improves lipid profiles by decreasing LDL and apo-B while increasing HDL.<sup>40</sup> By induction of lipoprotein lipase and stimulation of LDL receptor, rhGH attenuates the characteristic increase in VLDL-TG in CKD.<sup>40</sup> In addition, rhGH reduces visceral adiposity, increases lean body mass, and restores normal body composition in CKD.<sup>42</sup> However, it is yet to be seen if these favorable metabolic and biological changes will translate into a better long-term CV outcome in CKD. On the other hand, GH therapy may increase lipoprotein (a), an independent CV disease risk factor.<sup>40</sup> While it shares a common lipid fraction with LDL, lipoprotein (a) clearance is not influenced by the GH-induction of LDL-receptor activity.<sup>40</sup> Nevertheless, the clinical significance of the modest yet notable increase in lipoprotein (a) during rhGH treatment on CV health is not known.

### FOOD INTAKE AND ENERGY HOMEOSTASIS

Uremia promotes excessive transport of tryptophan across the blood-brain barrier and consequently increases neuronal synthesis of serotonin, an endogenous anorectic compound.<sup>43</sup> Adequate food intake may be further compromised in uremic patients by an accumulation of cholecystokinin, TNF- $\alpha$ , interleukin (IL)-1, leptin, and middle molecule toxins (eg, beta (2)-microglobulin, advanced glycation end products).

### Ghrelin and rhGH in CKD

Ghrelin, an endogenous ligand for GH secretagogue-receptor, is principally secreted by pancreatic alpha-like cells (designated Gr cells) from the stomach fundus, in

response to changes in nutritional status.<sup>44</sup> In addition to a potent pituitary stimulation for GH secretion, ghrelin increases food intake by activating agouti-related peptides and neuropeptide Y within the hypothalamus.<sup>45</sup> Experimental use of ghrelin in human subjects was shown to increase food intake, energy consumption, and visual analog scores for appetite.<sup>46</sup> Although the physiological consequence is unknown, there is often accumulation of biologically active (acylated polypeptide) and inactive (desacyl) ghrelin in CKD subjects because of impaired renal clearance. It may be speculated that ghrelin retention constitutes an adaptive mechanism to promote caloric intake in chronic uremia. Perhaps ghrelin's failure to correct the calorie deficiency state arises from the prevailing end-organ resistance to its orexigenic (appetite-stimulating) effects. Similarly, its role in promoting appetite may be physiologically counteracted by the anorexic forces from excessive accumulation of leptin, serotonin, and cytokines in CKD. It has yet to be determined whether the use of ghrelin as an adjunct to rhGH might be beneficial in overcoming anorexia in chronic uremia.<sup>45</sup>

It has been suggested that there may be a negative feedback control of ghrelin by the GH/IGF-I axis. Thus, a short-term rhGH induction of IGF-I causes a proportionate reduction in ghrelin with no alteration in plasma adiponectin.<sup>47</sup> On the other hand, a reduction in body fat mass from long-term use of rhGH may contribute to an increase in circulating levels of ghrelin and adiponectin.<sup>47</sup> The confounding effect of impaired filtration and/or catabolism of ghrelin in renal failure on the purported ghrelin-GH/IGF-I feedback axis is not known.

### Leptin and rhGH in CKD

Hyperleptinemia is a common finding in renal failure, and may result from decreased renal clearance, increased secretion from adipose tissue, and hyperinsulinemia. Leptin is a potent endogenous anorectic agent; its effect may be modulated by rhGH therapy. Thus, administration of rhGH in the Zucker obese rat (which is characterized by leptin and insulin resistance) induces lipolysis and down-regulates leptin gene expression in visceral fat mass.<sup>48</sup> However, as previously stated, the appetite-promoting effect of rhGH may be overcome by persistent hyperleptinemia in CKD subjects. Recent discovery of leptin receptor isoforms in multiple organs suggests that leptin is an important mediator of other unknown biological functions.<sup>49</sup> Therefore, further studies are required in defining the roles of leptin in the modulation of metabolic and nutritional derangements in uremic syndrome.

### SLEEP DEFECTS AND rhGH

About 50% to 70% of adults with end-stage kidney disease suffer from sleep apnea, insomnia, daytime somnolence, and restless leg syndrome.<sup>50</sup> In CKD the



high prevalence of sleep disorders may be confounded by co-morbidities of obesity and depression. However, there is often a strong positive correlation between blood urea nitrogen and indices of sleep dysfunction in patients with kidney failure.<sup>50</sup> Potential complications of sleep defects in uremia may include resistant hypertension, autonomic dysfunctions, and left ventricular hypertrophy.<sup>51</sup> Corroborating the role of uremic burden in sleep dysfunction is the remarkable improvement in symptoms with the administration of daily nocturnal hemodialysis. To date, there are no studies in humans on the therapeutic role of rhGH on sleep defects in CKD, although rapid eye movement (REM) sleep is restored by rhGH, and non-REM sleep is modulated by GHRH in GH-deficient (transgenic) animal models.<sup>52</sup> Similarly, use of ghrelin, a GH secretagogue, results in a preponderance of the more physiological pattern of slow and delta waves that occur during sleep.

Although there are case reports of sudden deaths from obstructive sleep apnea attributed to the use of rhGH in patients with Prader-Willi syndrome, scientific analysis has failed to confirm these assumptions.<sup>53-55</sup> On the contrary, there is potential for beneficial effects on respiratory physiology because of the favorable effects of rhGH on inspiratory drives, ventilatory muscle functions, respiratory quotients and resting energy expenditure.<sup>56-58</sup>

## IMMUNE FUNCTION

CKD is characterized by a persistent micro-inflammatory state with increased circulating levels of IL-1, IL-6, and TNF- $\alpha$  cytokines. Negative nitrogen balance may result from the reduced hepatic syntheses of albumin and apolipoprotein; however, increased release of fibrinogen and amyloid precursors by the liver may enhance vascular thrombogenicity.<sup>9</sup>

Immune deficiency in CKD results from a direct inhibition of uremic toxins and/or altered metabolic activities of immunological cells, including neutrophils, lymphocytes, and macrophages. One subset of T-helper cells, Th-1, is the effector of cell-mediated immunity and recruits new Th-1 cells by producing interferon-gamma while inhibiting Th-2 induced cellular differentiation.<sup>59</sup> The other subset of T-helper cells, Th-2, secretes inhibitory IL-4 and IL-10 cytokines and consequently attenuates the self-perpetuation of Th-1 cells. Uremia shifts the delicate regulatory balance between Th-1 and Th-2 cellular pathways in favor of the latter, thereby causing a depression of cell-mediated immunity.<sup>59</sup> In addition, the impaired expression of B7-2 (co-stimulatory) molecules on the surface of antigen-presenting cells may weaken activation of effector T cells.<sup>60</sup>

The capacity for B-cell antibody production and superoxide generation by polymorphonuclear leukocytes

are also reduced in a uremic milieu.<sup>9</sup> The defect may be due to elevated cytosolic  $\text{Ca}^{2+}$  resulting in poor ATP generation (impaired mitochondrial oxidative phosphorylation) and may be reversed by calcium-channel blockers.<sup>9</sup> Increase in neutrophil apoptosis is in part mediated by the Fas-Fas-L pathway in CKD; there is a positive correlation between Fas-mediated apoptosis and creatinine clearance in plasma obtained from uremic subjects.<sup>61</sup>

## rhGH Impact on Immune Dysfunction

GH stimulates T-cell cytotoxicity and releases superoxide anion from inflammatory cells. CD4 and NK-cell activities were shown to be restored in GH-deficient adults treated with rhGH, while phagocytic function was normalized.<sup>62</sup> In addition, rhGH was shown to prevent apoptosis of immunologic cells by inactivating the pro-apoptotic Fas-FADD pathway and increasing the anti-apoptotic expression of Bcl-2. The overall physiological impact was a down-regulation of Caspase 3, an intracellular effector of apoptosis.<sup>63</sup>

GH is a member of the cytokine super-family and has a similar structure to granulocyte colony-stimulating factor.<sup>64</sup> GHRs, which bind to GH, are found on a number of immunological cell surfaces. Use of rhGH in severe sepsis may exacerbate the ongoing inflammatory process by cross-activation with other cytokine-receptors and, thereby result in a higher fatality rate.<sup>65</sup> In a rat model of bacterial sepsis, increased expression of suppressors of cytokine signaling (SOCS)-1 and -3 inhibited intracellular signaling of GHR, resulting in a poor generation of IGF-I.<sup>66</sup> Thus, a relative IGF-I deficiency may contribute to the impairment of glomerular filtration rate that may result from septicemia. Although in normal circumstances IGF-I increases renal perfusion, its administration in a rat model of ischemic renal failure results in higher mortality, apparently by evoking adverse inflammatory processes.<sup>67</sup>

The pro-inflammatory activity of rhGH was initially postulated to be a potential cause of allograft rejection. However, clinical evidence suggests otherwise, and the safety and efficacy of rhGH was recently demonstrated in renal transplantation.<sup>68</sup> In pediatric renal allograft recipients, rhGH has also been shown to prevent steroid-induced protein catabolism, maintain skeletal mass, and improve linear growth rate. In addition, postoperative administration of rhGH in rats with small bowel transplant restores morphology of allograft mucosa and promotes a net positive nitrogen balance.<sup>69</sup> Furthermore, the perioperative use of rhGH in immunocompromised rats enhances surgical wound healing.<sup>70</sup> Given that post-transplant use of the immunosuppressant sirolimus may cause a delay in wound healing because of its antifibrotic property, a study of the role of rhGH in this regard may provide useful information.

## BONE MINERAL CONTENT and rhGH

Within a few weeks of initiation of rhGH therapy, the molecule interacts with the bone-forming unit by increasing the biochemical markers of bone formation and resorption. In general, short-term (3–6 months) rhGH therapy may reduce or maintain bone mineral density, while treatment of GH-deficient adults for 2 years results in a sustained increase in mineralization.<sup>71</sup> On the other hand, the common use of high-dose calcium and calcitriol in CKD subjects for the treatment of hyperphosphatemia may result in suboptimal skeletal response to rhGH. Calcium-containing phosphate binders and vitamin D inhibit chondrocyte proliferation and delay mineralization, thereby causing adynamic bone disease.<sup>72</sup> Resistance to GH effects is manifested by low expression of IGF-I protein and decreased bone morphogenetic protein-7 staining, despite an increase in GH concentration and higher density of GHR.<sup>72</sup> It may therefore be prudent to avoid calcium-containing phosphate-binders and ensure appropriate vitamin D doses in CKD subjects receiving rhGH.<sup>73</sup>

There is evidence to suggest that GH may play a modulatory role in the musculo-skeletal effects of parathyroid hormone. Administration of rhGH to GH-deficient subjects improves end-organ responsiveness with a decrease in urinary calcium excretion, increased tubular phosphate reabsorption, and increased markers of bone turnover (type I collagen C-telopeptide and procollagen type I amino-terminal propeptide).<sup>74</sup>

## QUALITY OF LIFE

Psychometric analysis and physical assessment of renal patients reveals a high prevalence of reactive depression, reduced physical performance, and cognitive deficits. However, psychosocial support, physical exercise, and anemia control may ameliorate many of these deficits. Administration of rhGH may also play a positive role as replacement therapy in GH-deficient adults; rhGH has been shown to improve quality-of-life indices.<sup>75</sup> Similarly, rhGH improves linear growth and physical agility, and reduces psychosocial burden in children with Prader-Willi syndrome.<sup>76,77</sup> Confounding variables such as anemia in CKD make studying the psychosocial impact of rhGH a difficult exercise.

## CONCLUSIONS & SPECULATION

This review describes and highlights the potential therapeutic impact of rhGH in CKD patients. In the absence of kidney transplantation, it is important to restore the profound metabolic and physiological defects arising from renal insufficiency. In many instances, studies in GH-deficient models have demonstrated the beneficial effects of rhGH therapy beyond the longitudinal

skeletal growth for which rhGH is commonly indicated. Additional problems in CKD patients for whom rhGH may play a significant role include modulation of nutritional inadequacies, altered body composition, immune dysregulation, and impaired sexual development and/or reproductive capacity. However, given the differences in their pathogeneses, it may be overly simplistic to project similar benefits of rhGH therapy to all the clinical settings of growth failure in CKD.

The multifaceted physiological effects of rhGH should still be taken into consideration in future studies of renal patients. Efforts must be made to broaden the scope of outcome measures to include cellular growth, cellular metabolism and function, neurocognitive development, psychosocial impact, sleep physiology, energy homeostasis, and anemia control. The beneficial role of rhGH in uremic cardiomyopathy, bone disease, anemia management, body composition, hospitalization requirements, and vascular diseases should also be examined. Co-morbidities are common in CKD and, therefore, multiple pharmacological agents are often needed to treat the disease. The physiological outcome of the combined use of erythropoietin, steroids, vitamin D, carnitine supplements, and other nutritional supplements with rhGH requires further study. Experimental studies in animals suggest a favorable role for rhGH in surgical wound healing; studies are therefore needed to examine the role of rhGH in ameliorating delayed wound-healing that may characterize the use of sirolimus after surgical transplantation.

Furthermore, the role of ghrelin (a recently discovered endogenous GH secretagogue) in CKD requires critical evaluation. Relevant questions for future studies are numerous. What is the role of ghrelin in food intake behavior in CKD patients? What are the metabolic effects of uremia on the capacity of Gr cells to produce ghrelin? What is the effect of uremia on the pituitary GH secretagogue receptor? What is the therapeutic impact of oral administration of ghrelin as a sole agent and/or combined therapy with rhGH/rhIGF-I, GH releasing peptides, exogenous IGFBP-3, and IGF-I analogs? What are the relationships between ghrelin, leptin, cytokines, and UCP polymorphism in the regulation of food intake, energy balance, and body composition in CKD?

Finally, the essence of this review is to inform the scientific community of the need for operational research endeavors concerning the metabolic impacts of rhGH therapy. Therefore, efforts must be made to critically assess the risk and benefit of the continued use of rhGH beyond the traditional end-point of linear skeletal growth in children with CKD. Hopefully, an improved understanding of the roles of rhGH in restoring physiological disturbances in CKD will provide added value to the treatment of such patients throughout their lives.

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## ABSTRACTS FROM THE LITERATURE

## Hypothalamic Amenorrhea and Leptin

The authors assessed the effects of leptin treatment in 8 patients with hypothalamic amenorrhea, compared with 6 patients who were not treated. All 14 patients had secondary amenorrhea for 6 months or longer, coincident with increased exercise or low body weight and were otherwise healthy without acne, hirsutism or LH/FSH, TSH, and prolactin alterations. Basal and follow-up assessments in a clinical research center included comprehensive endocrine, body composition, metabolic rate analyses, bone densitometry and pelvic ultrasonography. The patients treated with leptin (r-metHuLeptin) received 0.08 mg/kg/day subcutaneously for 2 to 3 months, with 40% of the dose given at 8:00 AM and 60% given at 8:00 PM. If patients ovulated the study was terminated at 2 months. If no ovulation occurred the dose was increased to 0.2 mg/kg/day for a third month. Leptin treatment increased mean LH levels and LH pulse frequency after 2 weeks of treatment and increased maximal follicular diameter, the number of dominant follicles, ovarian volume and estradiol levels over the study period. Three patients had ovulatory menstrual cycles; 2 had preovulatory follicular development and withdrawal bleeding during treatment. Leptin treatment significantly increased levels of free  $T_3$ , free  $T_4$ , IGF-I, IGFBP-3, bone alkaline phosphatase and osteocalcin but not cortisol, corticotropin, nor urinary N-telopeptide. Untreated control patients did not have any significant changes in any of these variables. Body weight did not change in the control patients; however it decreased slightly among the treated ones, owing to a small decrease in body fat without changes in lean body mass. No significant changes in metabolic rates or food intake occurred. The authors concluded that the relative leptin deficiency in women with hypothalamic amenorrhea is improved with leptin treatment. This results in improved reproductive, thyroid, growth hormone axis and markers

of bone formation, suggesting that leptin is required for normal reproductive and neuroendocrine function.

Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 2004;351:987-97.

**Editor's Comment:** Hypothalamic amenorrhea, also called functional amenorrhea, is frequently seen in women who are athletic, underweight and/or stressed. It is usually preceded by irregular menses, weight loss or increase in physical activity and it is considered to be the result of energy deficiency. In non-athletic women of normal weight it may be associated with psychosocial stress also related to subtle deficits in calorie and macronutrient intake. The central energy-related hormone, leptin, is the common factor underlying the pathogenesis of this entity. The study by Welt et al adds data substantiating the importance of leptin in mediating the neuroendocrine abnormalities of hypothalamic amenorrhea, a leptin deficiency condition. They demonstrated an improvement with leptin treatment, without other medications to induce menstruation, while the patients maintained their usual dietary intake, exercise habits and lifestyle. However, let's not tread into new expensive treatments without correction of nutrient deficiencies or without first attempting to modify the dietary intake to meet all the energy and nutrient needs of the patient. The accompanying editorial by Ahima<sup>1</sup> addresses the distinguishing features of this condition from anorexia nervosa, as well as an erudite explanation of the pathophysiology of the disease as it relates to body fat, leptin and hypothalamic amenorrhea.

Fima Lifshitz, MD

## Reference

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## Statin Therapy in Hypercholesterolemic Children

Wiegman and associates report findings of a 2-year randomized placebo-controlled efficacy and safety trial of pravastatin for the treatment of familial hypercholesterolemia in children ages 8 to 18 years. Two hundred fourteen children (100 boys), mean age 13.0 years, were studied. Inclusion criteria were: one parent with a definite clinical or molecular diagnosis of familial hypercholesterolemia; at least 3 months on a fat-restricted diet (<30% of total calories from fat, 10% saturated fat); 2 fasting LDL-C levels of at least 155mg/dL; no current drug treatment or use of plant sterols.

The primary efficacy variable was change from baseline of carotid intima-media thickness (IMT) as measured by ultrasound. Blood samples were measured for total cholesterol, HDL-C, LDL-C and triglycerides at 3-6 month intervals over the 2-year study. In addition, ALT, AST, and CPK were measured for safety reasons, and levels of sex steroids, gonadotropins, cortisol, and TSH were determined to survey for potential side effects of the drug on growth and sexual development. Height and weight were measured and Tanner staging was performed at baseline, 1, and 2 years.



Baseline characteristics were similar in both groups. The mean carotid IMT was attenuated after 2 years of treatment, while there was a trend towards an increase in the placebo group. The overall change between the 2 groups was statistically significant. LDL-C levels were reduced in the treatment group, while HDL-C, triglyceride and lipoprotein(a) levels remained unchanged. All hormone levels (corticotropin, cortisol, LH, FSH, DHEA-S, TSH, estradiol, testosterone) were similar in both groups at 2 years. Height and weight increased similarly in both groups, as did stages of sexual development. AST, ALT, and CPK levels were also similar, although one child in the placebo group had an asymptomatic but marked increase in CPK, which returned to normal.

The authors point out that this is the first long-term safety and efficacy trial of a 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitor (statin) in children with familial hypercholesterolemia. The drug was both effective and well tolerated with minimal observable side effects. There were no effects on growth or sexual development. Despite these encouraging findings, they caution that even longer studies are needed to establish

the safety of this class of drugs.

Wiegman A, Hutten B, de Groot E, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA* 2004;292: 331-7.

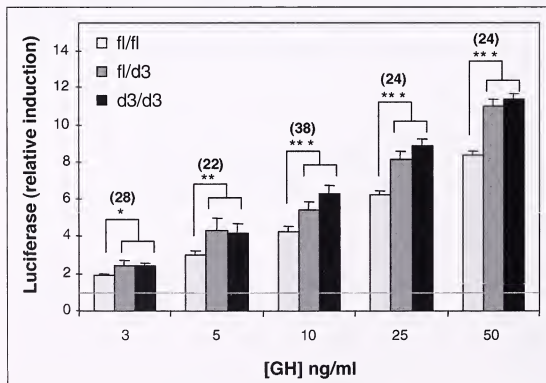
**Editor's Comment:** *This is a welcomed study. Although the efficacy of statins in reducing LDL-C has been reported in several studies, this safety study is reassuring. More and more frequently pediatric endocrinologists are faced with younger and younger children with obesity and hypercholesterolemia, or diabetes and hypercholesterolemia, and need to recommend effective and safe therapy. Diet and exercise, unfortunately, are rarely practiced with sufficient adherence to be considered effective and realistic treatment options. Furthermore, resins are poorly tolerated in this age group. The use of statins is therefore an obvious therapeutic choice, but information regarding their long-term safety and side effects has been lacking. We would encourage these authors to continue their study of these children with the anticipation that further data will establish safety over an even longer time period.*

William L. Clarke, MD

## Growth Hormone Receptor and Responsiveness to Growth Hormone

Intrigued by the clinical observation that the linear growth response to a similar dose of growth hormone (GH) in GH deficient (GHD) and in non-GHD children with idiopathic short stature (ISS) or children with intrauterine growth retardation (IUGR) varied substantially, the investigators correlated the biological effectiveness of recombinant human GH (rhGH) with 2 known isoforms of the GH receptor (GHR). The human gene GHR consists of 9

coding exons with exons 3–7 encoding the extracellular domain of 246 amino acids; there is one full-length isoform of GHR and a second isoform in which the 22 amino acid sequence coded by exon 3 is omitted by alternative splicing during transcription (d3-GHR). In prepubertal children with ISS or IUGR (defined by short birth length), the frequency of the d3-GHR isoform was comparable to that of normal subjects. The GHR genotype (GHR/GHR, GHR/d3-GHR, d3-GHR/d3-GHR) did not affect



*In vitro* bioactivity of full-length GHR and d3-GHR. HEK 293 cells transiently expressing full-length GHR, d3-GHR or both were stimulated by increasing concentrations of GH for 8 h. Relative induction of LHRE-luciferase reporter gene is expressed relative to unstimulated cells (value of 1, horizontal line).

Number of experiments in ( ), \*P < 0.005, \*\*P < 0.0005, \*\*\*P < 0.00001

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basal growth rate. When treated with rhGH, subjects with at least one d3-GHR isoform grew more rapidly in response to a standard dose of rhGH (0.36 or 0.23 mg/kg/week in 2 separate trials) than did those with the GHR/GHR genotype during the first 2 years of therapy. There was no difference in growth response to rhGH between children with 1 or 2 d3-GHR alleles or between those with ISS or IUGR. Expression of the GHR and d3-GHR isoforms in HEK fibroblasts *in vitro* demonstrated that in response to hGH the transcriptional activity of the luciferase reporter gene was ~30% greater in cells with d3-GHR than GHR (Figure). The authors concluded that analysis of the GHR genotype may permit more appropriate individualization of rhGH dosage (pharmacogenetic dose selection) in clinical conditions in which administration of rhGH is appropriate.

Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougnères P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 2004; 36:720-4.

**Editor's Comment:** The mechanism(s) by which the shorter d3-GHR transmits a more potent signal in response to ligand bind than does the full-length GHR is not known. The 22 amino acid sequence of exon 3 is not near the interface of ligand and receptor, and the mechanism by which its loss leads to increased receptor activity is unknown at present. It does not affect hGH/GHR binding or internalization. The d3-GHR polymorphism might permit more rapid propagation of signal to the intracellular signal transduction systems that mediate the cellular responses to hGH. In this regard,

it would be of interest to study the dynamics of this system in cells expressing either the full-length or shortened GHR isoforms. The report also raises the question that if a polymorphism that increases responsiveness to hGH exists, might there not also be a subtle polymorphism that mildly depresses GHR transduction of the hGH signal? Might this be another pathway through which the "genetic" regulation of growth and adult stature is mediated?

Allen W. Root, MD

## Therapeutic RNAi for Genetic Skeletal Disease?

RNA interference (RNAi) is a gene silencing phenomenon first identified in the nematode, *C. elegans*, but was subsequently found to occur in higher organisms including humans. It probably evolved as an ancient defense mechanism for cells to fend off mobile genetic elements, such as RNA viruses and transposons, but today it has been implicated in a growing number of cellular processes.

As discussed by Stevenson, RNAi involves sequence specific degradation of target RNAs triggered by the formation of double stranded RNA (dsRNA). When it occurs naturally, long dsRNA is processed to short interfering RNAs (siRNAs) 21-24 bases in length by a dsRNA-specific endonuclease named Dicer (Figure). They are incorporated into a nuclease complex referred to as the RNA-induced silencing complex or RISC. Unwinding of the siRNAs activates and directs RISC to the target RNAs, which are cleaved and degraded. The complementarity between the siRNA and the target RNA determines the sequence specificity of RNAi.

An important advance in the RNAi field was the discovery that exogenous synthetic siRNAs or endogenously synthesized siRNAs driven by viral vectors could be incorporated into RISC and induce sequence-specific degradation of target RNAs. This created an extremely powerful tool for scientists to "knock down" expression of genes of interest simply by adding synthetic RNA duplexes to the medium of cultured cells, introducing viral vectors that express siRNAs into cells or even generating transgenic animals that synthesize siRNAs.

RNAi is much more complex than outlined here, and there are many technical difficulties that complicate the use of RNAi to knock-down gene expression in experimental systems. Nevertheless, RNAi has stimulated considerable interest in the pharmaceutical/biotech industry as a potential therapeutic agent for human disease. The best examples to date have to do with treatment of infectious diseases, such as those caused by HIV, hepatitis viruses and poliovirus, as well as cancers that are mediated in part by overactive oncogenes. In the case of viral infections, interfering RNAs could be targeted to viral transcripts required for viral replication or survival. In the second case, using RNAi to silence expression of BCR-ABL, the fusion gene that results from the Philadelphia chromosome translocation in chronic

myelogenous leukemia or mutated RAS oncogenes that drive several types of cancer would be appealing.

Receiving less attention to date, but of probably at least as much interest to readers of *GGH*, is the potential use of RNAi to knock down expression of mutant alleles in dominantly inherited genetic disease. In concept, siRNAs could be tailored to distinguish mutant from normal (wild type) alleles and block only mutant allele expression. This could convert a dominant negative disorder, ie, a disorder in which the product of the mutant allele interferes with the function of the normal (wild type) allele product, to a disorder that results from haploinsufficiency or functional loss of one allele. For families in which both forms occur, manifestations are usually milder in the form resulting from haploinsufficiency, ie, osteogenesis imperfecta type I – haploinsufficiency vs osteogenesis type II – dominant negative. Thus, there is potential benefit from this therapeutic strategy.

Despite the excitement and promise of therapeutic RNAi, there are many obstacles, the greatest of which is delivery. Systemically delivered siRNAs face degradation by nucleases, and the use of viral vectors to target organs of interest is still in its infancy. A recent publication by Soutscheck and colleagues provides evidence that chemically modified siRNAs can successfully knock down endogenous genes in living mice. More specifically, they targeted expression of the gene encoding apolipoprotein B (*apoB*) in the mouse liver and jejunum where it is known to be expressed at high levels with 2 siRNAs known to silence *apoB* in cultured cells. They modified the *apoB* siRNAs by chemically stabilizing their backbone and also by adding cholesterol to their 3' end. The modified siRNAs were then compared to unmodified *apoB* siRNAs and other controls.

The results showed that the cholesterol-conjugated *apoB* siRNAs were significantly more stable in serum than their unconjugated counterparts. When administered intravenously, one of the conjugated *apoB* siRNAs was very effective at lowering *apoB* mRNA and *apoB* protein levels, as well as total cholesterol and LDL cholesterol. They observed no evidence of "off-target" effects, that is, effects attributed to silencing of genes other than *apoB* or other obvious complications from the injections. The authors concluded that exogenously administered chemically

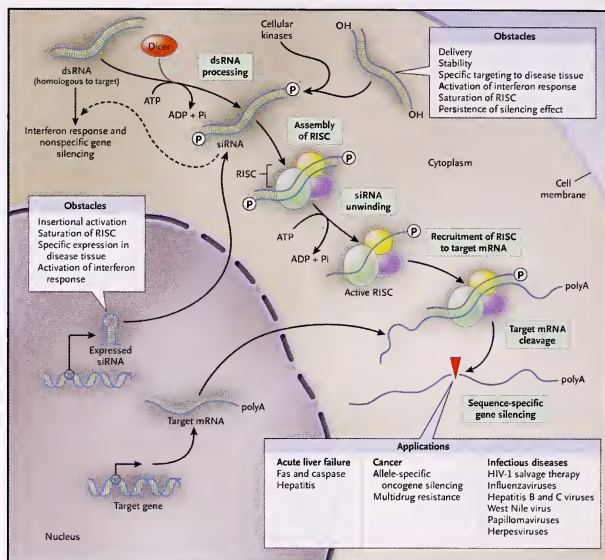
modified siRNAs can potentially be used to silence expression of endogenous genes involved in human disease.

Stevenson M: Therapeutic potential of RNA interference. *N Engl J Med* 2004;351:1772-7.

Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004;432:173-8.

**Editor's Comment:** RNAi has had a major impact on science since its relatively recent discovery. It is still not entirely clear how it works and there remain concerns about specificity and the so-called off target effects on genes other than specifically targeted genes. Nevertheless, it has great promise as a means to treat not only cancer and infectious diseases, but genetic diseases in which mutant alleles differing from their normal alleles by only a single base can be specifically targeted. It will probably be years before such treatment becomes realistic for humans, but the success of substantially knocking down apoB expression by systemically administering chemically modified apoB siRNAs in mice is very encouraging. One note of caution is that the growing skeleton may be difficult to target because the cartilaginous growth plate is relatively avascular compared to most tissues such as liver and gut.

William A. Horton, MD



Mechanism of Gene Silencing by RNA Interference. The double-stranded RNA (dsRNA) is processed and assembled into the RNA-induced silencing complex (RISC) and subsequently incorporated into target mRNA for the sequence-specific gene silencing application.

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## Variation in Expression in Human Genes

Medical genetics textbooks typically distinguish between continuous and discontinuous variation in (clinical) phenotype. The latter can often be traced to a single change in the DNA sequence of a gene, i.e., a "mutation" that serves as the basis of classic mendelian disorders. The genetic basis of continuously variable traits, such as height or blood pressure, is more difficult to explain. Variation in baseline expression of genes represents a mechanism that could contribute to continuous phenotypic variability. It is known to exhibit familial aggregation suggesting that it is heritable, but the tools to study the genetics of variation in human gene expression have only recently made it feasible to explore this notion. Morley and colleagues now document the existence of regulators of baseline gene expression.

The investigators utilized microarray technology to measure expression levels of genes, which they refer to as "gene expression phenotypes", in immortalized B cells from members of 94 Center d'Etude du Polymorphisme Humain

(CEPH) Utah families. Starting with approximately 8500 genes active in these cells, they found 3554 genes that showed greater variation of expression between individuals than between replicates from the same individual. They then carried out genome-wide linkage analysis using single nucleotide polymorphisms to identify the genetic determinants of this variation. The results showed that variation in expression of 984 genes was genetically linked to one or more regions of the genome.

They assumed that regions linked to expression levels were regulatory regions or "regulators". They examined the spatial relationship of the regulators to the 142 "target" genes that exhibited the strongest evidence for linkage. Twenty seven (19%) mapped to within 5 Mb of the target gene; they considered these to be *cis*-acting regulators because of their relatively close proximity to the coding sequence of the target gene. One hundred ten (77.5%) mapped further away and were designated *trans*-acting regulators. Both *cis*- and *trans*-acting regulators were



found for 5 (3.5%) of the variably expressed genes. Many of these genes (164/984, or 16%) had multiple regulators of expression.

In addition to genomic regions containing regulators that influence single expression phenotypes *in cis* or *in trans*, the authors also found genomic regions that contained transcriptional regulators of multiple expression phenotypes. To further characterize these regulators, they divided the genome into 5 Mb windows and searched for regulatory "hotspots" within these windows. Two hotspots were detected, one of which mapped to chromosome 14 (14q32) and the other to chromosome 20 (20q13). Further analysis showed that these 2 regulatory hotspots influence expression of 31 of the 984 target genes under investigation. The authors suggest that their existence provides evidence for master regulators of baseline gene expression in humans.

Finally, they asked if differential expression of target gene alleles could be explained by *cis*-acting regulators. Analysis of individuals in whom alleles could be distinguished by single nucleotide polymorphisms showed that some of the variable expression could be attributed to the influence of the *cis*-acting regulators.

Morley M, Molony CM, Weber TM, et al. Genetic analysis of genome-wide variation in human gene expression. *Nature* 2004;430:743-7.

Cox NJ. An expression of interest. *Nature* 2004;430:733-4.

**Editor's Comment:** This paper reminds us that the level of expression is an important aspect of gene action. Reduced or increased gene expression can influence quantitative traits, such as height. One can also envision a situation in which a mutation in a *trans*-acting regulator could cause disease by decreasing or increasing expression of its target gene(s). Take osteogenesis imperfecta type I for example; it typically results from mutations that cause transcripts from a mutant COL1A1 allele to terminate prematurely or undergo nonsense-mediated mRNA decay, functionally inactivating one of the 2 COL1A1 alleles. It is conceivable that a loss of function mutation of a *trans*-acting regulator of this locus could produce a similar adverse effect on type I collagen synthesis, especially if it were homozygous. Of note, such a mutation would not show linkage to the COL1A1 locus. There are several limitations of this investigation as noted by the authors and an accompanying news and views article. For instance, mRNA levels are only one determinant of the level of protein encoded by a given gene. Gene expression differs in different tissues, at different developmental stages and in response to physiologic and pathologic factors that are probably not reflected in immortalized B cells.

William A. Horton, MD

## IGF-I Receptor Signaling: Mechanisms of Growth Stimulation

Wu and colleagues used 2 cell models to study the effects of insulin-like growth factor-I receptor (IGF-IR) signaling via insulin receptor substrate (IRS)-1 on the upstream binding factor 1 (UBF1), a regulator of ribosomal RNA (rRNA) synthesis. 32D cells (myeloid cells dependent on interleukin-3 (IL-3) for growth)

express neither IRS-1 nor IRS-2. In complement, mouse embryo fibroblasts (MEFs) express IRS-1 but have a targeted disruption of the IGF-IR gene (R<sup>-</sup> cells).

Apoptosis normally takes place in 32D cells upon removal of IL-3. 32D cells expressing IGF-IR (32D IGF-IR cells) continue growing for 48 hours after IL-3 is replaced

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with IGF-I, and then undergo granulocyte differentiation. 32D IGF-IR cells ectopically expressing IRS-1 grow indefinitely without differentiation. R<sup>+</sup> cells were also used to develop sister cells for comparison. R<sup>+</sup>/T cells express the SV40 large T antigen, while R<sup>+</sup> cells have the IGF-IR reintroduced. IRS-1 is mostly nuclear in IGF-I-stimulated R<sup>+</sup> cells and in R<sup>+</sup>/T cells, but cytoplasmic in the parental R<sup>-</sup> cells.

Using these 2 systems, the authors showed that IGF-I increased transcription from the rDNA promoter (ie, activated UBF1) in a time course compatible with nuclear translocation of IRS-1. Since UBF1 activation generally occurs via phosphorylation, additional experiments showed that UBF1 phosphorylation, mainly in the C terminus, was IGF-I stimulated and IRS-1 dependent. Beyond that, UBF1 regulation in the 2 cell models differed. In the myeloid cells deprived of IL-3, 32D IGF-IR/IRS-1 cells died without IGF-I, but maintained high levels of UBF1 protein when stimulated with IGF-I. The 32D IGF-IR cells (ie, without IRS-1) had high UBF1 protein levels, which dropped at 48 hours (ie, while the cells were still growing exponentially and not yet showing any morphologic signs of differentiation) and completely disappeared by the time the cells were differentiated into granulocytes. The drop in UBF1 protein was due to both decreased synthesis and increased degradation, though UBF1 mRNA levels remained unchanged. In the MEFs, cells that do not differentiate, UBF1 protein levels were stable after IGF-I treatment in both R<sup>+</sup> and R<sup>-</sup> cells. Thus, the authors concluded that IGF-IR/IRS-1 signaling regulates UBF1 activity, and hence the rDNA promoter, through phosphorylation and in some cells, through changes in protein level. UBF1 protein loss may

be related to the differentiation process, which tends to involve nucleolar dissolution.

Wu A, Tu X, Prisco M, Basergo R. Regulation of upstream binding factor I activity by IGF-I receptor signaling. *J Biol Chem* 2005; 280:2863-72.

**Editor's Comment:** IGF signaling through the IGF-IR is understood to stimulate cellular survival and proliferation, and at the systemic level, growth. IGF-IR is a tyrosine kinase that is activated by ligand binding. Phosphorylation of tyrosine residues in IGF-IR recruits adaptor molecules like IRS-1 that then start kinase cascades, most notably the PI3 kinase/Akt pathways and the MAP kinase pathway (for reviews, see References 1-2). The paper by Wu et al adds another mechanism whereby IGF-IR signaling stimulates growth: activation of UBF1 through nuclear translocated IRS-1 and presumably PI3 kinase. UBF1 regulates RNA polymerase I activity at the rDNA promoter, thereby regulating the rate of ribosome biogenesis. Because ribosomes are required for protein synthesis, proliferating cells invest much energy in ribosome generation (reviewed in Reference 3). Without concomitant synthesis, proliferating cells would only become progressively smaller. Thus, growth involves increasing numbers of cells with maintenance of proper cell size, and IGF-IR is involved in regulating both these processes.

Adda Grimberg, MD

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3. Moss T. *Curr Opin Genet Develop* 2004;14:210-7.

## Long-term Effects of Estrogen Treatment on Fertility in Tall Girls

Venn and colleagues identified from medical records 1248 Australian women who had been assessed and/or treated with estrogens (3mg DES or 150µg ethinyl estradiol daily) for tall stature during the years 1959 to 1993, to assess the effects of this treatment on long-term fertility. A group of 184 self-referrals (members of Tall Girls Inc an Australian advocacy group) were included in the study. To be included subjects had to have had a bone age determination at the time of assessment. Subjects were invited to complete a written questionnaire and computer-assisted telephone interview. The interview included questions regarding reproductive history including whether or not they had ever seen a doctor due to difficulty becoming pregnant, whether they had ever tried unsuccessfully for more than 12 months to become pregnant, and whether or not they had ever taken fertility drugs as treatment for infertility. The time to pregnancy was analyzed for each month of attempting pregnancy. Data from the medical records included age at menarche, treatment type, duration of treatment, and first and last assessment of estimated

mature height by Bailey and Pinneau method.

The final sample size included 618 women (75% of the treated and 95% of the untreated). The mean age of these women was 39.8 years (treated) and 37.7 years (untreated). Both groups were similar in terms of marital status and highest level of education. Self-reported current height was greater in the treated women (179.0cm vs 176.8cm). Both groups were similar in terms of history of smoking, oral contraceptive use, age of first sexual intercourse and lifetime number of male sexual partners. There were no differences between the women treated with DES or ethinyl estradiol on any parameter. Women who had been treated with estrogen were more likely to report problems with fertility. When the data were adjusted for age, the women who had been treated were less likely to have ever been pregnant and to have ever had a live birth. Treated women were more likely to have tried unsuccessfully for 12 months to become pregnant, to have seen a doctor because of difficulty becoming pregnant, and to have taken fertility drugs. Height was not related to fertility problems and the differences between

the 2 groups remained when the self-referred women were excluded from the analysis. A significant, but weak duration of treatment effect was observed.

The authors state that the data were not sufficient to establish a pathophysiological cause for the reduced fertility. They also state that the likelihood of ever becoming pregnant and having a live birth, although statistically reduced for women who had been treated for tall stature, was only slightly lower than that for untreated women and that newer treatments for infertility may reduce that difference.

Venn A, Bruisma F, Werther G, et al. Oestrogen treatment to reduce the adult height of tall girls: long-term effects on fertility. *Lancet* 2004;364:1513-8.

**Editor's Comment:** Clearly there has been a significant drop in the number of girls seeking treatment to reduce

mature height potential over the past 20 years. However, the authors note that a recent survey of pediatric endocrinologists in the United States reveals that 23% have treated such girls over the past 5 years. Thus, although the absolute number of girls seeking treatment is low, such treatment is still being sought and is available. The current study, although not the first to show the possibility of adverse reproductive effects of estrogen treatment for tall stature, is perhaps the largest long-term follow-up to date. The information is interesting and important. Pediatric endocrinologists need to be able to discuss these facts with each family seeking to reduce their daughter's mature height potential. It is reassuring that no obvious safety concerns were identified through these interviews and chart data.

William L. Clarke, MD

## Micropenis: Long-term Follow-up

These authors report the long-term outcomes of 46,XY males with micropenis, but no other genital deformity, identified and treated intermittently with androgens or hCG during infancy, childhood and/or adolescence. Lee and Houk determined adult stretched penile length (SPL) and social adjustment in 20 patients with SPL  $<-2$  SD of normal at initial examination: 11 had hypogonadotropism and 3 primary testicular failure; in 6 patients no cause of the micropenis was identified. SPL increased in all subjects; adult SPL was  $>-2$  SD of the adult mean in 14 subjects and between  $-2.5$  and  $-2$  SD in 4; 2 patients had adult SPL  $<-2.5$  SD of the mean. Among these 20 patients and another 2 with micropenis first evaluated as adults, 21/22 were heterosexual; 8 were/had been involved in long-term heterosexual relationships. Relative to age-matched control subjects, those with micropenis (N=12 studied) had comparable findings in regard to heterosexual dating and sexual functioning, male friendships, education, employment, sports/leisure activities; none had a psychiatric illness. Despite normal adult SPL, 5 primarily obese patients stated that their penises were small. The investigators concluded that in adult men who had micropenis as children/adolescents: 1) 90% had adult SPL within the broad range of normal; 2) there was "reasonable social adjustment," no psychological pathology, and gender-appropriate sexual functioning.

Husmann evaluated adult SPL in 20 men with micropenis (here defined as SPL  $<-2.5$  SD of normal) diagnosed and treated during infancy in whom SPL did not increase appreciably despite multiple courses of testosterone. Five patients had a mutation in the androgen receptor, 6 had hypogonadotropism, and 9 had no known cause of the micropenis. Mean pretreatment SPL was  $-3$  SD (range  $-5.5$  to  $-2.6$ ) for age/race and mean adult SPL was  $-3.4$  SD (range  $-5.9$  to  $-2.2$ ).

All patients considered their penises to be small, and 5 had undergone (unsatisfactory) surgery to enlarge their penises; 19/20 were heterosexual; 12/20 men were sexually active, but 4 were incapable of vaginal penetration; 5 patients had mental illnesses requiring professional therapy. Despite these findings, Husmann concluded that these patients accept a male gender identity and many engage in a "satisfying heterosexual relationship."

Lee PA, Houk CP. Outcome studies among men with micropenis. *J Pediatr Endocrinol Metab* 2004. 17:1043-53.

Husmann DA. The androgen insensitive micropenis: Long-term follow-up into adulthood. *J Pediatr Endocrinol Metab* 2004.17:1037-41.

**Editor's Comment:** In the report of Lee and Houk, in 5/20 patients (1 hypogonadotropic subject, 1 with primary testicular failure, and 2 with "idiopathic" micropenis) SPL SD score did not appreciably increase between diagnosis and adulthood, but these subjects are not specifically discussed further, and their psychosocial status is unknown. It would have been of interest if Husmann had also reported his experience with the outcome of patients with micropenis responsive to testosterone. These data are reassuring in that they further demonstrate that there is no basis to consider sex reversal in the 46,XY male with micropenis as their gender identity is firmly masculine. Furthermore, with current surgical procedures for penile reconstruction, the opportunity for satisfactory penile enlargement has improved substantially.<sup>1</sup>

Allen W. Root, MD

## Reference

1. Jordan GH, Rosenstein DI, Gilbert D. *Growth Genet Horm* 2002;18:33-8.

## Growth Hormone Sensitivity in Obesity

These authors sought to explore the observation that insulin-like growth factor (IGF)-I levels remain normal in obesity despite reduced growth hormone (GH) levels. Ninety-one healthy adults (mean age about 50; range 21-82 years) were subdivided by body mass index (BMI) and gender; there were 19 normal weight men, 23 normal weight women, 15 obese men and 34 obese women (obesity defined as BMI > 30). Fat mass and percent body fat were measured by bioimpedance. GH sensitivity was assessed by an IGF-I generation test, with IGF-I levels measured before and 24 hours after a single, standard 7mg dose (21IU) of GH. The increment in IGF-I was greater in obese than normal-weight equivalents, negatively correlated with baseline IGF-I concentration, positively correlated with GH binding protein (GHBp) level, and seen in both men and women (pre- and post-menopausal). GHBp concentrations were higher in obesity, and also correlated with BMI, fat mass and percent body fat. The authors concluded that their study provides evidence of increased GH sensitivity in obesity. The fact they used a single, standard GH dose makes the result cleaner than earlier studies that employed a weight-based GH dosing scheme; IGF-I levels were higher in obese subjects, but in those studies, the obese subjects also received a greater GH dose. Because GHBp is the extracellular domain of the GH receptor (GHR), it is sometimes used as an indirect measure of GHR number. The finding of a positive association between GHBp level, markers of obesity and IGF-I increment led the authors to hypothesize that the enhanced GH sensitivity of obesity may be due to increased GHR density, itself resulting from the lower GH levels. Because the data are all associative, further studies are needed to test this hypothesis.

Gleeson HK, Lissett CA, Shalet SM. IGF-I response to a single bolus of growth hormone is increased in obesity. *J Clin Endocrinol Metab* 2005;90:1061-7.

**Editor's Comment:** *This paper clearly showed increased*

*hepatic sensitivity to GH in obesity, at least in terms of IGF-I generation, which helps to explain the discordance between the low GH but normal IGF-I levels seen in obesity. The pediatric correlate of this adult study is the enhanced growth frequently experienced by obese children who continue growing despite GH deficiency (classically, craniopharyngioma patients who develop hypothalamic obesity and GH deficiency); the growth without GH phenomenon is reviewed in Reference 1. Proposed mechanisms include hyperinsulinism-stimulated growth, decreased IGFBP-1 levels resulting in increased bioavailable (free) IGF-I, and increased growth plate stimulation by sex steroids (increased aromatization by the greater adipose mass). An interesting finding came from studies of a model of endochondral ossification, the chondrocyte population of the skeletal growth centers in the mouse mandibular condyle. The growth center chondrocytes expressed leptin receptors and when stimulated by leptin, increased expression of IGF-I receptor, increased both proliferation and differentiation processes, and had larger growth plate growth.<sup>2</sup> Furthermore, when mice were calorie-restricted by 40%, circulating IGF-I levels dropped by 70% and tibial growth decreased by 5%; leptin treatment corrected the growth deficit despite further reductions in circulating IGF-I levels.<sup>3</sup> Thus, the growth-promoting consequences of obesity are multi-factorial, and it will be interesting to see if enhanced hepatic GH sensitivity, perhaps due to increased GHR density, also plays a role in the growth of obese children.*

Adda Grimberg, MD

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### ASSESSMENT OF PSYCHOSOCIAL ASPECTS OF SHORT STATURE

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#### INTRODUCTION

The evidence is clear that growth hormone (GH) therapy can virtually eliminate the predicted height deficit for individuals with classic GH deficiency (GHD) if treatment is initiated at a sufficiently young age.<sup>1</sup> The unlimited availability of biosynthetic growth hormone (rhGH) has also made it possible to extend treatment to children who do not have GHD, but nonetheless exhibit short stature (SS) or poor growth. Consequently, the treatment of SS has become dissociated from its causes. Conditions for which rhGH is efficacious in promoting faster growth and taller stature include a diverse set of conditions: Turner syndrome,<sup>2</sup> chronic renal insufficiency,<sup>3</sup> Prader-Willi syndrome,<sup>4</sup> children born small for gestational age<sup>5</sup> and, most

#### From The Editor's Desk

Dear Colleague:

The increased number of abstracts and editorial comments published online has been very well received by readers of *GGH* journal. The feedback was praiseworthy, and there were a large number of viewers who accessed the e-abstracts. Both of these aspects are very rewarding to the Editorial Board. This issue also includes an expanded format; there are 8 abstracts published in the print version of the journal, plus one letter to the editor pertaining to the lead article dealing with pregnancy in T1DM patients (published in Volume 20, Number 4 of *GGH*). In addition, there are 6 papers published in the e-version, (accessed at [www.GGHjournal.com](http://www.GGHjournal.com)). Altogether the Editorial Board canvassed and reviewed some of the most pertinent papers in the current literature. Finally, the lead article in this issue addresses a most important topic, one that pediatric endocrinologists deal with on a daily basis; namely, the evaluation of children with short stature. The paper by Sandberg and Colman is an erudite review of the facts and pitfalls of the reports dealing with the psychosocial issues of short stature. They discuss the science and evidence and/or the lack of it, regarding the "heightism" prejudice that is so prevalent in our society. It constitutes an important contribution for those in practice dealing with short children, as well as for those interested in psychosocial research.

In May, 1985, I received an urgent call alerting me to the CJD association with the growth hormone that was used to treat hypopituitary patients. This hormone, extracted from cadaver pituitary glands, was immediately pulled off the market and we were left without any options to treat these patients. Fortunately, recombinant human growth hormone was in the pipeline and was soon available for clinical use. The 20th anniversary of this landmark accomplishment by Genentech is worthy of recognition.

Fima Lifshitz, MD

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recently, idiopathic short stature (ISS), ie, short, but without diagnosable pathology.<sup>6</sup> In addition to eliciting improved growth velocity, rhGH has also been shown to produce metabolic benefits in particular conditions, eg, GHD, Prader-Willi syndrome, and chronic renal insufficiency.

The primary rationale for rhGH treatment has traditionally rested on the assumption that SS, in the extreme, may constitute a physical disability, and otherwise serves as a significant psychosocial burden for the individual. Furthermore, treatment is predicated on the belief that rhGH-induced increases in height will improve quality of life (QOL). Allen and Fost<sup>7</sup> infer from the growing number of conditions for which rhGH is prescribed that "the cause of short stature is not morally relevant in deciding who is entitled to treatment." These authors proposed that rhGH therapy is indicated when a "disability" in adaptation attributable to SS is identified (rather than by virtue of a medical diagnosis), and that treatment should be aimed at correcting this disability up to the point that an adult height within the "normal range" is attained, ie, 5<sup>th</sup> percentile.

This review summarizes what is known about the psychosocial aspects of SS and the QOL benefits of rhGH treatment. Stereotypes and assumptions about SS are evaluated in light of empirical findings. As described elsewhere,<sup>8</sup> studies and reviews were identified on MEDLINE<sup>®</sup> and PsychINFO<sup>®</sup> and The Cochrane Database of Systematic Reviews<sup>®</sup> using the terms "short," "stature," "height," or "growth hormone" combined with "psychological," "psychosocial," or "quality of life."

## EVALUATING PSYCHOLOGICAL RESEARCH ON SS Analogue versus "real world"

Research on stereotypical beliefs about those with SS is often conducted by assessing participants' perceptions in "analogue" studies. Social scientists employ analogue studies to answer well-defined research questions by isolating aspects of everyday life and assessing them within a controlled setting. The validity of findings stemming from such research designs has been questioned when used to investigate complicated social phenomena. Analogue studies of the psychosocial concomitants of SS that constrain information about the individual, or which place emphasis on stature, may unwittingly tap the stereotypes held by participants, but may be poor predictors of how participants perceive or treat an individual in the "real world."<sup>9</sup>

## Descriptive cross-sectional studies

A common strategy is to use standardized questionnaires or interviews to assess psychological characteristics of individuals with SS, and then compare findings to those of individuals of average height. Such descriptive studies typically assess research participants at only

one point in time and do not include an evaluation of an intervention, eg, response to a treatment such as rhGH. Validity of cross-sectional studies can be threatened by sample selection biases and participant reactivity.

a) Sample selection biases. Ascertainment of the psychosocial adaptation of individuals with SS depends on the composition of the targeted group. To evaluate the generalizability of the findings to all individuals with SS, research must provide details regarding the representativeness of the sample, ie, the proportion of those individuals eligible for study, based upon anthropometric criteria, who participate relative to those who do not. Factors resulting in an over-representation of better or poorer functioning individuals would bias the findings. Examples of clinic-based studies in which sample representativeness cannot be ascertained, and which report greater behavioral dysfunction among children and adolescents with SS, include 2 large studies.<sup>10,11</sup> Investigations that have more carefully studied clinically representative samples of referred short youths have shown these groups to be similar in behavioral adjustment to population-based norms<sup>12,13</sup> and classmates.<sup>14</sup>

b) Comparison samples. The composition of comparison or control groups for individuals with SS is no less important than the selection of the target group when the goal is to make statements regarding the prevalence of problems. Factors contributing to recruiting a comparison sample that is functioning better than the general population would result in the SS group appearing less well-adapted.<sup>15</sup> Participant recruitment techniques which result in generally better functioning individuals include reliance on volunteers who are generally better adapted than those in the general population.<sup>16</sup> As an alternative to recruiting a control group, it is common to compare the target group (ie, youths with SS) with "norms" for the standardized method(s) administered. This practice is fraught with risks, including differences in inclusion and exclusion criteria and demographic characteristics that are related to participants' scores.<sup>17</sup>

c) Reactivity of assessment. An additional potential threat to the validity of a study stems from the subject's awareness of being studied.<sup>9</sup> The individual's motives and interpretations of the study can influence responses. For example, participants in clinic-based studies of the psychological adaptation of individuals with SS might assume that their role is to describe the liabilities associated with diminutive size, since they are being evaluated for short stature. If the participant's awareness of the assessment leads to a different response from usual, the measure is said to be *reactive*. Studies that have masked the examination of participant's height have failed to detect an association between height and psychosocial adaptation.<sup>14,18</sup>

d) Sources of information about the individual's psychosocial adaptation. Limited concordance in the reports of psychological adaptation across informants (child, parent, peers, others) is common and serves as a caution to readers of psychosocial literature regarding SS.<sup>19</sup> Stronger research designs involve the collection of data from multiple sources.<sup>20</sup> The most valid source of information about the social relationships of youths with SS would derive from studies utilizing peers as informants.<sup>21</sup> This strategy has been adopted in only 2 studies, one examining the social status of clinic-referred youth<sup>14</sup> and a community sample.<sup>18</sup>

### Treatment studies

Studies that examine the influence of medical treatments (such as rhGH therapy) on psychological outcomes are vulnerable to threats stemming from evaluation bias introduced through either the informant's (often the parent) or examiner's knowledge that the patient is receiving the treatment, or placebo effects. In most research, the minimal experimental conditions include one group that receives an intervention and another group that does not (control group). The purpose of adopting a no-intervention group is to rule out alternative explanations for change in the intervention group, eg, placebo effects or regression toward the mean.

To the best of our knowledge, there has been only one clinical trial of the psychological effects of rhGH in children and adolescents that employed a randomized, placebo-controlled research design.<sup>22</sup> A recent meta-analysis suggests that placebo effects are stronger in clinical trials employing continuous *subjective* outcomes (such as measures of psychosocial adaptation) as compared to large trials employing dichotomous *objective* outcomes.<sup>23</sup>

The "regression toward the mean" should also be considered. This concept refers to the tendency of extreme scores on any measure to regress toward the mean of the distribution when the measure is re-administered. If individuals are selected for a study in a manner that they are more likely to generate extreme scores on a given measure, one can predict on statistical grounds that scores will tend to revert toward the mean on subsequent retesting.<sup>15</sup> To rule out this phenomenon as an explanation, changes observed in the treated group need to be compared with changes seen over the same time interval in a sample with similarly elevated baseline scores.<sup>24</sup>

Expectation biases may also be introduced into the data by relying on parent reports of

children's behavioral adaptation. Parents' worries about their children's psychological adjustment to SS likely contribute to the referral to a pediatric endocrinologist and acceptance of a recommendation for rhGH therapy. These same worries may also be associated with an expectation (bias) that rhGH therapy results in reduced behavior problems. It is important to validate parental reports of psychological problems against other sources of information (eg, patients, teachers, or peers). Studies that have adopted this approach have demonstrated few differences between patients with SS and comparison or control groups.<sup>12,18,20</sup>

### STATURE-RELATED STEREOTYPES

Stereotyping refers to a process in which identical characteristics are assigned to all individuals within a group, regardless of the actual variation among group members. Negative stereotypes regarding experiences and characteristics of individuals with SS are plentiful and categorized as: accompanying psychological characteristics, differential treatment by others, social relationships, and education/occupation (Table 1). Children's and adults' beliefs about height reliably demonstrate a bias toward the notion that "taller is better." With few exceptions, both children and adults attribute significantly less favorable characteristics to short individuals compared to those of tall or average height.<sup>25-28</sup> It is thus not surprising that youths and adults of both genders prefer to be taller.<sup>29-31</sup>

It has been suggested that individuals with SS experience disadvantages in the way they are treated due to stature-related societal perceptions.<sup>32</sup> As early as preschool age, mothers differentially treat girls based upon height.<sup>33</sup> Two studies in adults investigated the relationship between a person's height and "personal

Table 1. Empirical status of stature-related stereotypes

| Stereotype  | Evidence   |
|---|--|
| Children and adults with SS are more poorly adjusted psychosocially       | Generally supported by analogue (laboratory-based) research <sup>25-28</sup><br>Not supported by general population- or clinic-based studies <sup>12,13,20</sup>   |
| Children and adults with SS are treated poorly due to their stature       | Mixed results from analogue studies <sup>33-35</sup><br>Evidence of teasing and juvenilization from clinic-based studies <sup>13,45</sup>  |
| Short men are less attractive and desirable to women as dates or husbands | Generally supported by analogue research <sup>27,36,37</sup><br>Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables <sup>53,58</sup> |
| Children and adults with SS do less well at school/are less intelligent   | Generally supported by analogue studies <sup>33,40</sup><br>Not supported by general population- or clinic-based studies of children <sup>47-49</sup> or adults <sup>52,62</sup>                         |
| Adults with SS hold lower status occupations and are paid less            | Supported by analogue studies <sup>37,41,42</sup><br>Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables <sup>52,57</sup>            |

space.” Results were mixed: in one, the taller individual was afforded more space<sup>34</sup>; in the other, differences were not found.<sup>35</sup> Research on the effects of height on social relationships focuses on heterosexual dating and partner selection. For dating relationships, findings support the conventional notion that taller is more attractive, and this appears particularly true for males,<sup>27,36-38</sup> but less so for females.<sup>27,38</sup> Regarding the importance of height in partner selection, the man's height is more important a consideration for women than the reverse.<sup>28,39</sup>

When asked to evaluate classmates' competence, preschool boys rated small boys as better at “art” than tall boys; girls rated tall boys as smarter than small boys; but girls' height did not correlate with ratings.<sup>33</sup> Mothers rated tall boys and girls as more competent than small boys in the majority of domains,<sup>33</sup> and had greater expectations for mastery and achievement from taller children.<sup>40</sup> With regard to adults' occupational status, undergraduates judged individuals who have more prestigious occupations as taller than those of less prestige.<sup>41,42</sup> They also expected taller people to have a higher professional status than shorter people.<sup>27</sup>

#### QOL ASSUMPTIONS REGARDING SS

Assumption 1: Patients with SS experience chronic psychosocial stress (Table 2). Early studies showed that SS is associated with teasing and juvenilization.<sup>43</sup> These investigations were generally restricted to patients with complex medical conditions with little attention directed toward bias introduced by subject selection factors.<sup>44</sup> Two relatively recent clinic-based studies found that approximately 60% to 70% of patients referred to pediatric endocrinologists for a growth evaluation had experienced teasing or juvenilization, and that these stressors were experienced with some regularity.<sup>13,45</sup> Contrary to expectations, however, the child's relative height

(−3.1 to −0.2 height SD) was not significantly related to the incidence of these negative experiences.<sup>13</sup> Furthermore, the presence of psychosocial stress does not imply that SS constitutes a “disability.”<sup>7</sup> To rise to this threshold, it would be necessary to provide clear evidence that these stressors are associated with clinically significant impairment in social, academic, or occupational functioning.

Assumption 2: Patients with SS exhibit clinically significant problems of psychosocial adaptation. It is commonly believed that patients with SS exhibit clinically significant behavioral or emotional problems.<sup>43</sup> Implicit in this assumption is the expectation that psychiatric problems are significantly more common among patients with SS than in the general population (rates of childhood psychiatric disorders fall between 18% and 22%).<sup>46</sup> However, this does not appear to be the case when selection biases in participant recruitment are minimized. For example, self-esteem scale scores for short youths referred for evaluation of SS were higher (ie, more positive) than questionnaire norms, despite reports that the majority of these individuals experienced teasing and juvenilization.<sup>13</sup> The same was true for behavior disturbance: patients reported significantly fewer problems than questionnaire norms, and parental reports indicated that patients were indistinguishable from the norms in behavioral and emotional functioning.<sup>13</sup> Similar findings were reported in other clinic-based studies.<sup>12,20</sup> In contrast, other studies report significantly more behavioral and emotional problems among children with SS relative to norms as measured by self- and parental-report.<sup>10,11</sup> Unfortunately, key details essential to gauge the representativeness of these samples<sup>10,11</sup> were not provided, such as the total number of eligible patients and the method of targeting participants for behavioral studies.<sup>24</sup> Studies featuring clinically representative samples show behavioral adjustment is comparable to classmates<sup>14</sup> and to population-based norms.<sup>12,13</sup>

Table 2. Assumptions underlying growth-promoting therapies

| Assumption  | Evidence  |
|---|---|
| Patients with short stature experience chronic psychosocial stress  | Supported by clinic-based studies <sup>13,45</sup>  |
| Patients with short stature exhibit clinically significant problems of psychosocial adaptation            | Not generally supported <sup>10,12,13,20,22</sup>   |
| Short youths and adults in the general population are similarly at risk for problems of social adjustment | Not supported in children, adolescents <sup>18,48,50,51</sup> or adults <sup>52,56,58</sup>   |
| Stature-related social stress results in significant problems of psychosocial adjustment                  | Limited support: though teasing and juvenilization were related to behavior problems, <sup>45</sup> overall psychosocial adaptation was equivalent to community norms <sup>12</sup> |
| Increases in growth velocity and height induced by rhGH therapy result in an improved QOL                 | Not supported <sup>20,22,31,62</sup>  |

Corollary of Assumptions 1 and 2: Individuals with SS in the general population also exhibit significant problems of psychosocial adaptation. Although rarely articulated, it follows from both preceding assumptions that short youths who are *not* referred for a medical evaluation are similarly at risk for psychosocial adaptation problems. In the prospective, longitudinal Wessex Growth Study, in which the sample comprised short, healthy children from the general population, no evidence of serious psychosocial or academic disadvantage was found.<sup>47-50</sup> Although individuals in the SS group preferred to be taller, and reported more bullying than their taller peers,<sup>29</sup> neither the desire for physical change nor bullying



had measurable effects on school performance or self-esteem,<sup>47,48,50</sup> suggesting that stigmatized individuals use self-protective cognitive mechanisms that allow self-esteem to remain intact.<sup>12</sup>

In the largest study of its type, and the only one conducted on a national probability sample of the U.S. population, Wilson and colleagues<sup>51</sup> assessed the relationship between stature, IQ, and academic achievement. Statistically controlling for potentially confounding background characteristics, subjects' height contributed significantly (approximately 2%) to the prediction of both indices. The Wessex Growth Study replicated this general finding. However, as in the U.S. study, height explained only 2% of the variance in IQ. Socioeconomic factors, rather than stature, best predicted psychosocial and academic outcomes.<sup>48</sup>

In a recent study using a novel research design, the influence of height on students' (N=956, grades 6–12; approximately 11–18 years old) psychosocial adaptation was assessed using peer informants.<sup>18</sup> Statistically significant relationships were not detected between height and measures of friendship, popularity, or most aspects of reputation among peers, despite substantial statistical power. Findings did not vary by participant gender, peer- or self-report, whether data from the entire sample were used, or when subgroups of very short ( $\leq -2.25$  height SD; 1<sup>st</sup> percentile) or very tall students ( $\geq 2.25$  height SD; 99<sup>th</sup> percentile) students were contrasted with average height (25<sup>th</sup>–75<sup>th</sup> percentile) classmates. In the lower grades, classmates perceived shorter students as younger than their age. However, this perception was not meaningfully related to measures of social acceptance or other aspects of reputation among peers. The authors concluded that extremes of stature in the general population—either short or tall—have minimal detectable influence on peer perceptions of social behavior, friendship, or acceptance.<sup>18</sup>

A statistically significant relationship between men's heights and the likelihood of completing college was not found.<sup>52</sup> Taller men were not more likely to achieve higher professional status when analyses controlled for educational attainment.<sup>52</sup> Studies of the relationship between height and income often report that tall men and women earn more than their shorter colleagues.<sup>52–56</sup> However, when potentially confounding variables such as age, health, education, and family of origin characteristics are controlled for statistically, the relationship between height and income is attenuated.<sup>52,56</sup> In a cohort study of all healthy Swedish military conscripts in 1994, short conscripts ( $< -2$  height SD) exhibited more physical and mental health problems and scored lower on tests of intellectual performance than taller men.<sup>57</sup> The investigators raised the possibility that the association between height and physical and

psychological adaptiveness are indirectly linked. For example, biological factors that contribute to poorer growth may also be responsible for poorer physical performance and more limited intellectual aptitude.

The relationship between height and marriage rates varies by study. In the National Child Development Study (a longitudinal study of British citizens), the probability of being married was 7% lower for short men ( $< 9^{\text{th}}$  percentile) and 5% lower for tall women ( $> 90^{\text{th}}$  percentile) than for adults of average height (20<sup>th</sup>–79<sup>th</sup> percentiles), when statistically controlling for social class, education, health, race, and region of residence.<sup>53</sup> Contrasting findings were derived from the U.S. National Longitudinal Survey of Youth, a study featuring a comparable research design. Although short men exhibited lower rates of first marriage than those of average height, this effect disappeared once family-of-origin variables (parental education, poverty status, and region of the country) were taken into account; no consistent relationship was found between women's height and marriage rates.<sup>58,59</sup>

#### Assumption 3: Height-related social stress results in significant problems of psychological adjustment.

As both teasing<sup>60,61</sup> and psychological adaptation problems<sup>46</sup> are relatively common among children and adolescents, support for Assumption 3 should come from a demonstrated statistical link between stressful stature-related experiences and psychosocial dysfunction. In the only study that specifically addressed this issue, parental report of stature-related teasing significantly predicted increased emotional problems.<sup>45</sup> The proportion of unique variance in problem scores attributable to teasing was approximately 2% and increased (to between 4% and 5%) when the frequency of teasing was taken into account. Juvenilization also contributed unique explanatory value, and summated with teasing as a negative influence on psychosocial adaptation.

To interpret the clinical significance of these effects, one must view them within the context of the mean level of behavior problems in this sample. As noted earlier, the psychological adaptation of short youths in this same clinic-referred cohort was comparable to community norms.<sup>12</sup> Thus, the possibility exists that stature-related stresses may contribute to variability in adaptation that falls within the "normal range."

Assumption 4: Increased growth velocity and height induced by rhGH therapy result in improved QOL. There are very few randomized, controlled trials of the QOL benefits of rhGH treatment (and only one randomized placebo-controlled trial<sup>62</sup>). In the Wessex Growth Study, rhGH-treated children with ISS were compared with those in an untreated control group at recruitment and after 3 and 5 years.<sup>62</sup> Despite a significant increase in



height in the treatment group, there were no differences between the groups on the behavioral measures at any of the 3 assessments. Comparable results were found in a more recent study in which, despite increased height in the treated group, no improvement on self- or parental-report measures of psychosocial adaptation and self-esteem were found.<sup>20</sup> In a recently published report on the psychological benefits of rhGH therapy, youths with ISS were randomly assigned to either treatment or a control group which received placebo injections.<sup>22</sup> At baseline, the behavioral/emotional adjustment and self-esteem scores for children with ISS were within the normative range. Furthermore, no systematic relationship was observed between attained height SDs, or the change in height SDs from baseline and annual changes in behavior problem or self-esteem scores. Finally, in a retrospective study of young adults who either had or had not been treated with rhGH therapy for ISS, no differences in education level or QOL were found,<sup>31</sup> though the treated patients had a romantic partner less often than participants who did not receive rhGH therapy in childhood.

Although the focus of the "treat or not to treat" debate is directed at rhGH therapy, androgen treatment of boys with constitutional growth delay has long been a strategy to accelerate growth velocity, hasten the onset of secondary sex characteristics and, thereby, ameliorate perceived psychological distress, without sacrificing adult height.<sup>63-65</sup> There has never been a comparison of the psychological benefits of rhGH versus androgen therapy. A direct comparison is tantalizing considering the differences in the objective

of treatment (ie, hastening pubertal progression versus achievement of taller adult height), duration of treatment, and cost.

## RECOMMENDATIONS

Practice guidelines for the use of rhGH therapy in children with SS state that decisions regarding "instituting or continuing therapy should be individualized...and be guided by the goal of improving the quality of life of the child and future adult."<sup>66</sup> These recommendations are echoed by Allen and Fost<sup>7</sup> who emphasize that access to rhGH therapy should be guided by the identification and amelioration of disability stemming from SS. Identifying those who experience SS as a "disability" is a challenging task. The fact that the child or adolescent experiences teasing or juvenilization, or that the family is seeking a consultation from a pediatric endocrinologist, are insufficient reasons to make this determination. Psychosocial stress is a common phenomenon in child development and, by itself, does not imply psychiatric dysfunction or even significant problems of psychosocial adaptation. Noeker and Haverkamp<sup>67</sup> developed a useful conceptual framework to guide the psychological assessment of SS which can be used to inform clinical management decisions. Three hierarchical levels of assessment are identified: stress exposure due to SS (Level I), quality of coping responses (Level II), and occurrence of psychopathology (Level III) (Table 3).

Clinical management is facilitated by a thorough psychosocial evaluation designed to delineate specific stressors experienced by the child, the pattern of coping, and psychosocial adaptation. Because of the salience of SS and its potential to serve as a lightning rod diverting attention from other stressors, clinicians must be watchful of misattributions by the child, parents, or others (including oneself<sup>20</sup>). This influence may direct attention away from prescribing psychosocial interventions for maladaptive coping.<sup>68</sup> This evaluation serves to assess individual characteristics (eg, intelligence, temperament) and social-ecologic factors (eg, degree of stress in the child's environment, salience of height to the family, social support from peers) that could moderate the influence of height on psychosocial adaptation. Finally, identifying adaptive coping strategies as an

Table 3. Psychological assessment of the short child (adapted from reference 67)

| Target of Assessment  | Information Collected   |
|---|---|
| <b>Level I</b>  |   |
| <ul style="list-style-type: none"> <li>Stress associated with condition</li> </ul>  | <ul style="list-style-type: none"> <li>Stigmatization and juvenilization associated with SS</li> <li>Other stressors associated with the medical syndrome               <ul style="list-style-type: none"> <li>Experiences of stress (Level I) do not imply psychiatric dysfunction (Level III)</li> </ul> </li> </ul>  |
| <b>Level II</b>   |   |
| <ul style="list-style-type: none"> <li>Quality of adaptive coping responses</li> </ul>  | <ul style="list-style-type: none"> <li>Behavioral and emotional propensities in response to stresses</li> <li>Individual and family characteristics serving to attenuate or amplify maladaptive responses to stress (ie, risk and protective factors)</li> </ul>  |
| <b>Level III</b>  |   |
| <ul style="list-style-type: none"> <li>Occurrence of behavioral or emotional adaptation problems</li> <li>Impairment in family, peer, or educational functioning</li> </ul> | <ul style="list-style-type: none"> <li>Range and intensity of problems and their coalescence into psychiatric syndromes</li> <li>Presence of "impairment" in key psychological development domain: family, peer, or educational functioning               <ul style="list-style-type: none"> <li>"Dissatisfaction" with height does not imply impairment in function</li> </ul> </li> </ul> |

alternative (or adjunct) to rhGH therapy is an additional goal. Gathering such detailed information is prudent in view of the clinical evidence showing that the adult height of formerly treated GH-sufficient individuals often remains substantially below average.<sup>6,69-71</sup>

The comprehensive nature of this evaluation implies that it should be conducted by a mental health professional—ideally by a member of the pediatric endocrinology team, knowledgeable in both the medical and psychosocial aspects of SS. The team member is in a position to delineate predictable psychosocial experiences related to SS and to offer anticipatory guidance to patients and families. The entire team should reassure parents that SS does not have to limit their child's current or future happiness, success, or productivity. However this is an ideal model that most often is not applied in clinical practice, even in most academic centers. Thus, the practicing physician caring for children with SS needs to balance the "do's and don'ts" (Table 4) before casting assumptions for the consequences of SS and the recommendations for treatment.

Parents may evaluate factors for and against rhGH therapy differently from physicians.<sup>72,73</sup> Factors parents consider (in order of descending importance) include risk of long-term side-effects, out-of-pocket costs, the child's attitude toward wanting rhGH therapy, the likelihood of a height increase, the magnitude of the height increase, and the route of rhGH administration.<sup>72</sup> Given the importance of these to families, it is prudent to gear interactions toward addressing these priorities. To this list, we would add the importance of making explicit the assumptions that the child, family, and physician hold concerning the liabilities of SS and the expected benefits of rhGH therapy (Table 4).

## CONCLUSION AND SPECULATION

Commonly held beliefs and attitudes serve as implicit assumptions in the QOL rationale for applications of rhGH therapy beyond the traditional role of hormone replacement. In view of the findings on stereotypes, particularly research findings gleaned from laboratory studies, it is understandable that parents of children with SS may be concerned about their child's psychosocial and educational adaptation. However, findings from clinic- and general population-based research on the real-world experiences of youths and adults with SS do not generally support the view that SS is associated with psychological dysfunction, ie, constitutes a "disability".<sup>7</sup> Similarly, research on the QOL benefits of rhGH therapy does not demonstrate efficacy for this outcome.

What might account for the stability of negative stereotypes and assumptions regarding SS despite contradictory evidence? Schkade and Kahneman<sup>74</sup> proposed that a "focusing illusion" potentially accounts for such a phenomenon. Assuming (with considerable evidence to support it<sup>8,26</sup>) most believe that SS is associated with multiple negative characteristics, it follows that evaluations of an individual's QOL that focus on this isolated trait would be overly negative. The focusing illusion occurs "when a judgment about an entire object or category is made with attention focused on a subset of that category, . . . whereby the attended subset is overweighted relative to the unattended subset."<sup>74</sup> Schwarz and colleagues (as cited in <sup>74</sup>) described one instance of the focusing illusion. In their study, college students were asked 2 questions: "How happy are you?" "How many dates did you have last month?" The correlation between responses to the questions depended on which question was asked first. When the happiness question came first, the correlation was

0.12. However, when the dating question preceded the one on happiness, the correlation rose to 0.66. Thus, focusing on one aspect of life to the exclusion of others results in overweighting of that factor in the experience of well-being. In the case of the individual with SS who is being queried about social experiences they believe are linked to height, the context of questioning encourages the respondent to focus on this one aspect of their life to the exclusion of others. Under these circumstances, responses are likely to be overweighted in the negative direction because of the shift of focus away from compensating factors. The focusing illusion thus serves as a potential explanation for why our perceptions of the QOL of others—in this case those with SS—seems to be off the mark. The existence of a focusing illusion may also serve as a cautionary

**Table 4. Recommendations for clinicians**

| Do's   | Don'ts   |
|--|--|
| Conduct a comprehensive psychosocial assessment <sup>14,67</sup>   | If problems of psychosocial adaptation are detected, do not assume that these are attributable to SS       |
| Recommend psychosocial strategies to directly address predictable social challenges associated with SS <sup>68</sup> | Do not neglect the psychosocial implications of features other than SS associated with particular syndrome |
| Balance medical recommendations with suggestions to address any psychosocial stress associated with SS <sup>67</sup> | Do not assume the parent or patient wants rhGH therapy   |
| Discourage the expectation that taller stature is associated with changes in QOL <sup>18,20,31,62</sup>              | Do not restrict discussion of side effects (known and unknown) while emphasizing safety                    |
| Be aware of and address factors the parent and patient use in making their decision <sup>2,73</sup>                  | Do not minimize potential monetary costs of rhGH therapy; discuss these prior to initiating therapy        |
| Discuss treatment efficacy in terms of the degree of certainty and magnitude of effects <sup>6</sup>                 |  |

note for parents and clinicians. The possibility exists that by focusing on height, this characteristic becomes overvalued relative to less salient ones. Ironically, the treatment with rhGH of individuals who are destined to be shorter than average, and the attendant focusing of attention and energy over years, may potentially amplify the negative influence of this cognitive phenomenon.

In conclusion, the data summarized indicate that most individuals with SS adapt psychologically to the common psychosocial stresses associated with height. These positive findings notwithstanding, family and physician concerns for the child may be influenced by prevalent stature-related stereotypes and prejudices. Furthermore, the conclusion that individuals generally make positive adaptations to difficult circumstances should not be used as a justification to ignore stresses that may be remediable. It is worth remembering that subgroups of children (and households) are already facing multiple challenges to healthy psychological function, and that the burden of teasing or juvenilization may push the balance from adaptive to maladaptive coping. Valid "remedies" for children experiencing stress (and distress) related to SS will likely come about through individualized treatments involving both psychosocial and medical interventions, including the use of growth-promoting medications.

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## ABSTRACTS FROM THE LITERATURE

### Islet Cell Transplantation in T1DM

Islet cell transplantation has succeeded in restoring insulin independence in type 1 diabetes (T1DM) patients. However, islet allografts from 2 to 4 donors have been required to transplant an appropriate cell mass. This paper described the safety and efficacy of a single-donor, marginal-dose islet transplant protocol in 8 women with T1DM, nocturnal hypoglycemia, and advanced secondary complications. Each patient received a small dose of islet cell allotransplants from a single cadaver donor pancreas after antithymocyte globulin, daclizumab, and etanercept, and were immunosuppressed with mycophenolate mofetil, sirolimus, and no- or low-dose tacrolimus. All 8 patients achieved insulin independence and freedom from hypoglycemia; 5 remained insulin-independent for longer than 1 year. Graft failure occurred in 3 patients preceded by sub-therapeutic sirolimus trough levels (<9 ng/mL) in the absence of tacrolimus trough levels (<9 ng/mL). The authors concluded that improved islet cell engraftment was secondary to the peritransplant administration of antithymocyte globulin and etanercept.

Hering B J, Kanadaswamy R, Ansit JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA*. 2005; 293:830-835.

**Editor's Comment:** Transplanting insulin producing cells from fresh cadavers into T1DM patients is known to reverse the disease, but the procedure has been too costly and fraught with difficulties for widespread use. The authors of this study showed that their protocol was effective, safe, and less costly, as a single donor cadaver was sufficient to produce an appropriate dose of islet cells for transplantation. These allografts took residence in the liver of the patients and started producing insulin. Although 3 patients rejected the transplant, they achieved insulin-independence and freedom from hypoglycemia for 127, 76, and 7 days. In previous trials there was a need to utilize 2 to 4 cadavers, and each infusion of cells cost about \$75 000, including follow-up treatments. In the new trial there was a cost saving, since only one pancreas was needed and there was a need for less diabetogenic immunosuppressants. These findings are of interest and may have implications for a not very distant day when this type of therapy will be routine in clinical care of T1DM patients.

Fima Lifshitz, MD

### Growth Hormone Receptor: In Vivo Analysis of the Cytoplasmic Signaling Domains

*In vitro* studies of the growth hormone receptor (GHR) have identified multiple post-receptor signaling pathways including JAK2 tyrosine kinase, STAT5, ERK1/2, PI3-kinase, a JAK2-independent calcium signaling element, SHP2 phosphatase, SOCS and CIS. Although STAT5 is primarily responsible for GH-induced expression of insulin-like growth factor (IGF)-I, STAT5b<sup>-/-</sup> mice have less severe growth retardation than GHR<sup>-/-</sup> mice, indicating a physiologic significance of alternative pathways.

Rowland and colleagues undertook the impressive task of teasing apart the GHR signaling domains *in vivo*. They created 2 knockin mice bearing truncated GHR mutants: m569 was truncated at residue 569 (wild-type GHR contains 650 amino acids) and had site-directed mutations of tyrosines 539 and 545 in order to delete 70% of the STAT5 docking sites, while m391 was truncated at residue 391, thereby also deleting the proximal STAT5 sites (0% STAT5 signaling left) while retaining 100%



## LETTER TO THE EDITOR: PREGNANCY IN T1DM ADOLESCENTS

Thank-you for your comprehensive article that highlighted the potential complications of pregnancy in teenagers with type 1 diabetes (T1DM). We were surprised at your findings that "chronically ill adolescents are less likely to receive contraceptive counseling and sexual education than their healthy counterparts".<sup>1</sup> The clinical practice guidelines in Canada and the United States clearly require that adolescents with T1DM receive counseling on contraception and sexual health to avoid unplanned pregnancy.<sup>2,3</sup> Given that adolescents with T1DM see a physician 3 to 4 times per year, there is much more opportunity to discuss sexual health and birth control issues compared to adolescents without chronic disease.

We are a multi-disciplinary team in a pediatric diabetes education and care program for children with diabetes. Pediatric endocrinologists, a masters-prepared social worker, nurses, and dietitians make up our team. As a quality assurance activity, we surveyed adolescents age 12 to 18 years with diabetes in our program from January to August 2001 to determine rates of smoking and sexual activity and their recall of teaching on these subjects.<sup>4</sup> We found 11.8% of adolescents with T1DM were sexually active compared to the Canadian national average of 44% for females age 15 to 19 years.<sup>5</sup> Only 5.9% of our adolescent males with T1DM reported sexual activity compared to 43% of Canadian boys age 15-19 years.<sup>6</sup> Sixty percent of adolescent girls and 40% of adolescent boys in our clinic reported having been involved in a discussion about sexuality in the previous year. We understand that our rates may be low due to reluctance of the patients to divulge this information and because we included younger adolescents in the survey.

Our team begins education about adolescent issues including sexuality, birth control, and preconception counseling at approximately age 12 years. Oral contraceptives are encouraged as well as barrier methods to prevent sexually transmitted diseases. We know of only one pregnancy in more than 500 females with T1DM in our program since 1985. Our concern lies in the difficulties that adolescents face when they are transferred to adult care at age 18.<sup>6</sup> The rate of dropout of diabetes care in young adult years has been found to be 25%,<sup>7</sup> and this is alarming considering the risk of pregnancy without early care, as you clearly state in your paper. Our focus needs to be on supporting young adults through the stress of transition while remaining ever vigilant in the care of our adolescents in preventing pregnancy.

Sincerely,

Gillian Toth, RN, CDE; Heather Dean, MD, FRCPC;  
Elizabeth Sellers, MD, FRCPC; Janet Grabowski, MD,  
FRCPC; Louise Rawluk, RN, CDE; Nicole Aylward,  
RD, CDE; Norma VanWallegghem, RD, CDE; Gen  
Henderson, MSW, CDE; Catherine MacDonald, BFA(H)  
Diabetes Education Resource for Children & Adolescents  
Department of Child Health  
Winnipeg, Manitoba

## Author's Response

*We appreciate your letter and kind words concerning our review on pregnancy in adolescents with T1DM, and we are grateful to learn of your comprehensive care program for adolescents with diabetes.<sup>4</sup> Such programs are ideal and hopefully will translate into a decrease in the rate of pregnancies in diabetic adolescents. You are correct in quoting the guidelines in Canada and the United States that mandate education on contraceptive counseling and sexual education.<sup>2,3</sup> Unfortunately, these guidelines are not a guarantee for appropriate contraception. The quality assurance activity that you reported<sup>4</sup> may not reflect the true prevalence of sexual activity among T1DM adolescents. Your comparison group of non-diabetic adolescents (1994-95 National Population Health Survey) reported an estimated 43% of girls aged 15 to 19 years had at least one sex partner in the previous year. In addition, among sexually active 15- to 19-year-old adolescents, 51% reported having sex without a condom in the past year and less than 50% of the adolescent females who admitted to sexual activity reported using oral contraceptives.<sup>5</sup> These surveys are not available for comparison in T1DM.*

*You reported that 69% of adolescent females in your clinic have been involved in a discussion about sexuality in the last year. The impact of these discussions to lower the pregnancy rate in T1DM patients is yet to be shown. Intensification of methods that prevent pregnancy in this high-risk population need to be implemented. Offering effective contraception, even without consent of the parents, may be the only means to decrease the pregnancy rate in adolescents. Hopefully, your program will provide the evidence and strategies that are applicable to diabetic adolescents and thus spur increased efforts to focus on pregnancy prevention.*

Lois Jovanovic, MD  
Director & Chief Scientific Officer  
Sansum Diabetes Research Institute  
Santa Barbara, California

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of JAK2 and ERK1/2 signaling. Radioreceptor assays confirmed normal levels of specific binding of [<sup>125</sup>I]-labeled GH, expressed per milligram of membrane protein, in the 2 mutant mice. Comparison to wild-type mice showed 44% of GH-dependent growth in the m569 mice and 11% growth in the m391 mice. Serum IGF-I levels were 16% to 21% of wild-type in m569 and less than 10% in m391. However, hepatic IGF-I transcript levels were not depressed as much, suggesting additional IGF-I protein clearance due to decreased ternary complex formation from reductions in IGFBP-3 and ALS expression. Both mutants developed obesity in males after 4 months of age, as well as associated hyperglycemia.

The authors took their characterizations one step further: microarray analysis of the 2 mutant mice compared to wild-type and GHR<sup>-/-</sup> mice revealed domain-specific regulation of different target genes. Four hundred three transcripts (398 genes) were differentially expressed across all groups, 20 were common to all, 13 unique to m569, 59 unique to m391 and 268 unique to GHR<sup>-/-</sup>. Interestingly, only 5 genes were regulated exclusively by residues 569-650; thus the distal 70% STAT5 binding played a minor role in mediating the genomic effects of GH. IGF-I was one of 20 STAT5-regulated genes, and the proximal 30% STAT5 binding was important for inducing IGF-I. The majority of regulated transcripts related to the more proximal GHR domains, where JAK2 leads to ERK1/2 and PI3-kinase signaling and SOCS proteins play an inhibitory role. These included many metabolic genes and genes related to hepatocyte function such as signaling, proliferation, translation, and transporter proteins. I refer the reader to the paper and the associated website (<http://research.imb.uq.edu.au/~mwaters/ghr/>) for detailed listings.

Rowland JE, Lichanska AM, Kerr LM, et al. In vivo analysis of growth hormone receptor signaling domains and their associated transcripts. *Molec Cell Biol*. 2005; 25: 66-77.

**Editor's Comment:** *This tremendous piece of work significantly advances our knowledge of GHR function;*

*not only is GHR signaling dissected to a sharper degree than before, but new GH functions are suggested by the target genes identified in the microarray analyses. How does all this correlate clinically? GHR mutations cause GH-insensitivity syndrome, or Laron syndrome, characterized by severe postnatal growth retardation, low circulating IGF-I levels despite elevated GH levels, and lack of IGF-I response to rhGH. The majority of reported mutations occur in the extracellular part of the protein; defects in the cytoplasmic domains of GHR, studied in this paper, are rare. However, 2 recent papers described patients with distal cytoplasmic GHR mutations resulting in selective loss of STAT5 pathway. Two siblings, in their 50's, had homozygous deletions that encoded GHRs truncated at amino acid 449; loss of STAT5 binding, despite retention of normal JAK2 phosphorylation, STAT3 and ERK2, was sufficient to cause severe growth failure (height z scores of -8.7 and -6).<sup>1</sup> A 17-year-old girl was identified with a height z score of -5.28 and classic features of Laron syndrome. She was a compound heterozygote for novel GHR mutations: C83X (lack of GHR expression due to mRNA decay or defect in cell membrane anchoring) and 1776del (GHR truncated at 581 amino acids). The 1776del GHR had significant impairment of STAT5 activation despite intact extracellular, transmembrane and more than 80% of the cytoplasmic GHR domains. STAT3 activation was normal.<sup>2</sup> The clinical importance of STAT5 signaling was further confirmed in a 16.5-year-old girl whose Laron syndrome was caused not by a GHR mutation, but by a homozygous missense mutation in the STAT5b gene; her height z score<sup>3</sup> was -7.5.*

Adda Grimberg, MD

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## Anthropometry, Metabolic Control, and Thyroid Autoimmunity in Type 1 Diabetes with Celiac Disease: A Multicenter Survey

The DPV-Wiss database is a central registry for the documentation of treatment processes and outcomes in children with type 1 diabetes (T1DM) in Germany and Austria. Data are gathered and available for analysis from 150 pediatric departments and 19 796 subjects (ages 0.1 to 19.9), representing approximately half the children with T1DM in Germany. Kaspers et al report their findings regarding the association between T1DM and celiac disease (CD) and anthropometrics and metabolic control. Although there is no consensus for measuring celiac and thyroid antibodies in children with diabetes, in Germany these determinations are

commonly performed every 1 to 2 years.

Three groups of diabetic children were identified: Group 1 comprised no clinical or biochemical signs of CD (n=11 470); Group 2 had 1 or more significantly elevated CD-associated antibodies (IgG and IgA antibodies to gliadin, endomysium, or tissue transglutaminase-IgA antibodies), but no jejunal biopsy performed or recorded (n=1 119); Group 3 exhibited biopsy-proven CD (n=127). At least one CD associated antibody elevation was present in 6.7% of the cohort, while 0.6% had histologically confirmed CD. The mean age of diagnosis of CD was lower than that of the Group 1 subjects (12.2 ± 4.6 years vs 13.4 ± 4.3 years,

$P < 0.05$ ) and the onset of T1DM was at an earlier age in Group 3 children ( $5.8 \pm 4.0$  vs  $8.2 \pm 4.0$  years,  $P < 0.001$ ). The average age of diagnosis of CD was  $4.3 \pm 3.8$  years. In 13 children, CD was diagnosed prior to the diagnosis of T1DM; 57% of Group 3 children were female.

Standing height was significantly reduced in Group 3 children ( $-49 \pm 1.1$  vs  $-0.06 \pm 1.0$  height-SDS,  $P < 0.001$ ), and this difference increased over time. Growth of children less than 11 years of age was more affected than that of older children. BMI was also lower in the CD children and did not improve over time. Daily insulin requirements, number of daily injections, and number of severe hypoglycemic episodes did not differ among groups, but HbA1c levels were significantly lower in the Group 3 children at CD diagnosis ( $8.1\% \pm 1.8\%$  vs  $8.8\% \pm 2.4\%$ ,  $P < 0.001$ ) and remained lower during the observation period. The incidence of thyroid disease was greater in the group with T1DM and CD ( $6.3\%$  vs  $2.7\%$ ,  $P < 0.02$ ).

The authors noted that the actual incidence of CD in their population may be greater than recorded, since all children with positive antibodies did not undergo jejunal biopsy. The failure of children with CD to exhibit catch-up growth may have been the result of poor compliance with the gluten-free diet. The growth reductions seen in these children would account for a loss of 5.2 cm in final height. The lower HbA1c values in the Group 3 children could have been the result of malabsorption of nutrients or perhaps more meticulous attention to carbohydrate amounts and source when using the gluten-free diet. The authors concluded that the recommendations for regular screening of children with T1DM for CD beginning at disease onset is both rational and important, as untreated CD is associated with significant long-term health risks such as osteoporosis and intestinal lymphoma.

Kaspers S, Kordonouri O, Schober E, Grabert M, Hauffa B, Holl R and the German Working Group for Pediatric Diabetology. Anthropometry, metabolic control, and thyroid autoimmunity in type 1 diabetes with celiac disease: A multicenter survey. *J Pediatr*. 2004;145:790-795.

**First Editor's Comment:** This paper presents important data confirming the relationship between CD and T1DM; it lends evidence for the need to screen children with T1DM for celiac associated antibodies at the time of diagnosis and throughout childhood. The findings of reduced

height and BMI, not related to poor glycemic control, are of concern. The implementation of a gluten-free diet is difficult and compliance is even more difficult to assess than that of the more routine carbohydrate-counting caloric recommendation diet usually prescribed for children with T1DM. It will be important for studies such as the current one to continue in order to assess the long-term impact of living with both diseases. Information regarding the development of osteoporosis and lymphoma in asymptomatic children is especially important. The current database may assist in the collection of such information.

Currently, some third-party payors in the United States are reluctant to reimburse for the laboratory determinations of celiac associated antibodies in children with T1DM who do not exhibit typical symptoms and signs of CD. The data from this German database can be used to help justify such screenings.

William L. Clarke, MD

**Second Editor's Comment:** It is important to distinguish CD from celiac autoimmunity (CA). The former presents with symptoms and abnormalities on the intestinal mucosa and function which improve with a gluten-free diet; the latter presents no alterations other than positive IgA transglutaminase antibodies and endomysial antibody immunofluorescence IgA. The natural history of CD is well known, including chronic intestinal malabsorption and long-term risks of osteoporosis and lymphoma. However, no such data exist for CA patients who have no symptoms or alterations in jejunal morphology and function; however, monitoring height and weight progression is warranted. Since adherence to a gluten-free diet is poor even in the CD patients, the added burden to diabetic individuals would need to be considered before recommending it when CA is encountered. The reader is referred to GGH<sup>1</sup> for a review and commentary of a paper by Hofferberg<sup>2</sup> on the natural history of CA.

Fima Lifshitz, MD

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## Movement and Energy Expenditure in Obesity

The authors quantitated the movement and energy expenditure of 20 healthy self-proclaimed "coach potatoes." Ten lean ( $BMI = 23 \pm 2 \text{ kg/m}^2$ ) and 10 moderately obese ( $BMI = 33 \pm 2 \text{ kg/m}^2$ ) volunteers donned and wore a physical activity monitoring system (PAMS) for 10 days while continuing their normal occupations, hobbies, and day-time and night-time activities, and while consuming a diet designed to maintain a constant body weight. Energy expenditure related to purposeful exercise and

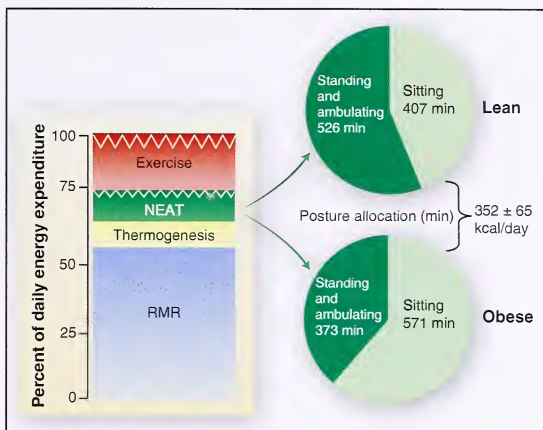
that related to routine activities of daily living, non-exercise activity thermogenesis (NEAT), was determined. NEAT was further divided into energy expended in relation to posture (lying, sitting, standing) and energy utilized for movement (ambulation). The findings revealed that both groups slept (lying) for similar intervals, but that obese subjects sat 164 minutes per day longer and stood 152 minutes less per day than did control volunteers (Figure). This translated into a mean lower daily energy expenditure



of 352 kcal. Neither supervised weight loss (8 kg) in 7 obese subjects nor weight gain (4 kg) in 10 lean volunteers altered the distribution times of posture and movement, suggesting that these activities were "intrinsic" to the individual rather than environmentally determined.<sup>1</sup> However, the mechanism(s) that regulates posture and movement distribution are not known. The investigators suggest that if an obese subject were to increase daily caloric expenditure by 350 kcal (without corresponding increase in calorie intake, of course), over the course of 1 year there would be a 15 kg weight loss!

Levine JA, Lanningham-Foster LM, McCrady SK, et al. Interindividual variation in posture allocation: Possible role in human obesity. *Science*. 2005;307:584-586.

**Editor's Comment:** It has long been known by clinical observation that very obese subjects move imperceptibly when sitting (ie, they do not fidget) and choose to sit when others in the vicinity are standing; by inference they must be conserving every calorie. However, present data provide quantitative proof on this propensity even in only modestly obese individuals. The method for measurement of PAMS was designed by Levine and consisted of 6 sets of sensors embedded in special underwear, 4 "inclinometers" attached to the trunk and thighs, and 2 "triaxial accelerometers" fixed to the base of the spine.<sup>2</sup> Each subject wore this unit 23:45 hours daily (15 minutes for showering) for 10 days. These instruments recorded data every half-second providing information on body position and motion 1 728 000 times over 10 days per subject! Experimentally, injection of orexin into the paraventricular nucleus of rats increases NEAT, implying that posture and movement may be



The components of daily energy expenditure are depicted (left) and the differences between lean and obese subjects are shown (right). The pie charts show the cost of unplanned physical activity (NEAT).

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modulated by neural transmitters. Clearly, efforts to increase NEAT in our obese patients are worthwhile—primarily by substituting physical activity such as walking for television viewing and game playing.

Allen W. Root, MD

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## The Many Faces of *PTHR1* Mutations

Gain-of-function mutations of the gene encoding the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) type 1 receptor (*PTHR1*) gene cause the severe, dominantly inherited metaphyseal chondrodysplasia, type Jansen. Loss-of-function mutations of this receptor are associated with osteosclerosis and advanced skeletal maturation of the recessively inherited Blomstrand chondrodysplasia. Eiken syndrome is a rare autosomal recessive bone dysplasia with a skeletal phenotype quite different from the other 2 conditions, most notably exhibiting multiple epiphyseal dysplasia with extremely delayed ossification.

Duchatelet et al mapped Eiken syndrome in an informative family to the region *PTHR1* locus. Mutation analysis revealed an ARG485STOP nonsense mutation in the last exon that predicts truncation of the last 108 amino acids from the receptor's cytoplasmic tail. This domain contains several elements critical to the function of the receptor. These include several serine residues that are phosphorylated upon ligand binding and docking sites for proteins that propagate *PTHR1* signals including G-protein receptor kinases (ie, adenyl cyclase(AC)/protein kinase A (PKA), phospholipase C (PLC), protein kinase C (PKC)) and  $\beta$ -arrestin as well as residues that participate in the receptor internalization and down regulation.

The authors did not carry out functional studies, but they speculated based on what has been previously reported from knockin mouse and cell culture investigations in which the receptor was genetically modified to alter kinase-mediated signaling pathways. Specifically, they propose that the truncation creates an imbalance between AC/PKA versus PLC/PKA activation. They acknowledge that other mechanisms could be involved.

Duchatelet S, Ostergaard E, Cortes D, Lemaninque A, Julier C. Recessive mutations in *PTHR1* cause contrasting skeletal dysplasias in Eiken and Blomstrand syndromes. *Hum Molec Genet.* 2005;14:1-5.

**Editor's Comment:** This is an interesting paper, not so much because of any firm conclusions about the mechanism involved since the authors provide no biological data; rather, it is because there are 3 distinct clinical phenotypes associated with mutations of the same gene. This study underscores the fact that many proteins have multiple functions that reflect different domains of the protein and that mutations of genes encoding these proteins can have quite different consequences depending upon which of these domain functions they disturb.

William A. Horton, MD

## Developmental Expression Patterns of Human Thyroid Transcriptional Regulators

To further the understanding of thyroid dysgenesis, the most common cause of congenital hypothyroidism, Trueba and colleagues examined the developmental expression patterns of human thyroid transcriptional regulators by *in situ* hybridization and immunohistochemistry in tissues obtained from legally terminated pregnancies. They focused on 3 factors: *PAX8*, *TITF1* (also known as *Nkx2a*, *Ttf-1* or *Tebp*) and *FOXE1* (*Ttf-2* or *Ttf-2*). These 3 factors lead to thyroid dysgenesis in knock-out mouse models and have been found to cause congenital hypothyroidism when mutated in humans. The *PAX8* gene was strongly expressed in median thyroid anlagen (from pharyngeal primordium) and laterally ectodermic region of the fourth pharyngeal arch, thyroglossal duct cells, ultimobranchial body and in later fetal follicular cells. It maintained follicular cell phenotype by activating thyroperoxidase, sodium/iodide symporter and thyroglobulin genes. The expression in thyroglossal duct cells suggested that the track was created by the migrating thyroid anlagen (rather than a pre-established pathway through which the thyroid migrated). This may explain why cells of thyroglossal duct remnants can differentiate into follicular cells to create follicle- and colloid-containing cysts. Additionally, *PAX8* gene had an extra-thyroid expression on the otic vesicle, central nervous system (midbrain-hindbrain boundary, spinal cord) and the developing kidney (metanephric blastema, ureteric bud and their derivatives). The clinical correlates of the *PAX8* gene have been found in congenital hypothyroid patients who also presented either unilateral renal agenesis

or left-sided ureteropelvic obstruction. No *PAX8*<sup>-/-</sup> humans have been reported with bilateral renal agenesis (? lethal phenotype form). However, no CNS defects were seen in knock-out mice and heterozygote *PAX8*<sup>+/-</sup> humans have been detected.

The *TITF1* gene was weakly expressed in the median thyroid primordium and later fetal thyroid. The extra-thyroid expression was limited to the forebrain (hypothalamic floor and infundibulum, developing basal ganglia territory) and lung epithelial cells which became progressively restricted to distal branches, reducing surfactant production. The clinical correlates of the *TITF1* mutations were hypotonia and dyskinesia, changes in basal ganglia and pituitary gland and postnatal respiratory distress syndrome.

The *FOXE1* gene had a weak expression in thyroid primordium and gland throughout development and the extra-thyroid expression was seen in the thymus and in the oropharyngeal, tracheal and esophageal epithelium. The clinical correlates were seen in patients with thyroid dysgenesis and cleft palate as well as knock-out mice. There were no thymic or immunologic abnormalities yet reported. The thyroglobulin protein promoter contained binding sites for *PAX8*, *TITF1* and *FOXE1*, but thyroglobulin was not produced until the thyroid gland reached its final position.

Trueba SS, Auge J, Mattei G, et al. *PAX8*, *TITF1*, and *FOXE1* gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. *J Clin Endocrinol Metab.* 2005;90:455-462.

**Editor's Comment:** This paper is an excellent example of bench-to-bedside applications. It also points out that, despite the power of knock-out mouse models for explaining physiology, caution should be taken in extrapolating to humans as species differences occur. For a recent review of congenital hypothyroidism and its etiologies, see reference 1. Another genetic cause of congenital hypothyroidism, not listed in this paper or the review, is inactivating mutation of the gene encoding the TSH receptor; 2 siblings with compound heterozygosis had severe congenital hypothyroidism with

apparent athyreosis, while their non-consanguineous, hemizygous parents had either normal thyroid function or compensated hypothyroidism with mild thyroid hypoplasia.<sup>2</sup>

Adda Grimberg, MD

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## Sex Differences in Patients Referred for Evaluation of Poor Growth

This study examines the sex difference in the rate of referral to a pediatric endocrinology center for evaluation of short stature or poor growth. The source of data was medical charts from all patients initially evaluated during 2001. After exclusion of those with a prior evaluation by a pediatric endocrinologist for treatment with growth hormone (GH) (n=4), referral for evaluation of pituitary function secondary to brain disease or abnormality (n=15), and girls with known Turner syndrome (n=6), the medical records of 278 patients were available for analysis.

The table indicates multiple statistically significant disparities in anthropometric characteristics of boys and girls at the time of the initial visit to the pediatric endocrinologist.

Grimberg A, Kutikov JK, Cucchiara AJ. Sex differences in patients referred for evaluation of poor growth. *J Pediatr.* 2005;146:212-216.

**Editor's Comment:** This is not the first epidemiologically-oriented study that has detected a sex difference in referral patterns.<sup>1</sup> Similarly, a survey demonstrated that pediatric endocrinologists were more likely to recommend GH therapy for boys with idiopathic short stature than for a girls with identical auxologic characteristics.<sup>2</sup> Converging evidence from these and additional studies replicate the societal bias that taller stature is more important in boys/men than in girls/women.<sup>3</sup> The fact that this bias is reflected in pediatric care is worrisome however. The under-representation of girls receiving growth evaluations raises the possibility of missed or late diagnoses. Alternatively, the over-representation of short boys in pediatric endocrinology referrals raises the possibility that health care has become complicit in societal prejudices along with the added burden to the patient of potential medical and psychological risks (recognized and unknown) as well as economic costs.

This study raises an additional cause for concern: the majority of patients (59%) referred to one pediatric endocrinology clinic for a growth evaluation, arrived without plotted growth measurements. Other studies have shown that inaccurate height measurement tools are often used in primary care.<sup>4</sup> What is needed is a return to fundamental practice, recommended by the American Academy of Pediatrics<sup>5</sup> of routine growth monitoring in primary care to

Referrals for growth evaluation by gender (selected findings)

| Variables  | Boys<br>(n = 182) | Girls<br>(n = 96) | P       |
|--|-------------------|-------------------|---------|
| Gender   | 65%               | 35%               | <0.0001 |
| Median height at referral *                                  | -1.9              | -2.4              | <0.01   |
| Median deficit from mid-parental target height *             | -1.3              | -1.9              | <0.001  |
| Median time to referral (months) **                          | 24                | 35                | 0.30    |
| Age at referral  |                   |                   |         |
| < 9 years  | 57%               | 43%               | <0.05   |
| ≥ 9 years  | 71%               | 29%               |         |
| Median height deficit at referral *                          |                   |                   |         |
| < 9 years  | -2.1              | -2.4              | 0.48    |
| ≥ 9 years  | -1.9              | -2.4              | 0.01    |
| Deficit from mid-parental target height *                    |                   |                   |         |
| < 9 years  | 1.5               | 1.8               | 0.34    |
| ≥ 9 years  | 1.2               | 2.1               | <0.001  |
| Organic disease+   | 15%               | 41%               | <0.0001 |
| Normal height referrals                                      | 38%               | 20%               | <0.01   |
| Familial short stature, constitutional growth delay, or both | 72%               | 48%               |         |

\* z scores

\*\* Calculated by subtracting age at first deviation across major percentiles from the age at first visit to pediatric endocrine center (growth curves were available for only 115 of 278 patients)

+ Difference remains statistically significant after excluding girls with Turner syndrome (n = 9)

differentiate healthy from pathological growth. Evidence of a strong sex bias in referral to a specialist suggests that clinicians (and parents) are possibly over-valuing "height" and possibly devaluing "growth" to the detriment of girls, in particular, and society at large.

David E. Sandberg, PhD

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## Visfatin – A New Visceral Fat Adipokine

Employing the method of differential display of expressed genes (by analysis of 8800 gene products utilizing cDNA probes) in paired samples of subcutaneous and visceral fat donated from 2 female volunteers, the investigators identified an adipokine that is synthesized primarily by visceral fat and termed this molecule "visfatin." They subsequently found that the visfatin had been previously identified as "pre-B cell colony-enhancing factor" (PBEF). This is secreted by the liver, bone marrow, and muscle, is a growth factor for early stage B lymphocytes, and down-regulates apoptosis of neutrophils. The investigators demonstrated that: 1) expression of PBEF/visfatin increased during adipocyte differentiation *in vitro* with increased secretion of this protein into medium; 2) plasma concentrations of visfatin correlated with the volume of visceral fat in humans and mice but not the quantity of subcutaneous fat; 3) plasma levels of visfatin increased as the amount of fat accumulated in a mouse model of obesity; and 4) visfatin values rose rapidly in mice ingesting a high-fat diet. Subsequently, the authors observed that intravenous administration of visfatin led to a dose-dependent decline in glucose concentrations without affecting insulin values in intact and diabetic mice. Complete knock-out of the visfatin gene was lethal. In heterozygotic (visfatin<sup>+/-</sup>) animals, basal plasma glucose values were elevated, glucose tolerance was impaired, while there was no difference in size or insulin levels. *In vitro*, visfatin had several insulin-like actions including: enhancement of glucose uptake, suppression of glucose release, accumulation of triglycerides, and induction of gene markers of adipocyte differentiation (PPAR $\gamma$ , fatty acid synthase, adiponectin, and so forth). The investigators also showed that visfatin bound to the insulin receptor and induced its autophosphorylation, as well as phosphorylation of a number of downstream products consistent with induction of the insulin/insulin

receptor signal transduction pathway. Most interestingly, they demonstrated that visfatin did not bind to the same segment of the insulin receptor as insulin (extracellular  $\alpha$  subunit), although the binding site on the insulin receptor to which visfatin adheres was not identified. The authors concluded that visfatin has insulin-like effects and may be of physiological significance in the regulation of glucose homeostasis.

Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science*. 2005;307:426-430.

**Editor's Comment:** *This exciting discovery adds yet another factor to the many that regulate glucose and lipid homeostasis and to the list of adipocyte products that includes leptin, adiponectin, resistin, tumor-necrosis factor- $\alpha$ , and interleukin-6.<sup>1</sup> Although visfatin has many insulin-like qualities, its serum concentrations are lower than those of insulin and do not change acutely after eating. Inasmuch as visfatin is primarily secreted as the quantity of visceral fat increases, it may serve as a (less than optimal) compensatory mechanism for the deleterious effects of increased visceral adiposity. It is of interest that visfatin, like other non-peptidic small molecules, such as modified benzoquinones, can cross the plasma membrane and interact with and activate the insulin receptor tyrosine kinase.<sup>2</sup> These agents are active orally in animal models of type 2 diabetes mellitus; they increase insulin sensitivity and also exert other central and peripheral effects.*

Allen W. Root, MD

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## GROWTH AND GROWTH HORMONE IN CHILDREN WITH NEUROFIBROMATOSIS TYPE 1

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### INTRODUCTION

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant, commonly inherited disease that affects one of every 3000 individuals.<sup>1</sup> The gene responsible for this condition has been isolated by positional cloning to chromosomal region 17q11.2. It spans over 350 kb of genomic DNA and encodes neurofibromin, a protein product of 2818 amino acids that is expressed in various tissues.<sup>2</sup> According to the National Institutes of Health Consensus Development Conference (Bethesda, Maryland, July 13-15, 1987), there are 7 key components of the disease (Table 1), at least 2 of which must be present in order to establish the diagnosis.<sup>3</sup>

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### From The Editor's Desk

In this issue the international contributing editors of GGH began abstracting papers for the readership. They reviewed the literature, selected important publications, abstracted them, and made insightful and important comments. Thus, with these contributions we continue to expand the reach of the journal. On behalf of everyone I thank them for their contributions and welcome them.

The lead article covers an important area that clinicians often encounter, namely evaluating the growth of patients with neurofibromatosis type 1. Growth alterations affect 13% to 24% of children with this disease, with more than 40% of adult NF1 patients reaching a decreased final height. Drs. Karantz and Geffner have written a notable review outlining the most pertinent issues of growth and growth hormone in NF1 patients. I am sure that this lead article will be tremendously useful and will constitute an important reference.

Additionally, the highlights of the 87th annual meeting of the Endocrine Society are summarized and 8 abstracts are presented in this printed GGH. There are 7 more abstracts posted on the web at www.GGHjournal.com. I trust you will enjoy and treasure this issue.

The next issue will include a historical review of growth hormone that commemorates the 20th anniversary of the FDA approval of recombinant human growth hormone and the launching of GGH—both made possible by Genentech.

Fima Lifshitz, MD

### BACKGROUND

Endocrine disorders have been reported in approximately 1% to 3% of all NF1 patients. Pheochromocytoma is the most common endocrinopathy in adults with NF1, occurring in approximately 1% of patients.<sup>4</sup> In children with NF1, the most prevalent hormonal disorder is central precocious puberty (CPP), with a frequency of 3% compared to 0.06% in the



general pediatric population.<sup>4-6</sup> Delayed puberty has also been described, but its exact incidence has not been reported to date. Short stature (defined as a height that is equal to or more than 2 standard deviations [ $\geq 2$  SD] below the population mean) has long been known to be a feature of NF1, affecting approximately 13% to 24% of prepubertal patients and >40% of adults.<sup>7,8</sup> Although short stature is the most common growth disturbance seen in patients with NF1, tall stature has also infrequently been described as a result of growth hormone (GH) hypersecretion linked to brain tumors.<sup>9-15</sup> It is the primary aim of this paper to summarize current knowledge on growth disturbances and GH secretion in children with NF1.

**Table 1. Diagnostic Criteria**

|  |
|--|
| 1. Six or more café-au-lait macules, the greatest diameter of which is >5 mm in prepubertal patients, and >15 mm in post-pubertal patients |
| 2. Freckling in the axillary or inguinal region  |
| 3. Two or more neurofibromas of any type or one plexiform neurofibroma   |
| 4. Two or more Lisch nodules in the iris   |
| 5. Optic glioma  |
| 6. A distinctive osseous lesion such as sphenoid dysplasia or pseudoarthrosis  |
| 7. A first-degree relative with NF1 diagnosed according to the preceding criteria  |

## GENETICS OF GROWTH

The known molecular functions of the *NF1* gene and its protein, neurofibromin, could account for the short stature phenotype of NF1-affected individuals (Figure 1). Neurofibromin is a major regulator of the Ras pathway, a key signal transduction pathway which transmits mitogenic signals to the nucleus, and is expressed in many different tissues, including the brain. It contains a central domain related to Ras-specific guanosine triphosphatase-activating proteins (Ras-GAPs). It stimulates the intrinsic activity of Ras-GTPase and is involved in control of cellular growth and differentiation through down-regulation of Ras activity.<sup>16</sup> Mutations in the GAP-related domain of the *NF1* gene lead to increased levels of activated Ras and, thus, to increased downstream mitogenic signaling.<sup>17</sup> The NF1-conserved *Drosophila* homologue acts as a negative Ras regulator. Homozygous NF1 *Drosophila* mutants with 2 different mutations that result in lack of expression of NF1 protein are 20% to 25% smaller than flies of the parental strain, but are otherwise patterned normally. Their growth defect is rescued by expression of an *hsNF1* transgene, as well as by increasing cAMP-activated protein kinase A (PKA) expression, implying that both Ras and PKA interact in a pathway that controls overall growth.<sup>18</sup> Activated PKA has also been shown to play a critical role in stimulating proliferation of some cell types<sup>19</sup> and may physiologically contribute to body growth. Based on the *Drosophila* model, it could be postulated that alterations

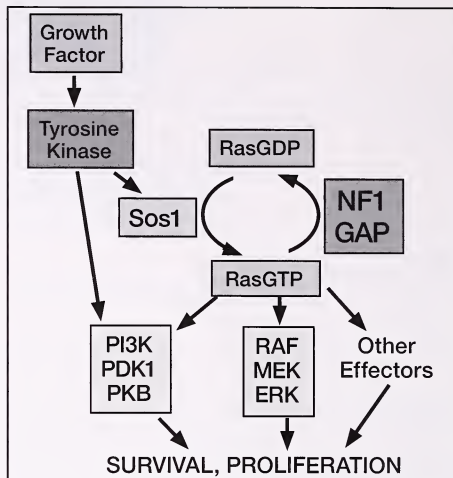


Figure 1. Cross talks among signaling pathways linked by GAPs. Neurofibromin (NF1) and p120RasGAP (GAP) control the hydrolysis of RasGTP. RasGTP is activated by growth factors via the exchange factor Sos1 and activates a number of effectors such as RAF/kinase and PI3K, which in turn activate and phosphorylate the ERK and PKB kinases. In this way, GAPs can serve as key integrators of distinct signaling pathways. Reprinted with permission Donovan S, Shannon KM, Bollag G. *Biochim Biophys Acta*. 2002;1602:23–45. Copyright © 2002. Elsevier. All rights reserved.

in these pathways could result in smaller phenotypes in humans with NF1 as well.

## GROWTH PATTERNS IN CHILDREN WITH NF1

### Short Stature

Short stature associated with NF1 usually affects the skeleton symmetrically.<sup>20</sup> The etiology of short stature in patients with NF1 does not correlate with disease severity and is multifactorial, stemming from the disease itself or its complications. These complications may include problems that interfere with normal skeletal development, such as scoliosis<sup>21,22</sup> or deep plexiform neurofibromas, or the use of psychostimulant medications<sup>23</sup> for the treatment of attention deficit disorder,<sup>24</sup> which is a frequent behavioral problem in children with NF1. Risk factors for suboptimal growth are listed in Table 2.

Riccardi<sup>20</sup> suggested that short stature was an “all-or-none” phenomenon that affected only a subset of NF1 patients. Contrary to this suggestion, the National Neurofibromatosis Foundation International Database (NFDB) cross-sectionally analyzed the distribution of heights in 569 Caucasian North American children<sup>25</sup> with NF1 (Figure 2). Of note, the mean height SD score (SDS) among their patients was lower than that of the reference population. Thirteen percent of the NF1 patients fell >2 SD below the reference population mean, compared to only 2% of controls. They concluded that the distributions of stature

**Table 2. Risk Factors Associated with Short Stature in Children with NF1**

|  |
|--|
| Suprasellar lesions                                |
| Surgery or radiotherapy for intracranial lesions   |
| Growth hormone deficiency                          |
| Thyroid-stimulating hormone deficiency             |
| Central precocious puberty                         |
| Delayed puberty                                    |
| Scoliosis  |
| Plexiform neurofibromas                            |
| Familial NF1                                       |
| Familial short stature                             |
| Methylphenidate use for attention deficit disorder |

are shifted and unimodal among NF1 patients. The NFDB provided NF1-specific growth charts (Figure 3). From a clinical standpoint, it is important to realize that deviations from the NF1-specific standards may indicate the additive effect of a specific disease feature, such as an optic glioma.

Clementi et al<sup>26</sup> also constructed NF1-specific growth charts in a study of 528 Italian patients with comparable stature centile curves to those of the NFDB. In this study, height velocity was normal during childhood for both sexes, whereas the pubertal growth spurt was slightly reduced in boys, but not in girls. During and post-adolescence, the 50<sup>th</sup> centile for NF1 patients overlapped with the 25<sup>th</sup> centile for normal subjects, but the 3<sup>rd</sup> centile was much lower in NF1 subjects than in normal subjects. There was no association of height impairment to disease severity. Carmi et al<sup>7</sup> prospectively evaluated parameters of growth, puberty, and final height in 89 children with NF1. Short stature was observed in 25.5% of patients during the prepubertal period, with a significant gradual reduction of relative height for age during puberty. Forty-three percent of patients had short adult height; of these, 58% had short stature attributable to familial NF1. Short adult height was more often attributed to central nervous system (CNS) pathology when the father was the affected parent, less when both parents were affected, and rarely when neither parent was affected. There was also a four-fold higher frequency of CPP among their patients compared to that observed in the general population, but the frequency of short stature remained the same even when patients with CPP were excluded. GH deficiency (GHD) as the cause of short stature was found only after neurosurgery and irradiation in a minority of short patients.

### Tall Stature

Short stature is a cardinal feature in NF1; however, based on the stature distribution analysis of the NFDB, 24% of NF1 patients reside >2 SD above the reference population mean<sup>26</sup> (Figure 2). Carmi et al<sup>7</sup> reported tall stature in 4 of 89 patients with NF1, all without evidence of abnormalities in the GH axis. GH hypersecretion presenting as gigantism

has rarely been described in children with NF1, and has always been associated with the presence of optic pathway gliomas (OPG).<sup>9-15</sup> In some of these patients, elevated prolactin was also observed.<sup>12-14</sup> Treatment of the OPG with surgery, radiation, and/or chemotherapy has resulted in a reduction in growth velocity and improved basal and stimulated GH levels in all cases. Bromocriptine<sup>11</sup> and, more recently, the somatostatin analogue, octreotide,<sup>15</sup> have also been successfully used in some tall NF1 patients. The mechanism of excess GH in these patients is not clear. There does not appear to be a direct secretory role of the tumor itself. Infiltration of the somatostatinergic pathways by the tumor leading to loss

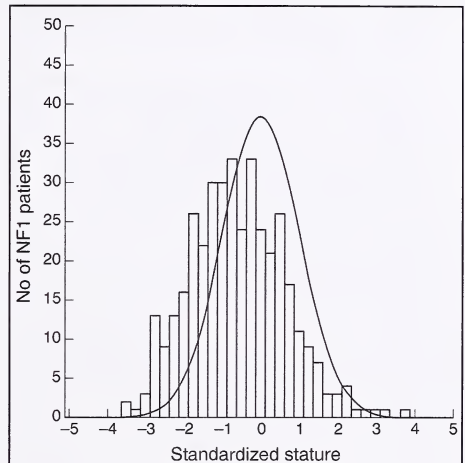


Figure 2. Distribution of sex and age standardized stature. NF1 patient measurements are from the National NF Foundation Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute. Reprinted with permission Szudek J, Birch P, Friedman JM. *J Med Genet.* 2000;37:933-938. Copyright ©2000. *British Medical Journal.* All rights reserved.

of somatostatinergic tone and, subsequently, increased GH release and loss of pulsatility, appears to be a possible mechanism in some cases.<sup>9,10</sup>

### SUPRASellar LESIONS

Malignancy accounts for the development of significant morbidity and mortality in patients with NF1, including intracranial lesions, particularly suprasellar neoplasms. The first 6 years of life appear to be the period of highest risk for development of symptomatic tumors, the median age of detection being 4.2 years.<sup>27</sup> OPGs are the most frequent neoplasms, with an overall 19% incidence on routine magnetic resonance imaging (MRI) of the brain, but with a 7% symptomatic incidence.<sup>28</sup> Gliomas of the optic chiasm are reported to cause endocrinological disorders, especially CPP and GHD.

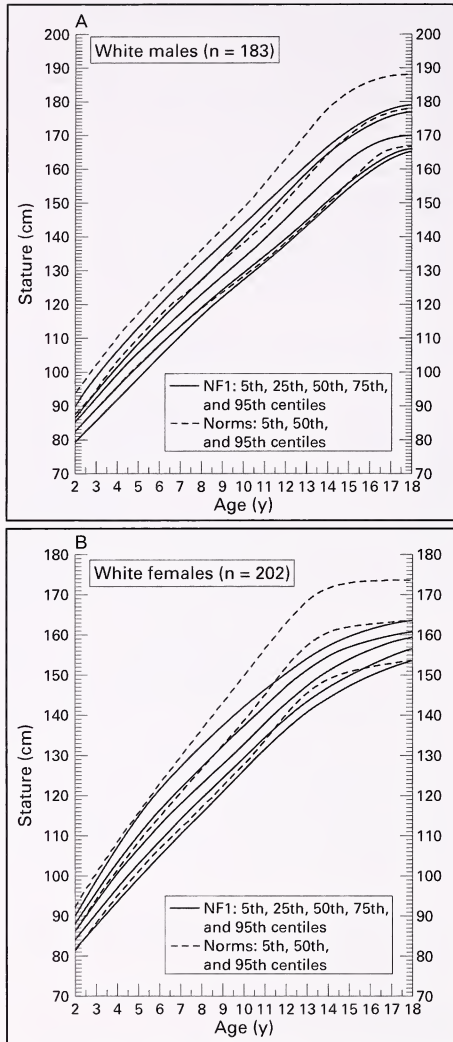


Figure 3. (A) Stature centiles in males 2-18 years. (B) Stature centiles in females 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and are denoted by dashed lines. Reprinted with permission Szudek J, Birch P, Friedman JM. *J Med Genet.* 2000;37:933-938. Copyright ©2000. British Medical Journal. All rights reserved.

### Central Precocious Puberty

In a study by Habiby et al.<sup>29</sup> of 219 children diagnosed with NF1, 3% had CPP, all associated with OPG. This

association also held true in all CPP patients in the study by Carmi et al.<sup>7</sup> However, in a study by Cnossen<sup>30</sup> of 122 children with NF1, the prevalence of CPP was the same as that previously reported; however, there was no evidence that OPG was a prerequisite for CPP, since only 1 of 3 children with CPP had an OPG at the time of diagnosis. Listernick et al.<sup>31</sup> reported CPP in 5 of 17 children with an OPG and NF1, in contrast to no cases of CPP in a group of children with OPG and no features of NF1. Virdis et al.<sup>32</sup> reviewed the records of 412 NF1 patients and also concluded that CPP is frequently—but not exclusively—associated with OPG. The above studies support an independent association of CPP and NF1 that cannot be solely attributed to OPG. A distinct feature of NF1-associated CPP is its slower rate of pubertal progression compared to CPP not associated with NF1. Whether treatment with gonadotropin-releasing hormone (GnRH) agonists is mandatory and/or efficacious in improving final height in the NF1 population remains under debate. However, there is general agreement that treatment should be offered in children manifesting signs of CPP at a young age and/or in those with a progressive decline in predicted final height.<sup>33</sup>

### Growth Hormone Deficiency

GHD is an important complication in children with NF1, with the etiology in some patients remaining unclear. In the majority of children with NF1, GHD occurs primarily in those with an intracranial tumor who undergo intracranial surgery and cranial irradiation therapy. Indeed, in the study by Carmi et al.<sup>7</sup> using clonidine or insulin-induced hypoglycemia as GH secretagogues, all children diagnosed with GHD had a history of cranial surgery or irradiation. In a study by Pierce et al.<sup>34</sup> of 24 patients with OPG, half of whom had NF1, GHD was found in 15 of 18 patients who were evaluated following treatment with radiotherapy. Huguenin et al.<sup>35</sup> evaluated the relationship of adult height after cranial radiation for OPG to NF1, CPP, and GHD caused by the tumor itself or its management. Cranial irradiation resulted in GHD in 100% of cases. Reduced adult height resulted when there was GHD and CPP in the presence of NF1. In a retrospective review of the Pfizer International Growth (KIGS) database,<sup>36</sup> which is a database monitoring recombinant human GH (rhGH)-treated children, a total of 102 children with NF1 were identified, 43 of whom had an intracranial tumor. Ninety-two percent and 80% of the GH-tested patients with a cranial tumor had peak GH responses below 10 and 5  $\mu\text{g/L}$ , respectively. Eighty-one percent and 56% responded below 10 and 5  $\mu\text{g/L}$ , respectively, in the non-tumor group. The median GH peak response to stimuli (most commonly insulin-induced hypoglycemia or arginine) was significantly lower in the tumor group compared to the non-tumor group (3.0 vs 4.6  $\mu\text{g/L}$ ;  $p < 0.001$ ). However, Cnossen et al.<sup>30</sup> reported a 2.5% prevalence of GHD in children with NF1 without an intracranial mass and before



surgical or radiation therapy for OPG, a frequency that is significantly higher than the 0.03% observed in the general pediatric population. An OPG was detected in 1 of 3 children with GHD, suggesting that GHD appears independently of the presence of OPG. In a study by Vassilopoulou-Sellin et al,<sup>37</sup> the incidence of GHD was investigated in 19 poorly growing children with NF1 and without other identifiable risk factors for shortness. Seventy-nine percent were diagnosed as having GHD on the basis of a peak GH response <10 µg/L after clonidine stimulation, and 42% had a peak GH level <5 µg/L, indicating a high frequency of profound GHD in this cohort. The causal mechanism of increased frequency of GHD in patients with NF1 remains to be elucidated. It is still plausible that despite the high-resolution capability of current MRI neuroimaging, cerebral abnormalities responsible for GHD are present, but not readily identifiable. Another possible explanation could be that there are abnormalities occurring at the cellular level, implicating the known molecular function of neurofibromin in signal transduction.<sup>17</sup>

#### Other Anterior Pituitary Hormone Deficiencies

Deficiencies of other anterior pituitary hormones such as thyroid-stimulating hormone (TSH) and adrenocorticotrophin (ACTH) have also been described in subjects with NF1 as a result of surgery and/or irradiation for intracranial tumors. Unrecognized hypothyroidism can account for poor growth, and unrecognized adrenal insufficiency can have potentially fatal consequences. Carmi<sup>7</sup> described 3 out of 6 children with NF1 and OPG who required thyroid hormone replacement after surgery and/or cranial irradiation. In the review by Huguenin et al,<sup>35</sup> no subject had TSH or ACTH deficiency prior to irradiation. However, 80% were found to be TSH-deficient and 17% were found to be ACTH-deficient after irradiation. Gonadotropin deficiency was variable with delayed or even arrested pubertal development in 43% of the patients, and low gonadotropin responses to GnRH were found in 60% of the patients evaluated.

#### GROWTH HORMONE REPLACEMENT Efficacy

In a retrospective review of patients with NF1 from the KIGS database,<sup>36</sup> the outcome of 102 children treated with rhGH, at a mean dose of 0.18 mg/kg/wk with a mean duration of treatment of 2.7 years, was assessed. These included pre- and post-pubertal patients with and without intracranial tumors. The pretreatment median height SDS was -2.4 and the median height velocity was 4.2 cm/year. The median height velocity increased to 7.1 cm/year during the first year of treatment and remained above the baseline value during the next 2 years. The median height SDS increased from -2.4 to -1.9 in the first year and remained stable thereafter. There was no significant difference in the response to treatment between the tumor

and the non-tumor groups, nor between those who had received radiation and those who had not. It is notable that the response to treatment was modest and less than that observed in patients with idiopathic GHD. However, the dose of rhGH given to patients with GHD was lower than that in other studies where an average dose of 0.30 mg/kg/wk was used, and it is likely that the growth velocity would have further declined if the patients had been left untreated. Vassilopoulou-Sellin et al<sup>37</sup> reported their experience with rhGH replacement therapy in a cohort, including children with NF1 and GHD without suprasellar lesions. This group of patients increased their annual growth rate (from a pre-treatment average) to 5 cm/year to 9 cm/year the first year, 8.3 cm/year the second year, and 6 cm/year during years 3 to 5 of rhGH therapy.

#### Safety

While therapy with rhGH has been shown to be safe, theoretical concerns remain that rhGH treatment may potentially increase an individual's risk of developing cancer *de novo* or increase the risk of recurrence of primary tumors and/or the incidence of second tumors in cancer survivors. Analysis of the KIGS database revealed recurrence of a primary CNS tumor and/or appearance of a second tumor in 5 of 102 rhGH-treated subjects<sup>36</sup> with NF1. Unfortunately, MRI neuroimaging was not performed in all patients prior to the start of rhGH treatment and, hence, definitive conclusions on the timing of malignancy presentation and its relation to rhGH therapy cannot be drawn. The natural history of OPG in children with NF1, as reported in previous studies,<sup>39</sup> suggests an incidence of tumor recurrence of 11% to 14%. There are also reports of a 30% recurrence rate of OPG after 10 years in NF1 patients under the age of 20 treated with surgery.<sup>40</sup> The occurrence of second intracranial tumors has also been frequently reported in children with NF1 and OPG. Hochstrasser<sup>41</sup> and Kuenzle<sup>42</sup> reported second tumors in 21% and 52%, respectively, during 9 years of follow-up. Based on the results of the above studies and clinical observations, there does not appear to be an increased risk of primary tumor recurrence nor development of a second malignancy in children with NF1 treated with rhGH.<sup>43</sup> However reassuring the data may be, continuous surveillance for all NF1 individuals treated with rhGH is mandatory.<sup>44</sup>

#### Progression of NF1 Features

It is well documented that café-au-lait macule size increases during puberty.<sup>45</sup> It is also known that neurofibromas increase both in size and in number in pubertal patients. Superficial growth of neurofibromas can lead to underlying segmental hypertrophy, whereas deeper structure invasion of the spine and paraspinal areas can create anatomical problems, the most dangerous of which is spinal cord compression. Whether rhGH treatment can accelerate or augment the growth of these lesions with harmful sequelae remains of concern. Indeed, 13% of the NF1 patients in the KIGS database,<sup>36</sup> many of whom



were pubertal, had changes in café-au-lait macules and neurofibromas. There are no reports that the increase in disease progression was accelerated secondary to rhGH, although one patient had an increase in the size of a pre-lumbar mass thought to be a neurofibroma. Cnossen et al<sup>30</sup> reported no growth of neurofibromas that could be ascribed to rhGH replacement in their patient population. The above results are reassuring; however, until larger-scale observations become available, close monitoring of the growth of neurocutaneous lesions is still warranted in rhGH-treated NF1 patients.

## CONCLUSION AND SPECULATION

Short stature is a well-recognized manifestation of patients with NF1, although its etiology is not fully understood. Insight as to what represents a normal pattern of growth for individuals with NF1 has been gained through the generation of NF1-specific growth charts using information from the NFDB. It is apparent that most children with NF1 grow normally until puberty. Thereafter, their height velocity is diminished compared to their healthy peers, leading to a final height significantly below their predicted genetic target. Disease-specific features, such as scoliosis and extensive neurofibromas, can further compromise final adult height. Suboptimal growth (using the NF1-specific growth charts) is also a compelling argument to look for disease-related complications such as malignancies, the most common being OPG. These tumors are frequently the cause of CPP which if present, may further compromise final height. It is also important to be aware that the increased incidence of CPP in patients with NF1 cannot solely be attributed to the presence of OPG, as CPP may occur in this setting without any tumor. Treatment of symptomatic OPG with radiation, surgery, or chemotherapy may result in decreased final height by causing damage to the hypothalamic-pituitary region and connections thereof, resulting in one or multiple pituitary hormone deficiencies, most often GH. Recent evidence of an increased incidence of isolated GHD without identifiable risk factors in children with NF1 suggests that screening is mandatory when no plausible alternative explanation accounts for a suboptimal growth velocity. Children with NF1 have a modest, although significant response to GH treatment. Current knowledge suggests that such treatment does not influence the progression of any of the features of NF1, including the incidence of recurrence of primary or the development of secondary intracranial tumors. Hence, it appears that the use of GH is efficacious and safe in children with NF1 and GHD, although continuous vigilance is necessary. The discovery of neurofibromin, with its multiple actions on signal transduction and control of cellular growth, has shed light onto aspects of the molecular biology of the disease. Further analysis and exploration of the *NF1* gene action and the effects of its mutations may help to elucidate the cellular pathways leading to the phenotypic features (including growth disorders) of neurofibromatosis.

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## ABSTRACTS FROM THE LITERATURE

### Summary Highlights: Endocrine Society 87th Annual Meeting in San Diego, June 4-7, 2005

The full ENDO 2005 program may be viewed online at [www.endo-society.org](http://www.endo-society.org). Summarized here are some highlights of interest to the editor.

#### Growth

A new cause of short stature was reported by Olney et al from Jacksonville, Cleveland, and Christchurch (OR43-4). They described **acromesomelic dysplasia**, Maroteaux type (AMDM), a form of dwarfism characterized by marked short stature with short arms and legs. These patients had a mutation in the natriuretic C-type peptide (CNP) receptor B gene that prevents cells from exerting the action of this peptide to enhance growth, despite very elevated CNP levels. Patients with AMDM were homozygous for the gene mutation, whereas family members with one single gene copy mutation did not exhibit the syndrome but had short stature and were about 9.5 cm shorter and had an elevated CNP level 4 times higher than other family members who did not have the CNP mutation. Researchers estimated that approximately 1 in 700 people have a mutation in this gene. Thus, this alteration may be present in up to 1.3% of short stature children.

Naturally conceived children have different growth patterns and lipid profiles than those of children conceived by **in vitro fertilization (IVF)**, as reported by Miles et al from Auckland (OR54-6). They studied 110 children, ages 4 to 10 years, 50 of them were born following IVF. The IVF children were taller and had higher growth-promoting hormones IGF-I, IGF-II and IGFBP3 and had a more favorable cholesterol profile than those conceived naturally.

Countering previous data, Maghnie et al from Pavia, Milano, Parma, Rome, and Cagliari (P1-504) studied patients with **multiple pituitary hormone deficiencies (MPHD)** and those with **isolated growth hormone deficiency (IGHD)**. They reported that both types reached the same adult height. They studied 49 patients with MPHD and 39 with IGHD who were diagnosed at a median age of 7 years and treated with GH. The median adult height did not differ among the 2 groups. Adult height of MPHD patients was positively correlated with both the time period of GH treatment and with height at the time of diagnosis. The adult height of IGHD patients was positively correlated with height at diagnosis and with pubertal height gain.

A simple, compact **inhaler** showed promise in easing **delivery of human growth hormone (hGH)** as safely and effectively as by injection. Chipman et al from Indianapolis, Cambridge, and London (OR33-3) treated 12 healthy

males, 21 years to 36 years of age, in a cross-over design, with hGH given subcutaneously or by inhaler. Blood levels of GH achieved with either method of administration were similar among both groups. Mild side effects were similar and infrequent in the 2 groups. This study showed evidence that inhalation of large proteins may produce blood concentration-time profiles and variability levels similar to those obtained by hGH injection.

Geffner et al from Los Angeles, Stockholm, Prague, London, and Antwerp (P2-505) reported patients with **childhood-onset growth hormone deficiency (CO-GHD)**, who discontinued treatment after reaching adult height, but later needed additional GH therapy. They stated that GHD patients should resume treatment as soon as possible. Data from 210 patients with severe CO-GHD followed in the Pfizer International Growth and Metabolic database (KIGS and KIMS), who were off GH for more than 6 months during transition from childhood to adulthood therapy were studied. There was a significant difference in IGF-I, cholesterol, and triglyceride levels and quality-of-life scores after GH was discontinued. The poorest results were among those with longer intervals without GH. Thus, after retesting to confirm persistence of GHD, GH treatment should be resumed promptly in adults with a history of CO-GHD.

#### Obesity

Westphal et al from Ulm, Leipzig, Luebeck (P2-149) reported a novel link describing the connecting signaling pathways in adipose tissue with arterial blood pressure. The connection was the **JAK/STAT pathway** that inhibited 3-adrenergic crosstalk which down-regulated expression of angiotensin II, thus contributing to the regulation of the renin-angiotensin system. This crosstalk may represent the molecular link between **obesity and hypertension**.

Flint et al from Philadelphia (P1-705) determined that overweight children need repeat evaluations of their **glucose tolerance**, as their ability to metabolize glucose changes over time. Overall, 6 of the 44 children studied demonstrated deterioration in glucose tolerance in 15 months. In contrast, there was no significant change in the body mass index, HOMA-1R, cholesterol, and triglyceride levels of these 6 children over time. Longitudinal evaluation by oral glucose tolerance testing was necessary to detect worsening glucose metabolism.

According to de Zegher et al from Barcelona, Cambridge, and Leuven (OR34-4), **small for gestational age (SGA)** infants who have rapid **catch-up weight gain**, present with excess body fat by 2 to 3 years of age, and

may therefore already be on the path to type 2 diabetes (T2DM) later in life. The authors compared a group of toddlers with body composition measurements by DEXA, who were born small (SGA) and who normalized their weight during infancy, with another group of toddlers of average size at birth (AGA) and with normal weight in infancy. The SGA infants accumulated more adipose tissue than lean body mass; fat was deposited principally in the abdominal region and noticed shortly after completion of their catch-up growth. The path from early growth restraint to insulin resistance and later T2DM emerges early in life.

### Diabetes

For the first time a developmental gene was discovered capable of reversing **autoimmune diabetes mellitus** in mice by Yechoor and Chan et al from Houston and Otsu (P1-10). The researchers treated mice with type 1 autoimmune diabetes (NOD/LtJ) with a single intravenous infusion of an **islet cell developmental gene** (HDAd-ND and HDAd-BTC). This reversed the disease and normalized the blood sugar levels. The *in vivo* gene with *neuroD* along with betacellulin reversed the autoimmune process and restored insulin secretion. This is a promising and interesting alternative to islet cell transplantation.

According to Milanesi et al from Winston-Salem and Padova (P1-14), **amniotic fluid stem cells** showed promise

in treating **type 1 diabetes** by functional regeneration of pancreatic islets. The researchers found that mouse amniotic fluid stem cells, taken from pregnant mice, could induce pancreatic differentiation and formation of islet-like clusters that expressed insulin in streptozotocine (STZ)-treated NOD/SCID mice. When given the stem cells, these mice showed the same number of islets as the healthy mice. Furthermore, the treated mice maintained normal blood glucose levels and their pancreas' showed normal islets that co-expressed insulin.

### Steroids

This report by Yazawa et al from Fukui and Saitama (P1-330) showed that **stem cells from adult bone marrow** were able to create **cells in the testis and in adrenal glands**. Mesenchymal stem cells or marrow stromal cells were found to be engrafted and differentiated into steroidogenic cells that were indistinguishable from Leydig cells when transplanted into immature rat testes. Because adult stem cells can be easily obtained from adult bone marrow by simple aspiration, these findings may be of great potential as a stem cell resource that may be used clinically for diseases associated with steroid hormone-producing alterations.

Fima Lifshitz, MD

## Caffey Disease is a Type I Collagenopathy

Caffey disease (OMIM 114000), also known as infantile cortical hyperostosis, is characterized by spontaneous episodes of subperiosteal new bone formation typically involving the diaphyses of long bones, mandible, and clavicles in young children. It is associated with acute inflammation of soft tissues and can lead to profound alterations in the shape and structure of affected bones. It often exhibits an autosomal dominant pattern of inheritance with substantial variation in severity.

A group headed by Jüppner undertook genome-wide linkage studies to map the Caffey disease gene locus in 3 unrelated families. Their search led them to chromosome 17q21 and eventually—to their surprise—to the *COL1A1* locus, which encodes the  $\alpha 1$  chain of type I collagen. Affected individuals in all 3 families had the identical mutation: an arginine to cysteine substitution at position 836 (R836C) placing it in the carboxy portion of the triple helical domain of the collagen molecule. About one-fifth of family members in whom the mutation was detected had no clinical features consistent with previous reports of reduced penetrance for the condition.

Mutations of the *COL1A1* are typically associated with osteogenesis imperfecta (OI) and, to a lesser extent, with Ehlers-Danlos syndrome (EDS). The affected members of these families did not display clinical signs of OI. They lacked gray-blue sclerae, dentinogenesis imperfecta, premature hearing loss, and short stature. Although bone fractures

were relatively common in one family, they were considered within the range of normal. One affected member in this family had normal bone densitometry studies.

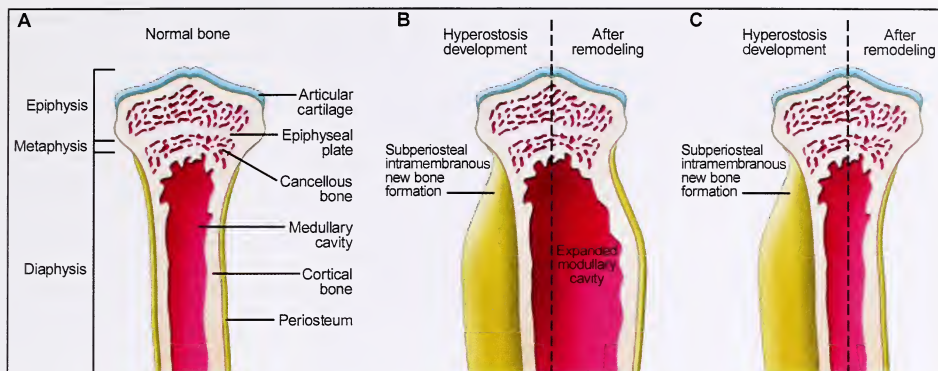
Several affected family members had joint hypermobility and abnormally soft and hyperextensible skin suggestive of mild EDS. Electron microscopy of a skin biopsy from one of these patients revealed that collagen fibrils varied more in size and shape and were less densely packed than normal. Collagen biosynthetic studies of fibroblasts from this patient showed abnormalities consistent with the presence of cysteine residues in the mutant type I collagen chains.

Perhaps most interesting, as addressed by both Gensure et al and in an accompanying comment by Glorieux,<sup>1</sup> is how one explains the episodic nature of this condition by a mutation in an extremely abundant structural protein present in bone and neighboring tissues (Figure). Although both raise several interesting possibilities, they also conceded that the question remains open and will require further investigation.

Gensure RC, Mäkitie O, Barclay C, et al. A novel COL1A1 mutation in infantile cortical hyperostosis (Caffey disease) expands the spectrum of collagen-related disorders. *J Clin Invest*. 2005;115:1250-1257.

**Editor's Comment:** It was not surprising to learn that OI and some forms of EDS are allelic disorders given the overlap in some of their features. However, it is surprising





Schematic illustrating normal and exuberant bone formation. (A) Representation of a growing bone. Growth in length is achieved by endochondral bone formation adding cancellous bone in the metaphyseal area. Gain in diameter comes from subperiosteal new bone apposition by intramembranous bone formation. The periosteum is an envelope of fibrous connective tissue that is wrapped around diaphyses. The size of the marrow cavity is controlled by a combination of bone apposition and resorption at the endocortical surface. (B and C) In ICH/Caffey disease, hyperostosis develops by exacerbated subperiosteal intramembranous bone formation triggered by local inflammation (left side of B and C). In the remodeling phase, the excess of bone tissue is resorbed either at the endocortical surface, leading to an expansion of the marrow cavity and a more persistent deformity (right side of B), or at the exocortical surface, with no effect on the size of the marrow cavity (right side of C). Reprinted with permission Glorieux F. *J Clin Invest.* 2005;115:1142–1144. Copyright ©2005. ASCL. All rights reserved.

to find Caffey disease in this group. Even though there appears to be some clinical overlap with mild EDS, the inflammatory and episodic nature of Caffey disease makes it quite distinct. As both Gensure and Glorieux<sup>1</sup> point out, some of the differences in clinical phenotype may be due to the fact that the mutation reported here does not involve a glycine residue as do most OI mutations. Collagen glycine mutations are thought to disrupt the formation and stability of the collagen triple helix, which is responsible for the structural properties of collagen. The Caffey disease mutation affects an arginine residue, which interestingly has been reported in 2 unrelated patients

with classic EDS. As both also note, administration of prostaglandin E to infants with congenital heart disease sometimes causes local hyperostosis similar to episodes of Caffey disease, raising the possibility that this substance somehow mediates the pathologic events. If so, it remains to be determined how abnormal type I collagen sets the stage for inflammation and reactive bone formation.

William A. Horton, MD

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## Final Height in SGA Children Treated with Growth Hormone

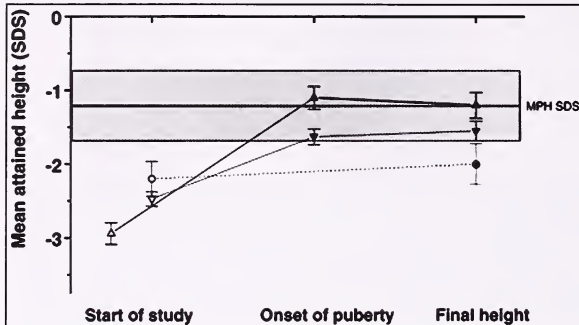
Dahlgren and Wikland report for the Swedish Study Group for growth hormone (GH) treatment of short children born small for gestational age (SGA). The final height (FH) achieved in 77 patients treated with exogenous GH (33 µg/kg/day starting prior to puberty and continuing until growth was less than 1 cm/year) was compared with data from GH treatment trials. Data were compared with data from a group of 34 short untreated SGA children. All children were born SGA (–2 SDS from mean for gestational age) for weight, height, or both, during the years 1973 to 1984. Only data from prepubertal children were analyzed. Two groups were identified: those who received GH more than 2 years before the onset of puberty (Group 1) and those who received GH beginning less than 2 years from the start of puberty (Group 2). A subset of 28 children were randomized to receive either 33 or 66 µg/kg/day during puberty. Children were excluded from the analysis if they had chromosomal abnormalities,

serious malformations, chondrodysplasia, maternal history of alcohol or substance abuse, or a condition requiring chronic medical treatment. The projected FH was compared with height of the reference population in Sweden and the gain in FH as the projected adult height in SDS minus the achieved adult height in SDS. Maternal and paternal heights were compared with reference values and mid-parental height (MPH) in SDS. Arginine-insulin GH stimulation tests were performed in all but 2 children; 37% of patients failed to achieve maximal serum GH stimulation values of 5.3 µg/L (cut-off for severe growth hormone deficiency at the time of diagnosis).

The mean FH of the entire group was –1.2 SDS, reaching the mean MPH of –1.2 SDS, and 86% of the children achieved a FH within their target height (within 1 SDS from their MPH). In the untreated, comparison group, only 52% achieved a FH within their target height ( $p < 0.001$ ). Although the mean height gain for the entire



group was  $1.3 \text{ SDS} \pm 0.8$ , those treated for more than 2 years prior to the onset of puberty had a gain of  $1.7 \text{ SDS} \pm 0.7$ , while those treated less than 2 years prior to the onset of puberty had a smaller gain of  $0.9 \text{ SDS} \pm 0.7$ . The growth responses were most pronounced among those treated the longest prior to puberty. No differences were seen in FH among the subset of children who received the higher doses of GH during puberty (Figure).



The prepubertal and pubertal height gain (SDS) in the two GH-treated groups, expressed as mean and SE: regular triangles = treated >2 y before puberty, and inverted triangles = treated <2 y before puberty. Attained height in the untreated group is shown as circles and broken line, expressed as mean and SE. Mean MPH  $\pm 0.5 \text{ SD}$ , is shown as shadowed area.

Reprinted with permission from Dahlgren J, Wikland K. *Pediatr Res.* 2005;57:216–222. Copyright © 2005. International Pediatric Research Foundation. All rights reserved.

The authors discussed the importance of treating SGA children at as early an age as possible and the effects of continuing that therapy until growth is complete. They also noted that the therapy was well tolerated with no drug-related adverse events. They emphasize that differences between their study results and those of others may relate to the long duration of GH treatment in their cohort. They concede that a broad range of height gain was observed and that this suggests that individualized dosing may be appropriate. They conclude that younger, shorter, and lighter children at the start of GH treatment have better growth responses, are taller at the onset of puberty, and achieve a better FH.

Dahlgren J, Wikland K on behalf of the Swedish Study Group for Growth Hormone treatment. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57:216–222.

**Editor's Comment:** This is an interesting and potentially important study of the long-term effects of GH therapy on FH in children born SGA. Many pediatric endocrinologists are faced with the decision whether or not to recommend

GH therapy for young short children born SGA. Parents often ask if there is any harm in delaying treatment until the child is older, perhaps at an age when the benefits of daily injections might be more understandable. This manuscript suggests that delaying GH treatment in such children is not in their best interest and that maximal benefits are associated with early prepubertal therapy.

It would have been interesting to know whether or not the children who had a GH deficiency by stimulation testing (33%) grew better than those who made sufficient amounts of GH. Since 50% of the comparison group achieved FH at their MPH without any GH therapy, one wonders if the difference in outcomes between the comparison group and the treated group could be accounted for by the increased growth rates of treated GH-deficient SGA children.

Finally, it is important to note that although no adverse events related to GH therapy were reported, the authors did not report which potential side effects were screened for and what type of testing was performed to assure that they did not occur. Specifically, it would be very important to know how glucose intolerance and/or insulin resistance was monitored in these children. This editor would caution pediatric endocrinologists who opt for long-term GH therapy for short SGA children to monitor them carefully and repeatedly for potential side effects.

William L. Clarke, MD

## Is Growth Hormone Deficiency in Ectopic Neurohypophysis Permanent?

Anatomical abnormalities of the hypothalamic-pituitary axis, as detected by cerebral magnetic resonance imaging (MRI) in children with isolated growth hormone deficiency (GHD) or multiple pituitary hormone deficiencies (MPHD) are a landmark of a group of patients with hypopituitarism. Leger et al reported a prospective study of a group of such 18 patients who had in common MRI markers of ectopic neurohypophysis with defects of the pituitary stalk. The researchers followed the patients until adulthood, after completion of GH treatment. The initial diagnosis of GHD was based on a GH peak of  $<10 \text{ } \mu\text{g/L}$  after provocative

stimuli. At retesting, the same criteria were applied, but GHD was considered as severe if the peak value was  $<5 \text{ } \mu\text{g/L}$ . The important finding at reevaluation was the presence of normal or only partially deficient GH secretion with a peak value of  $>5 \text{ } \mu\text{g/L}$  in 7 patients; 6 out of whom had isolated GHD. Among the 11 patients with severe GHD at retesting, only one had isolated GHD. Therefore, MPHD, regardless of etiology, was a strong predictor of permanent GHD after adolescence.

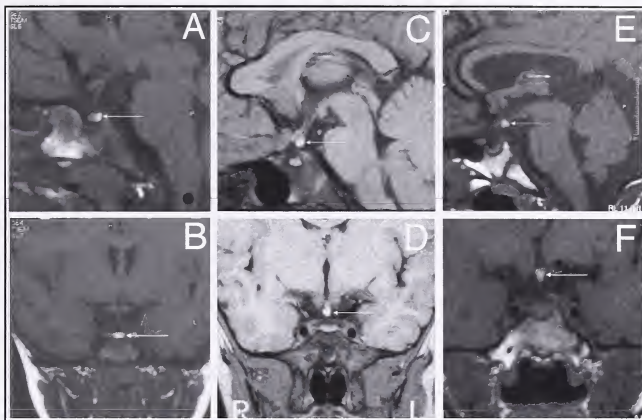
The MRI structure of the hypothalamic-pituitary axis differed among both groups. It should therefore be

recalled that the main anatomical finding in the so-called pituitary stalk interruption syndrome is the ectopic location of the bright spot of the neurohypophysis. This spot may be located in its upper position at the median eminence or at a lower level along the pituitary stalk with a hypoplastic anterior pituitary. The ectopic neurohypophysis was found at the median eminence level in 10 out of 11 patients with permanent, severe GHD. In contrast, it was located along the stalk in all but one of the patients with normal or partially reduced GH response at retesting.

The authors concluded that increased GH secretion may be observed in adult patients with less severe MRI anatomical defects. These individuals need to be retested at the completion of GH treatment. In contrast, the patients who persisted with severe GHD formed a subgroup with their neurohypophysis at the median eminence with lack of or poor visibility of the pituitary stalk. Retesting may not be necessary in these patients, especially if there is MHPD.

Leger J, Danner S, Simon D, Garel C, Czernichow P. Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab.* 2005;90:650–656.

**Editor's Comment:** The authors studied a group of non-acquired GHD patients identified by MRI-detectable anatomical defects of the hypothalamic-pituitary axis. This type of patient is of theoretical as well as practical interest. It was first hypothesized that all patients with such MRI-detectable defects would show permanent GHD and eventually be candidates for life-long GH therapy. It is now shown that a subgroup (approximately 40%) with isolated GHD in childhood may appear as normal, or moderately affected, at retesting after growth is completed. Furthermore, the patients with GHD appear to present an anatomical defect detectable in the MRI which can be considered as less severe: the pituitary stalk is eventually visible and the neurohypophysis has partly "migrated" downward. These findings may help predict a more favorable outcome. In contrast, those presenting with MHPD with an ectopic neurohypophysis located in the median eminence usually present persistent GHD into adulthood. However, a word of caution is necessary as some of the patients with isolated GHD may develop other pituitary defects at any age. They require a lifetime follow-up, even if GH secretion has apparently returned to normal. These data should be considered for future



Cerebral MRI (T1-weighted images). A, Sagittal slice; B, coronal slice; normal morphology of anterior pituitary and pituitary stalk is seen. The hyperintense signal of the posterior pituitary is in the normal location. C, Sagittal slice; D, Coronal slice; a normal anterior pituitary with a thin pituitary stalk is seen. The ectopic posterior pituitary hyperintense signal is located along the stalk (at a proximal level of the pituitary stalk; arrow). E, Sagittal slice; F, coronal slice; hypoplastic anterior pituitary with no visible pituitary stalk after gadolinium injection. The ectopic pituitary hyperintense signal is at the median eminence (arrow). Reprinted with permission Leger J, et al. *J Clin Endocrinol Metab.* 2005;90:650–656. Copyright ©2005. The Endocrine Society. All rights reserved.

#### guidelines of GH treatment.

The pathogenesis of the pituitary stalk interruption syndrome with pituitary insufficiency remains unknown in most cases. This defect has been reported in some patients with identified molecular defects of transcription factors controlling the early pituitary development.<sup>1–3</sup> Although it was not the scope of this study, it seems important to not consider pituitary dysfunction as a stable condition. As also shown in other studies, the switch from isolated GHD to multiple defects remains possible at any age.<sup>4,5</sup> It will be of great interest to follow the patients who had an apparent recovery reported by Leger et al to document their reproductive function. Finally, patients with this illness are candidates for genetic studies and long-term follow-up to provide a more complete and eventually significant description of their hypothalamic-pituitary function throughout life.

Raphaël Rappaport, MD

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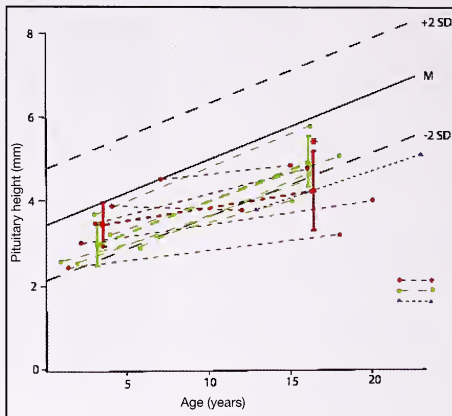
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## Familial Isolated Growth Hormone Deficiency Type II: Not So Isolated After All

Isolated growth hormone deficiency (IGHD) is thought to be familial in 5% to 30% of cases. Familial IGHD is categorized into 4 types: IA is autosomal recessive with absent endogenous GH; IB is autosomal recessive with decreased GH; type II is autosomal dominant with decreased GH; and type III is X-linked with decreased GH. Type II IGHD results from *GH-1* gene mutations<sup>1</sup> that lead to missplicing and subsequent loss of exon 3; the resultant 17.5-kDa GH variant acts as a dominant negative inhibitor of the normal 22-kDa GH isoform (from the wild-type allele) by disrupting the Golgi apparatus, impairing trafficking of GH and other hormones, and reducing stability of the 22-kDa GH isoform.

Mullis and colleagues studied 57 subjects from 19 families with type II IGHD resulting from different splice site and missense mutations in *GH-1*. Thirty-three had received GH treatment, and 24 were untreated. Those who had been treated during childhood stopped treatment for 2 months when reaching near adult height and underwent pituitary retesting; the untreated subjects underwent similar testing. Several interesting findings arose. First, subjects with a splice site mutation in the first 2 bp of the third intron (5' IVS +1/+2 bp) seemed to have a worse phenotype than those whose splice site mutation occurred in the 5th or 6th bps of the same intron (5' IVS +5/+6). The former had lower mean serum cortisol and ACTH concentrations and were more likely to have lower TSH levels. They also had a significantly smaller pituitary height (-2.59 SDS vs -1.56 SDS,  $P < 0.01$ ) when reaching adult height. One patient with a missense mutation (P89L GH) also presented with ACTH and TSH deficiencies, and another (R183H GH) had a small pituitary size at age 73 years.

The authors concluded that the phenotype was partially genotype-related. On one hand, children with splice site mutations were younger at diagnosis (mean



Pituitary height in affected GH-treated subjects at diagnosis as well as at the end of growth. The age-dependent heights of the adenohypophysis, which was determined in a strict midline positioned sagittal scan, are shown. Because MRI was performed at different ages and the size of the normal pituitary increases with age the -2.0 and +2.0 SDS are shown as lines. In each subject 2 measurements were performed: at the beginning/diagnosis and at near AH after the GH treatment was stopped for 2 months. Green/closed squares, Patients with 5' IVS-3 +5/+6 bp splice site mutation; red/closed circles/dots, patients with 5' IVS-3 +1/+2 bp splice site mutation; blue/closed triangle, R183H GH. \*,  $P < 0.01$ . Reprinted with permission Mullis PE, et al. *J Clin Endocrinol Metab.* 2005; 90:2089-2096. Copyright ©2005. The Endocrine Society. All rights reserved.

age 3 years) than those with missense mutations (mean age 9.3 years), and the splice site mutation in the first 2 bps of intron 3 presented with more pituitary dysfunction in adulthood than mutation in bps 5 or 6 of the same intron. However, there was still considerable phenotypic

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variability among individuals within the same family with the same mutation. Consistent with transgenic mouse models, it seems the phenotype is dose-dependent (ie, the ratio of mutant 17.5 kDa GH to wild-type 22 kDa GH). Transgenic mice with high-copy number IGHD II also developed pituitary hypoplasia and multiple hormone deficiencies (prolactin, TSH and in males only, LH).

Mullis PE, Robinson ICAF, Salemi S, et al. Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. *J Clin Endocrinol Metab*. 2005;90:2089–2096.

**Editor's Comment:** I agree with the authors that the most important lesson from this study is the need for long-term monitoring of pituitary function in patients with type II IGHD. Interestingly, the hormonal deficiencies and pituitary hypoplasia manifested later. The difference in pituitary size among patients with the 2 splice site mutations (+1/+2 vs

+5/+6) was not significant at the time of diagnosis (–1.1 and –1.5 SDS, respectively), but became significant by the time near adult height was reached (–2.59 and –1.56 SDS) (Figure). Although type II IGHD is supposed to have isolated GHD by definition, the onset of additional pituitary deficiencies in adulthood warrants attention. This is reminiscent of the finding of central adrenal insufficiency in adults who had been treated for idiopathic GHD in childhood.<sup>2</sup> Unrecognized and under-treated adrenal insufficiency contributes to the increased mortality of individuals with GHD.

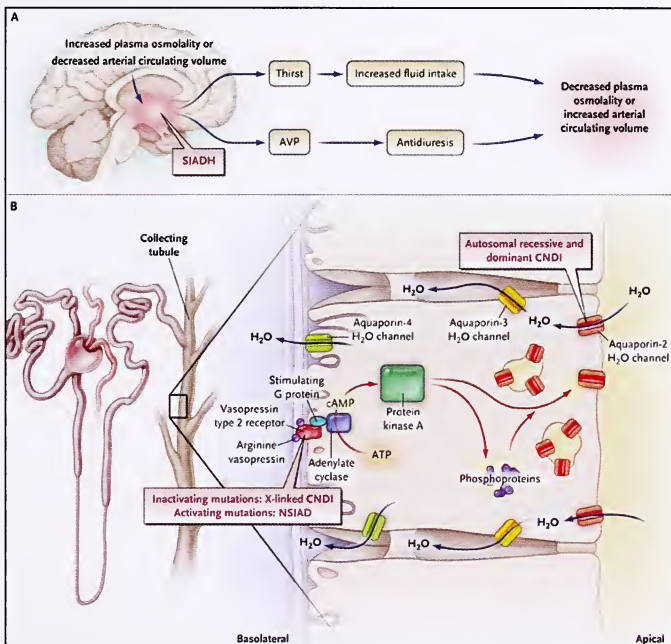
Adda Grimberg, MD

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## SIADH Due to Gene Mutation in Vasopressin Receptor

The authors described 2 unrelated male infants (2.5 and 3 months of age) with clinical and biochemical evidence of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) including irritability and/or seizures, hyponatremia, hypochloremia, and hypo-osmolality, with relatively increased urinary osmolalities and sodium concentrations. However, instead of measurable values of plasma ADH, concentrations of ADH were undetectable in both infants. In the absence of other causes of SIADH (neural insults, drug exposure), the investigators questioned the possibility of a constitutively active mutation in the gene (*AVPR2*, chromosome Xq28, OMIM 304800) encoding the vasopressin-2 receptor (V2R), a G-protein-coupled 7 transmembrane receptor (GPCR) that activates adenylate cyclase. Sequencing of *AVPR2* revealed hemizygous mutations in codon 137 (arginine) in both subjects: C770T resulting in Arg137Cys; G771T leading to Arg137Leu. Codon 137 is located in the second intracytoplasmic loop at the end of transmembrane domain III, a highly conserved region in all GPCRs. One of the asymptomatic mothers was heterozygous for the same mutation present in her



Physiology of Water Homeostasis in Humans (Panel A) and Pathway of AVP Signaling in Renal Collecting-Duct Cells Involved in Regulating Water Excretion (Panel B). Reprinted with permission Knoers NVAM. *N Engl J Med*. 2005;352:1847–1850. Copyright ©2005. Massachusetts Medical Society. All rights reserved.

son; the mutation in *AVPR2* in the other patient was apparently spontaneous. Expression of the mutated V2R in COS-7 cells revealed that basal levels of cAMP generation were 4- to 7-fold greater than that of cells



expressing wild-type receptor, consistent with a constitutively active V2R. The patients were treated successfully with oral urea to induce an osmotic diuresis. The authors suggested that the possibility of a gain-of-function (GOF) mutation in *AVPR2* be considered in other patients without an apparent cause of SIADH and with low serum concentrations of ADH.

Feldman BJ, Rosenthal SM, Vargas GA, et al. Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*. 2005;352:1884–1890.

**Editor's Comment:** The V2R now joins a number of other GPCRs with germline mutations that render them constitutively active and lead to dysfunction of the endocrine system including: *LHR*—familial male-limited isosexual precocity; *TSHR*—autosomal dominant nonimmune hyperthyroidism; *CaSR*—autosomal dominant hypocalcemia; *MC2R*—corticotropin independent hyperadrenocorticism;

*FSHR*—familial ovarian hyperstimulation syndrome; *PTH/PTHrPR*—Jansen's metaphyseal chondrodysplasia with hypercalcemia.<sup>1</sup> It is of great interest that in patients with nephrogenic diabetes insipidus, the substitution of histidine for arginine at codon 137 has been identified. Thus, different mutations of the same amino acid in V2R lead to functional or non-functional states, emphasizing the critical importance of this site and its effect on the receptor's 3-dimensional configuration. It would also be of interest to assess water homeostatic mechanisms (water loading or deprivation) in the woman who is heterozygous for the GOF mutation in V2R to determine whether there is a gene dosage effect for this protein (Figure).

Allen W. Root, MD

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## JNK vs Insulin/IGF Signaling: Mediating the Effects of Stress and Nutrition on Longevity

Insulin/IGF signaling (IIS) promotes growth and energy storage when nutrients are abundant, but the life span of different eukaryotic organisms (mice, *Drosophila*, *C. elegans*) is actually increased when IIS is reduced by calorie restriction or by mutations in its pathway components. It seems that resistance to oxidative stress underlies this paradox. Environmental insults such as UV irradiation and oxidative stress activate, among other molecules, Jun-N-terminal kinase (JNK), a component of mitogen-activated protein kinase (MAPK) cascade, that induces a protective gene expression profile and thereby confers tolerance to oxidative stress and prolongs life span.

Wang and colleagues studied the opposing effects of IIS and JNK on oxidative stress tolerance and longevity in *Drosophila*. The *Drosophila* genome contains 7 insulin-like peptides, of which *dilp2* most closely resembles human insulin. *Dilp2* is secreted by insulin-producing cells (IPC) that form a small cluster of neuroendocrine cells in the fly brain. Specific elimination of IPCs leads to growth retardation, developmental delays, and decreased late-life mortality. Similar to humans, *Drosophila* IIS leads to activation of PI3 kinase and Akt, which in turn phosphorylates the Forkhead transcription factor, causing its cytoplasmic retention and down-regulation of its target genes. The *Drosophila* Forkhead transcription factor *DFoxo* extends lifespan when over-expressed.

The authors provided evidence that *DFoxo* is required for JNK-mediated life span extension in *Drosophila*. Further, JNK signaling affected *DFoxo* function as shown by modulation of *DFoxo*-dependent phenotypes in eye development and expression of the *DFoxo* target genes *thor* (a translational repressor that suppresses growth when IIS is inactive) and *I(2)effl* (a small heat shock protein that enhances survival of cells exposed to oxidative damage). Finally, the authors found that JNK and *DFoxo*

restrict IIS systemically by repressing *dilp2* expression in IPCs. When JNK activity was specifically increased in the IPCs only, there was a *DFoxo*-dependent decrease in body size and increase in life span. Thus, the JNK-*DFoxo* effects on aging and lifespan occur at 2 levels. In peripheral tissues, JNK activates *DFoxo* to prevent senescence cell-autonomously through expression of genes protective against oxidative damage (eg, preventing age-related declines in cardiac or neurologic function). JNK activation of *DFoxo* in IPCs represses *dilp2* expression, thereby decreasing IIS systemically and coordinating cellular responses to environmental changes, which impacts the life span of the organism as a whole.

Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell*. 2005;121:115–125.

**Editor's Comment:** In this intriguing paper, the authors made a strong case for Foxo being the convergence point of the opposing effects of IIS and JNK activity on longevity and stress response in *Drosophila*. They speculated whether Foxo homologs may play a similar role in mammals, and they cited prior evidence that JNK can inhibit IIS by phosphorylating and inhibiting the insulin receptor substrate.<sup>1,2</sup>

Another unintended convergence point in mammals is the tumor suppressor, p53. Inactive JNK binds the N-terminus of p53, leading to p53 ubiquitination and degradation; this is one of the principal mechanisms by which the tumor suppressor is kept at very low concentrations under normal circumstances. However, when JNK is activated by radiation or oxidative stress, it phosphorylates p53 on threonine 81, thereby activating it.<sup>3</sup> By repressing transcription of IGF-II and the IGF receptor while activating transcription of IGF binding protein (IGFBP)-3,

*p53 directly inhibits IGF signaling.<sup>4</sup> As well, p53 has been implicated in issues of senescence, longevity, and responses to nutrition and stress. Mice harboring a carboxy-terminus p53 fragment that augments activity of the wild-type p53 allele displayed enhanced resistance to spontaneous tumors, but early onset of aging phenotypes, including osteoporosis, lordokyphosis, generalized organ atrophy, decreased stress tolerance and reduced longevity.<sup>5</sup>*

Adda Grimberg, MD

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## Congenital ACTH Deficiency, Hypoglycemia and TPIT Gene Mutations

Over the past decades congenital pituitary hormone deficiencies have been described in humans; corresponding animal models were also developed. Quite a number of transcription factor mutations have been reported. Of these, TPIT is the most cell-restricted transcription factor controlling the terminal differentiation of the corticotrophs. Mutations of the *TPIT* gene in humans are associated with congenital ACTH deficiency.

This is the first large report of a neonatal-onset form of congenital isolated ACTH deficiency (IAD). The authors described a series of patients (n=27) from 21 unrelated families. *TPIT* gene mutations, all of which affected coding sequences, were found in only 17 of the 27 patients. Ten different mutations were identified and their distribution indicated a recessive mode of transmission. It was also shown by functional studies of 4 missense mutations that there was a defect in the transcriptional ability with loss of DNA binding, a mechanism inducing a loss of function. The 10 remaining cases belonged to 8 different families who were consanguineous or had evidence of hereditary transmission of IAD.

In the group carrying *TPIT* gene mutations the diagnosis was made before the age of 2 years. Severe hypoglycemia led to the diagnosis of IAD. Furthermore, 11 out of 17 neonates presented prolonged neonatal cholestatic jaundice. These symptoms were suppressed by cortisol replacement therapy. Adrenarche did not occur at time of puberty. In patients without mutations the clinical picture was the same, however, there were some cases with milder disease who had evidence of some ACTH secretion, but it was insufficient to avoid hypoglycemia.

Therefore, congenital IAD, regardless of the molecular findings, presented with a homogeneous clinical phenotype. Consanguinity was observed in 5 of 13 families. Compound heterozygotes were also present, indicating that mutant alleles may be more frequent than expected in the population. It was concluded that the subgroup of IAD patients without mutations should be further investigated for loss-of-function of other genes.

Vallette-Kasic S, Brue T, Pulichino AM, et al. Congenital isolated adrenocorticotrophin deficiency: An underestimated cause of neonatal death, explained by *TPIT* gene mutations. *J Clin Endocrinol Metab*. 2005;90:1323–1331.

**Editor's Comment:** This group, led by Drouin, presented their first paper in 2001 on *TPIT*, a pituitary cell restricted T-box factor, showing that mutations in this gene were associated with neonatal IAD.<sup>1,2</sup> These researchers now report that a large number of patients and families (some followed-up until puberty) showed lack of adrenarche. The presenting symptoms are characteristic of profound neonatal cortisol deficiency combining hypoglycemia and cholestatic jaundice. Thus, this entity should be considered in the array of causes of early adrenal insufficiency and be considered a neonatal emergency, easily controlled by cortisol treatment. The more puzzling issue is the group of patients who have no mutations in the coding sequence of the *TPIT* gene. However, their clinical presentation and management were not different.

Another issue is the severity of hypoglycemia causing neonatal death or mental retardation in survivors. Death occurred in 5 infants belonging to 5 families regardless of the presence of *TPIT* mutations. Therefore, congenital ACTH deficiency should be rapidly recognized. In affected families prenatal diagnosis should be performed, as in other genetic diseases with adrenal hypoplasia, by measuring maternal serum estriol levels during the third trimester of pregnancy.

In addition, it is of interest that a late onset form of IAD has been described with a presentation of cortisol deficiency without skin hyperpigmentation during childhood.<sup>3</sup> In these cases, mutation of the *TPIT* gene could not be found. Here again, other genes contributing to this lineage differentiation and to ACTH secretion may be involved. We do not know whether these cases are somehow related to the early congenital form without identified mutations.

Raphaël Rappaport, MD

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# Neuropsychological Sequelae and Brain Function in Adults with Childhood-Onset Growth Hormone Deficiency

The researchers set out to further examine reports of cognitive dysfunction in adults with childhood-onset growth hormone deficiency (GHD) and to investigate potential causes in atypical brain metabolism. Eleven adults (7 male and 4 female) with childhood-onset GHD, who had been treated with GH during childhood for 4 to 16 years (mean duration 8.2 years), were evaluated by neuropsychological testing and magnetic resonance spectroscopy (MRS) at least 3 months after discontinuation of GH replacement. The GHD participants were compared to a health- and demographically-matched control group (n=9). MRS was used to assess brain *N*-acetylaspartate (NAA) and NAA/choline ratios, indices of hormonal density and integrity. The GHD group exhibited significantly lower performance on a delayed memory recall task (15-word delayed recall score), a measure of planning behavior, cognitive processing speed, and attention (Trail-making test, Part A). The GHD group also showed significantly lower NAA and NAA/choline levels, and increased choline levels compared to controls. Finally, IGF-I was significantly correlated with NAA levels, but not with choline levels or NAA/choline ratios. The investigators interpret their findings as corroboration of other reports indicating subtle neurocognitive deficits in adults with childhood-onset GHD. Moreover, these effects (in combination with evidence of reduced NAA level in the brain) resemble those observed in normal aging.

van Dam PS, de Winter CF, de Vries R, et al. *Psychoneuroendocrinology*. 2005;30:357-363.

**Editor's Comment:** Cognitive function in children and adults with childhood-onset GHD has been the topic of multiple studies. Neuropsychological testing corroborates clinical impressions that associations between GHD and deficits in cognitive performance are subtle; the report by van Dam et al demonstrates an altered brain metabolism while they were off GH treatment. Nevertheless, there is evidence that GHD, which can be a consequence of perinatal insult, cancer (and its treatment), and other pathologic states, may be associated with substantially increased rates of learning disabilities.<sup>1</sup>

Future studies of this topic will benefit from larger sample sizes and statistical analyses that adjust for gender, participant's global intelligence, and adequacy of hormone replacement in adulthood for those with multiple pituitary hormone deficits. Presently, the benefits of GH replacement in adulthood on cognitive performance remain unclear. Whereas, physiologic doses of GH in individuals with adult-onset GHD appear to be ineffective,<sup>2</sup> more promising findings derive from a study in childhood-onset GHD.<sup>3</sup>

David E. Sandberg, PhD

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## GROWTH HORMONE AS A THERAPEUTIC AGENT

### ROBERT M. BLIZZARD, MD

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Twenty years have passed since recombinant human growth hormone (rhGH) was approved by the FDA for clinical use in patients with growth hormone deficiency (GHD). This was a major breakthrough, as the only previous source of GH was naturally-occurring GH extracted and purified, to a variable extent from human pituitaries removed at autopsy. This human GH (hGH) was first prepared and studied by Raben<sup>1</sup> in 1958 and was shown to produce growth in a sexually undeveloped adolescent. The supply of hGH for investigation and/or therapy was very limited until rhGH became available in 1985, when the supply suddenly became unlimited and the new modern era of GH as a therapeutic agent began. Genentech developed the recombinant techniques to synthesize rhGH, and also developed the necessary testing leading to approval by the FDA of rhGH for human use.

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### From The Editor's Desk

This issue's lead article commemorates the 50 years of human growth hormone (hGH) as a therapeutic agent and the 20th anniversary of recombinant hGH (rhGH) for the treatment of hypopituitary children. The personal recollections of Dr. Robert Blizzard bring to the reader a clear historical perspective of the developments that brought about the rise and fall of hGH. It also highlights the synthesis and approval of rhGH and the major strides made with the unlimited availability of rhGH.

We also commemorate 2 decades of *Growth, Genetics & Hormones (GGH)*. This journal was established in 1985 to provide a high-quality educational resource to physicians. The journal accomplished its mission, and more. Dr. Blizzard's leadership, the hard work of the Editorial Board, and an unrestricted educational grant from Genentech made it all possible. In 2002 www.GGHjournal.com was launched. This enabled us to bring GGH to most pediatric endocrinologists around the world. From their comments, we know they treasure the content and erudite comments of the Editorial Board. The on line archives of the journal constitute the repository of the fundamental advances that have occurred in the field of growth since the beginning of GGH.

Each year we have given readers more material and added features without an increase in budget. However, GGH may cease publication next year as the educational grant that we have enjoyed since its inception will not be available after April 2006. Thus, we are searching for sponsorship and have requested grant support from all manufacturers of rhGH. The pharmaceutical companies that compete for market share have a common responsibility to provide high quality educational resources to physicians who prescribe rhGH. I challenge them to promptly fill the void so we may continue bringing state-of-the-art, unbiased, valuable information in the field of growth to our colleagues worldwide. It has been estimated that the annual sales of rhGH are \$1.5-\$2 billion; 30% of the sales being for FDA approved indications to treat children and adults (Perls TT, Reisman NR, Olshansky SJ. *JAMA*. 2005;294:2086-2090.) Thus, there must be funds available to be allocated for the continuation of GGH, a highly regarded educational journal.

We will continue to explore sources of support to enable us to provide you with GGH on a complementary basis—as it has been done since 1985. On line subscribers recently received a survey to evaluate their interest in helping shape the future of the journal. I am gratified by their response; more than 40% indicated a willingness to pay for a subscription to the journal. I urge all of you to complete the one question survey (www.GGHjournal.com) or to send me a note indicating your interest in a paid subscription (editor@GGHjournal.com) so we may plan the future of GGH.

Respectfully,  
Fima Lifshitz, MD



The 20<sup>th</sup> anniversary of FDA approval of rhGH occurs simultaneously with the 20<sup>th</sup> anniversary of the establishment of the journal, *Growth, Genetics & Hormones* (GGH, available at [www.GGHjournal.com](http://www.GGHjournal.com)). GGH has been supported by Genentech, Inc., via an educational grant. GGH was the first journal established for the purpose of assimilating published information, both domestic and international, on growth problems valuable to the pediatric community (endocrinologists, geneticists, matabolists, and generalists). The current editor-in-chief of GGH believes that a review of the historical aspects of the development and use of hGH and rhGH should be presented during this simultaneously occurring 20<sup>th</sup> anniversary before the details are lost in obscurity.

I undertake this task as one who has been privileged to be an observer and participant in the accomplishments brought about by Genentech in creating both rhGH and GGH. As stated in the first issue, "GGH is established as an educational journal by the Editorial Board to facilitate the flow of information and commentary which provide a close look at current, and often controversial, topics in endocrinology, metabolism, and genetics, and their potential applications."<sup>2</sup> This goal has been, and continues to be met, for 20 years. Similarly, the creation and production of rhGH have benefited many thousands of children with growth disturbances. I also undertake this task as one participating actively in the use of native GH in the 30 years preceding the launching of rhGH and GGH.

This review is a personal perspective and recall of the past 50 years. In that sense, it may not always be totally accurate and it does not cover all important aspects in the field. Furthermore, these historical comments are made pertaining to my own experience in the United States and, therefore, do not reflect the equally interesting experiences in Europe, South America, Australia, New Zealand, and elsewhere.

#### **VERY EARLY HISTORICAL PERSPECTIVES (Prior to 1958)**

The first human who received GH of any origin was a 3½-year-old patient with presumed GHD to whom I gave bovine GH (BGH) in 1956 (supplied by Choh H. Li). This patient received BGH daily over a 3-week period while 24-hour metabolic balance studies were performed. I personally handled all stool, urinary, and dietary samples, and performed appropriate nitrogen and calcium determinations. Neither positive nitrogen balance nor hypercalcaemia markers of GH reactivity were demonstrable. The conclusion was that either BGH did not act in humans or the patient was GH insensitive. Later, in 1963 when the immunoassay for hGH became available, high levels of GH and low levels of somatomedin or insulin-like growth-factor-1 (IGF-1) were found in this patient's serum.<sup>3</sup> At 10 years of age, the patient did not

respond to hGH. Thus, this was the first patient to be diagnosed with GH insensitivity (GHI), eventually named "Laron's Syndrome." Now at 53 years of age, she survives without hypoglycemia (post-pancreatectomy), is married, and has a normal-size son.

#### **EARLY HISTORICAL PERSPECTIVES (1958-1965)**

Prior to 1958 studies with GH were pursued primarily in rodents and lower mammals, chiefly in 3 laboratories (led by Choh H. Li, PhD, UC Berkeley; Alfred Wilhelmi, PhD, Emory University; and Maurice Raben, MD, Tufts University). By 1958, each utilized different extraction methods to retrieve hGH from human pituitaries. For example, Raben's procedure used hot glacial acetic acid which destroyed TSH, LH, and FSH. Li's method was the most elaborate as he strived to report the chemical structure of hGH, which he did twice (once incorrectly and subsequently, correctly). Wilhelmi's procedure produced a wide array of pituitary hormones in side fractions, which could be purified and used for clinical investigation.

Initially, the collection of human pituitaries was a diverse effort. Each of the above-mentioned extractors, many other endocrinologists, and even parents of short children solicited pathologists to collect pituitaries on all autopsied patients. Pituitaries from most unembalmed and all embalmed bodies at autopsy were placed separately in acetone, and a majority of those from unembalmed bodies were frozen *en mass*. The latter yielded greater amounts of hormone and the GH was less antigenic. Individual collection programs rapidly developed, usually under the leadership of an individual pediatric endocrinologist or a university group of pediatric endocrinologists. These programs tended to be geographically proximal to the location of one of the extractors. By 1962, Raben was receiving approximately 15 000 pituitaries per year, Wilhelmi was extracting approximately 3500, and Li a few less. Approximately half of the hGH extracted was kept for the extractor's scientific use and the other half was returned to pediatric endocrinologists for clinical investigation of their patients. By 1959, I and a few others were studying presumed GHD patients with native hGH collected and extracted by these methods.

Initially, about 1 mg of hGH was obtained per unembalmed pituitary. Since 1 mg of hGH was needed to treat one patient per day, 365 pituitaries were needed per patient per year. From 20 000 pituitaries extracted per year, about 10 000 mg were available for pediatric endocrinologists. Thus, only 30 patients could receive a full course of therapy. The fascinating story of the collection of pituitaries, for extraction of hGH initially and other hormones subsequently, is a tale of intrigue and secrecy. A black-market competition for pituitaries developed. Scientific collegiality and secrecy occurred simultaneously. Clinical investigation produced many successes and too many disappointments.

In 1961, The National Institutes of Health (NIH) asked me to establish the National Pituitary Agency (NPA) to collect pituitaries on a national basis to counter the ever-growing black market for pituitaries, and to nationally organize and guide the collection, extraction, and distribution of hGH initially and other hormones later. To sell the concept of establishing the NPA was no easy task. Understandably, the extractors and involved pediatric endocrinologists had concerns about collection turfs. After extensive discussions and persuasion, an agreement of extractors, endocrinologists, and pathologists was finally attained. Each participant would be entitled to receive the same amount of pituitaries and/or hGH as he/she had received the previous year. The National Institute of Arthritis and Metabolic Diseases (NIAMD) entered into a contractual agreement with The Johns Hopkins University (my base of operation) to support the necessary personnel (other than myself), office expenses, and payments to pathologists of \$2 for the services rendered to collect, store, and deliver each pituitary to the NPA.

Funding for this agency was not available until 1963 (approximately 2 years later). Thus, I had to locate funding from other sources to implement the program. The initial success was due to many dedicated persons including Alfred Wilhelm, PhD; William Daughaday, MD; Eugene Latimer, MD, physician coordinator; Ms. Dorothy Miller, executive secretary; and many others. The NPA was assisted by parents such as Fred and Gwen Mahler, who had 2 children with genetic GHD. Mr. Mahler, a TWA pilot, arranged transporting frozen pituitaries in the cockpits of planes from major cities in the US to the NPA in Baltimore. Mrs. Mahler, a retired TWA flight attendant, organized other retired TWA flight attendants on a national basis ("TWA Clipped Wings") to raise and donate thousands of dollars annually, for at least 6 years, to fund expenses of the NPA and the Human Growth Foundation which was created by parents of children with growth disturbances.

Of interest are the very crude methods (by today's standards) utilized for the collection and handling of the pituitaries and extracted GH. The hGH was received from the extractors at the NPA in small mason jars. It was transferred by a spatula to wax paper and placed on a simple analytical balance. One mg of hGH was weighed and placed in a small sterile screw cap vial which then was sealed. Multiple vials were then transferred via parcel to the physician investigators, along with 5- or 10-mL vials of various solvents, depending upon which hormone was dispensed. The most disagreeable solvent was 0.1% HCl, which was necessary to use in order for the Raben hGH to go into solution. Patients much preferred hGH from sources other than Raben.

In those early days, no bio-potency was determined and hGH was dispensed and injected on a milligram weight basis. Not until 1965 were potency estimates utilized.

Subsequently, assays utilized the growth rate of the tails of rats injected with hGH. The concentrations between batches varied from 0.5 to 2.0 units/mg of hGH. Reading the literature of that period is confusing since often only the milligram designation was used. The amount of hGH extracted per pituitary steadily improved. By 1977 when Albert Parlow, MD, became the single extractor of all human pituitaries in the US, the amount of hGH obtained per pituitary was several times greater than that obtained in 1960. Because of Parlow's efforts the supplies of hGH greatly increased. Remarkably, the hGH distributed never led to infections or adverse reactions until the occurrence in 1985 of the first case of Creutzfeld-Jacob disease (CJD) resulting from the injection of apparently prion-contaminated hGH given many years earlier.

The treatment of patients was on the basis of investigation proposed by clinical research protocols on grant applications submitted to the NPA. Board review was the mechanism used to assess the proposals and to fairly distribute the extracted hGH. By law, the NIH could not support clinical treatment but could support investigative therapy. By 1963, substantial investigative therapy had been accomplished. An Editorial Commentary<sup>4</sup> in 1963 by myself stated that: (1) hGH had been proven to be effective for periods up to 5 years, (2) in the first few months of therapy linear growth accelerated 6 or 7 times the pretreatment period, (3) the effectiveness of the hormone gradually waned, (4) there were no significant side effects detected, and (5) the dosage and schedules in therapy varied widely, but approximately 300 to 500 mg of hGH were required per year for each child treated. Therefore, widespread use was not possible even if a pituitary from each autopsy performed in the US was collected, as even this would only permit therapy in about 4000 patients. The editorial comment also stated that there was reason to believe that the short stature of Turner syndrome and other types of short stature were amenable to therapy. This fact was confirmed several years later. Also stated was the prediction that when hGH would become available in sufficient quantities it would have a breadth of application approaching that of cortisone.

#### **HISTORICAL PERSPECTIVES (1965-1975)**

In 1965, a Ross research conference on hGH was held at The Johns Hopkins Hospital, Baltimore, Maryland. The proceedings<sup>5</sup> summarized the state of knowledge at the time, including that in 1962 a radioimmune assay for hGH was published,<sup>6</sup> which permitted insight into GH's action in relation to diagnosis and treatment. By 1966, Alfred Wilhelm, PhD; Robert Ryan, MD, Mayo Clinic; and Brij Saxena, PhD, Cornell University Medical College, were extracting and purifying TSH, ACTH, LH, and FSH from pituitaries. This ultimately permitted immunoassays for each of these hormones to be developed. It was possible, therefore, to significantly extend investigation of normal and abnormal endocrine physiology, and the

interrelations of hormones of the pituitary, the gonads, and the adrenals at adolescence. In the late 1960s, the development of a constant withdrawal pump by Avinoam Kowarski, MD and his collaborators<sup>7</sup> made it possible to measure integrated concentrations of hGH over various periods of time, which advanced the capability to better understand GH physiology and production in relation to age, gender, and the effect of sex steroids.

The success of collection of pituitaries for hGH therapy, and the accumulation of knowledge derived from the use of hGH, was not without disappointments. In 1965, in a major US city the press learned that pituitaries were being collected by the medical examiner's office and shipped to the NPA. The diener was being paid the customary fee of \$2/pituitary for collecting, storing, and shipping the pituitary glands. However, he also collected gold from the mouths of autopsied corpses, and used the money gained from his supplemented income to build a swimming pool in his backyard called "the pit." The news transmitted by the United Press International and Associate Press did its damage. Grand Jury hearings were held in several cities, which affected the number of pituitaries collected that year. Unfortunately, there were other questionable occurrences in conjunction with the NPA's collection. One example involved an employee of the agency who executed questionable transactions for personal benefit. The tasks of the Director and the Board of Directors were not dull and were time-consuming.

#### GENE SPLICING AND RECOMBINANT DNA (rhGH) (1976-1985)

Based upon the laboratory demonstration that genes could be manipulated to produce useful new substances such as rhGH and rh-insulin, a remarkable story of a scientific revolution unfolded. This manipulation relied upon a controversial new area of research known as recombinant DNA engineering or, more popularly, as gene splicing. Stephen Hall has told the fascinating stories of the race to identify and duplicate the structures of genes (ie, insulin, GH, and somatostatin), the incorporation of these into bacteria, and by 1985 the production of these hormones in mass quantities. His book, *Invisible Frontiers* (Oxford University Press, New York, NY, 1987) is a "must read" for anyone interested in this field. Hall describes the molecular biology which challenged the accomplishment of making these hormones available as therapeutic agents, as well as the personal and professional interrelationships between the scientists. The result is a remarkable documentary of the multiple facets which transected medical science, therapeutic treatment, the pharmaceutical industry, and medical ethics into an entire new world in a 10-year period.

The mass production of specific hormones such as rhGH required identification of the gene structure of the desired hormone, duplicating that structure, determining

a way to mass produce the gene, splice the human gene into the gene structure of a bacteria so that the bacteria would produce the desired hormone in large quantity, purify the hormone, test the potency and possible toxicity in non-human mammals, and then test the hormone's potency, effectiveness, and possible toxicity in humans. The concept to accomplish this was clear prior to 1975, but the competitive race to develop the methodology began by scientists in 1976 when 3 groups of scientists in the US started the race to make insulin by recombinant technology. These groups were located at Harvard (Walter Gilbert, PhD, lab chief), at University of California at San Francisco (William Rutter, PhD; Howard Goodman, PhD; and Herbert Boyer, PhD, lab chiefs), and at Genentech (Herbert Boyer). Robert Swanson was a venture capitalist who, with Boyer, had a business goal, specifically, to make and sell human insulin. In August 1978, the Genentech group succeeded. The product was sold to Lilly and operating capital for further projects was available to the Genentech scientists. Peter Seeburg, PhD, a post-doctorate fellow with Goodman at UCSF, had been working with the hGH gene splicing system and joined the Genentech group that subsequently produced rhGH.

By 1981, several pediatric endocrinologists, including myself, were in the process of establishing the protocols for clinical trials of rhGH. A key person and the first physician employed by Genentech for the establishment of the clinical endocrine projects was Ann Johanson, MD, Professor of Pediatrics, University of Virginia. By October 1985, the clinical trials were successfully completed and the FDA approved rhGH for clinical use in patients with GHD.

Serendipity was manifest. In 1985, two explosive occurrences transpired. In March, a patient who had received hGH years previously was reported to have died of CJD. Native hGH had been given to patients for 27 years without significant side effects. The question was asked, "Should hGH investigation and therapy be discontinued?" Mortimer Lipsett, MD, Director of the National Institutes of Diabetes, Digestive and Metabolic Diseases (NIDDM) quickly called a meeting at NIH to discuss the question. Twenty plus prominent physicians of various specialties were present. I led the group who believed that "One is a series of nothing," and my calculation from the data generated by the NPA that 11 miles of height had been given to GHD patients over the years persuaded the consultant group and Dr. Lipsett not to stop distribution. Upon returning home after a follow-up meeting in New Orleans, I found a letter awaiting me from parents of an adult whom I had treated with native hGH as a child. Their son had succumbed to a neuropathological disease several months previously. A third patient also was quickly recognized. The comet truly had exploded and hGH distribution had to be stopped. The second event was the approval of rhGH by the FDA in October of 1985 for



treatment of GHD patients. This was the culmination of a phenomenal development: the creation of a synthetic rhGH that was accompanied by unlimited supplies of hGH for investigation and therapy.

### hGH AND rhGH FOLLOW-UP (1985-2005)

This timeframe comprises 2 major areas of interest: first, the follow-up to the use of native hGH during the prior 27-year period (1958-1985), particularly in respect to the status of CJD, and second, the legitimate and illegitimate use of rhGH.

As of January 1, 2003, CJD in the US was reported to have occurred in 26 of the approximately 7700 patients (an incidence of approximately 0.34%) who had received hGH between 1958 and 1985. The names and addresses of 6272 of these are known. The possibility exists that some of the remainder (1428) may have been lost to follow-up because of death from CJD. Distribution of the preparations used in the early years was not always from the same extractor because the NPA often had only one type of preparation to distribute, and by necessity many patients received different preparations while undergoing investigative therapy. By 1977, the Wilhelmi extraction procedure had been dramatically improved in purity and in yield by Parlow, who had assumed responsibility for purification of all hGH for the NPA. No patient started on hGH after this improvement was incorporated into the process has developed CJD. In retrospect, Wilhelmi's preparations most likely were the source of the prion contamination, but even if this is correct only a few of the preparations were probably contaminated. Multiple factors, including total dose of the contaminated preparation and genetic susceptibility undoubtedly affected whether an exposed patient developed the disease. More cases might be expected to be reported, but the pandemic projected by Daniel Gajdusek, MD, PhD, in 1985 never occurred. In April 2003, Allen Spiegel, MD, Director of NIDDK, distributed 2 reports (a comprehensive and a short form) updating the information concerning CJD and hGH. (Information can be obtained on the NIDDK website, [www.nidddk.nih.gov/health/endo/pubs/cruetz/update.htm](http://www.nidddk.nih.gov/health/endo/pubs/cruetz/update.htm)).

Other diseases which could possibly be transmitted via hGH—including HIV—have not occurred. Adrenal crisis, however, has allegedly resulted in more deaths in patients having received hGH and rhGH than has CJD. Adrenal crisis is probably not caused by hGH or rhGH, but is a result of associated ACTH deficiency in patients with hypopituitarism. The positive aspects of the follow-up to the use of native hGH are many, and most are known to the readers of *GGH*. Those wishing additional information are referred to multiple articles in the *GGH* archives ([www.GGHjournal.com/search.cfm](http://www.GGHjournal.com/search.cfm)).

In respect to the illegitimate use of rhGH, unequivocally the abuse by athletes is, and should be, of primary

concern to society and should be halted. The abuse of prescribing rhGH in an attempt to retard the aging process also should receive attention. My credibility to speak regarding the latter issue is gained from personal experience as I participated in a research protocol as proband (1982-1985) to assess if hGH could reverse the aging process. Specifically, I received daily injections of hGH for 2.5 years; 4 other men joined me for the last 2 years.<sup>9</sup> The study terminated in 1985 when CJD was reported in patients who had received hGH. As a result of these early studies and subsequent short-term reports by multiple investigators, I remain unconvinced that hGH can reverse the aging process. Unequivocally we should strive to eliminate the abuse of rhGH in attempts to reverse the aging process. Unfortunately, the much needed study to determine whether rhGH will *retard* the aging process probably will never be done, as it would require 30 years of rhGH administration to a large group of individuals beginning at the ages of 30-35 years, as well as administration of a placebo to a similar group.

### SUMMARY, CONCLUSION, AND COMMENT

This abbreviated history written by my recollection of 50 years of the use of hGH as a therapeutic agent is designed to expose young physicians and others to the use of hGH and rhGH over this extended period. With the exception of Stephen Hall's insightful presentation regarding how recombinant hormones came into existence, I am unaware of any historical accounting of the 50 years of GH. I thank Dr. Fima Lifshitz and the Editorial Board of *GGH* for the opportunity to relate these historical events and to share these with the readers of *GGH*.

In conclusion, now in my golden years, I am grateful to have had the opportunity to know and collaborate with so many giants working in the field of somatotropin investigation in the past, and I continue to meet and learn from the giants working in the field today. I am also grateful, and honored, to have had the opportunity to know and collaborate with my many former fellows and colleagues, all of whom were also my mentors. I cannot possibly record here the names of these wonderful people, but each former fellow and colleague can be assured that I am writing about you. My gratitude is also expended to former and current members of the Editorial Board of *GGH*, all of whom have shared significantly in making *GGH* an outstanding journal in bringing together physicians of multiple specialties to share knowledge of common need. The initial goals of Genentech and myself as first Editor in Chief have been exceeded, and continue to be exceeded beyond expectation.

I also wish to thank my former colleagues at NIH and others for the professional opportunities that have been given to me. To Genentech, this double 20<sup>th</sup> anniversary of the marketing of rhGH and the establishment of *GGH* is worthy of commemoration. Hopefully, this article



adequately recants the significant accomplishments and value of both.

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## ADDENDUM

After submitting the above manuscript I became aware that after the March, 2006 issue (Vol 22, No 1), *Growth, Genetics & Hormones* may no longer have funding and thus cease publication. This journal has accomplished the significant goals set forth 20 years ago to broaden sharing of knowledge across pediatric endocrinology, genetics, metabolism and general pediatrics. Furthermore, the same goals need to be continued, as there is still a great need for sharing of important knowledge to provide the highest level of patient care and research among geneticists, nephrologists, endocrinologists, gastroenterologists, general pediatricians and others. There is no other journal that fulfills the need. Hopefully Genentech will continue to take the lead as they have in the past in so many endeavors, and either support directly the educational grant or organize collegially collaborative support among other organizations or corporations, so that GGH continues to be published next year and thereafter.

## ABSTRACTS FROM THE LITERATURE

### Idiopathic Short Stature Children Are Poor Eaters and Are Thin

Data on the eating behaviors and nutritional status of children with idiopathic short stature (ISS) are lacking. The paper by Wudy et al assessed 214 patients with ISS from 123 families and recorded the BMI and eating behaviors with the Child Eating Behavior Questionnaire and the Food Frequency Questionnaire. Endocrine markers of body weight regulation (leptin and ghrelin) were also measured. The ISS patients had a decreased BMI (-0.33 SDS) as compared with population norms. Furthermore, they also had a decreased food responsiveness with a score of 1.9 on the Child Eating Behavior Questionnaire, as compared with a score of 2.4 for the population mean. They had reduced enjoyment of food (3.2 vs 3.9), emotional under-eating (2.6 vs 3.0), and showed increased fussiness over food (3.2 vs 2.9). "Poor" eaters showed more marked alterations in BMI and behavioral characteristics than those who were "good" eaters. Total serum ghrelin was not different among good and poor eaters, and serum leptin was moderately increased but did not differ between the groups. The authors concluded that ISS patients present altered eating behaviors that possibly contribute to their short stature.

Wudy SA, Hagemann S, Dempfle A, et al. Children with idiopathic short stature are poor eaters and have decrease body mass index. *Pediatrics.* 2005;116:e52-57.

**Editor's Comment:** There are countless papers dealing with ISS and other forms of short stature, but the nutritional status and eating behaviors of the patients are rarely addressed. Indeed, low IGF-I levels are most often analyzed and considered essential for diagnosis and treatment of short stature patients, as well as for the publication of scientific papers, often without addressing body weight, dietary intake, or nutritional status. Thus, I am delighted to note the paper by Wudy and colleagues showing ISS patients presenting with alterations in eating patterns and decreased BMIs. Hopefully, these data will stimulate an interest in evaluating the role of suboptimal nutrition on the growth patterns of children with ISS and other short stature patients. This assessment should be a must before embarking in other more costly medical interventions.

Fima Lifshitz, MD

### Compliance with Medication Recommendations

Compliance is defined as "the extent to which a person's behavior coincides with medical or health advice." Despite the importance of the medication in treatment, disease prevention, and health promotion, compliance rates range from 11% to 93%. The authors reviewed pediatric

medication compliance literature based on Medline searches of: medication compliance, patient compliance, patient dropouts, or treatment refusal combined with 45 other terms including drug therapy and specific formulations or methods of drug delivery. Additionally,

data were excerpted from an AAP Periodic Survey on primary care pediatricians' views on patient compliance with completing prescriptions for acute and chronic illness. The authors noted a caveat regarding compliance data reliability: parental reports of compliance have been shown to be markedly overrated (eg, in one study, mothers reported 60% compliance with obtaining prescribed refills, compared to only 12% according to pharmacy records).

The review yielded a number of principles pertaining to barriers to good medical regimen adherence. Limits on the time the physician can spend with each patient and family to negotiate a best-fit medication and discuss their ability to comply with the prescribed regimen represents a significant barrier. Lack of continuity of physician-patient interaction, particularly between and within multi-personnel office settings, is a strong predictor of poor compliance. Patient and family characteristics constitute additional sets of factors influencing compliance: the patient's and family's ability to understand the importance of following the prescribed treatment is an important element. Factors affecting understanding include health literacy, education, and culture. Patient/family knowledge, information, and misinformation or perspectives from outside sources (including the Internet) influence compliance, as can psychological function (eg, psychopathology). Such preexisting or emerging problems necessarily require attention in order to enhance compliance.

Practice setting characteristics and specific physician behaviors can influence compliance. Parents are more likely to be actively involved in the communication process if they are not distracted by restless children, their own time constraints, and annoyance over long waits. Enhanced communication skills have been known to shorten visit duration, improve patient adherence, and decrease the need for follow-up care.

Medication factors (eg, duration, schedule, formulation, palatability, cost, and adverse effects) were clearly associated with compliance. Longer duration of the medication regimen and increased complexity of the medication schedule represented risk factors to adherence, with mid-day dosings being particularly problematic. Personal preferences and aversions became evident in relation to forms of medication and palatability. Children expressed preferences for one form over another (eg, sprinkles vs syrup) whereas parents preferred oral liquid to solid forms (eg, powder, tablets, capsules). Medication cost for the uninsured or under-insured constituted an additional burden leading to compromised compliance. Cost also drove drug formulary decisions that restricted access to some useful medications that were more palatable and/or facilitated the dosing schedule. Finally, adverse effects from medications decreased compliance.

The authors outlined a set of *General Principles to Enhance Medication Compliance* that include: (1) improving communication between physician and patient/family, (2) modifying or negotiating regimens, (3) emphasizing patient self-management of disease or

illness, (4) using the simplest and most effective regimen available, and (5) using technology and devices to facilitate compliance. The authors stated the overriding issue for improved compliance is developing a one-on-one relationship between "1 doctor and 1 patient."

Winnick S, Lucas DO, Hartman AL, Toll D. How do you improve compliance? *Pediatrics*. 2005;115:e718-724.

**First Editor's Comment:** The term compliance is often used interchangeably with adherence, as it has been in this paper. However, compliance entails obedience to a directive from a physician (eg, "take this medication 3 times a day"), whereas adherence implies that the patient and family are active collaborators in the treatment process. The WHO defines adherence as "the extent to which a person's behavior—taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider."<sup>1</sup>

The average adherence to medication recommendations is approximately 50% in the pediatric population.<sup>2</sup> Despite intuitive expectations, adherence can falter even in life-threatening conditions such as type 1 diabetes (T1DM) and congenital adrenal hyperplasia. Winnick et al emphasized the critical importance of the one-on-one relationship between physician and patient as the key to improving adherence. Improved delivery systems (eg, pumps, transdermal patches, etc.) alone are unlikely to eliminate adherence problems. For example, a good collaborative relationship associated with clear communication would facilitate prompt discovery that the adolescent with uncontrolled T1DM has "broken" insulin pumps because he is embarrassed that his peers can see the device. Another example would be the young adult male with gonadotropin deficiency who fails to adhere to recommendations because the testosterone replacement dose is inadequate for normal erectile function. There is typically an explanation for poor adherence, but the remedy presupposes strong lines of communication between the physician and the patient and the family. The cost is time—the time to develop and maintain a relationship. While technological advances can facilitate adherence, when problems emerge, they cannot be confused with the solution.

Finally, the authors' recommendation to "emphasize patient self-management of disease or illness" should be interpreted cautiously. In the pediatric context, one needs to know *who* assumes responsibility for various aspects of medical care or *how* that responsibility is shared within the family.

David E. Sandberg, PhD

**Second Editor's Comment:** Coincidentally, Osterberg and Blaschke<sup>3</sup> published a review article, "Adherence to Medication" which denotes the importance of this issue across disciplines. As C. Everett Koop said, "Drugs don't work in patients who don't take them." The problem is of particular importance to pediatric endocrinologists

who treat patients with chronic conditions requiring long-term therapy, complex regimens, and frequent medication changes. Furthermore, patients are often asymptomatic and cannot care for themselves. These patient characteristics are typical of poor compliance and/or adherence to treatment. Lack of response to medication, missed appointments, presence of psychological problems, and/or cognitive impairment of the patient or caregiver may be indicators of poor adherence. High medication costs and third-party payor requirements including high co-payments and frequent refills compound the problem. These barriers are important and add to the time required to obtain

medications. Poor adherence contributes to worsening of disease, increased costs of care, and even death. New cost-efficient technologies that facilitate treatment adherence are needed to aid physicians and patients in meeting the goals of therapy.

Fima Lifshitz, MD

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## LDL Receptor-Related Protein Mutations in Primary Osteoporosis

The LDL receptor-related protein 5 gene (*LRP5* – OMIM 603506, chromosome 11q13.4) is a 1,615 aa transmembrane protein that interacts with the secreted glycoprotein WNT (wingless – OMIM 604663, chromosome 2q35) and its Frizzled receptor to enhance autocrine WNT signaling of osteoblast-induced bone formation. The interaction of LRP5-WNT-Frizzled receptor is inhibited by another protein termed dickkopf (*DKK* – OMIM 605189, chromosome 10q11.2) that binds to *LRP5* near its amino terminal and interrupts Wnt signaling, thereby modulating the extent of osteogenesis. When a mutation in this region of *LRP5* prevents its binding to DKK, there is further increase in WNT signaling and bone formation leading to high bone mass. Homozygous loss-of-function (LOF) mutations throughout other regions of *LRP5* have been identified in subjects with the osteoporosis-pseudoglioma syndrome (OMIM 259770), an illness characterized by developmental delay, seizures, impaired vision due to a pseudoglioma of the retina, and lax ligaments, as well as

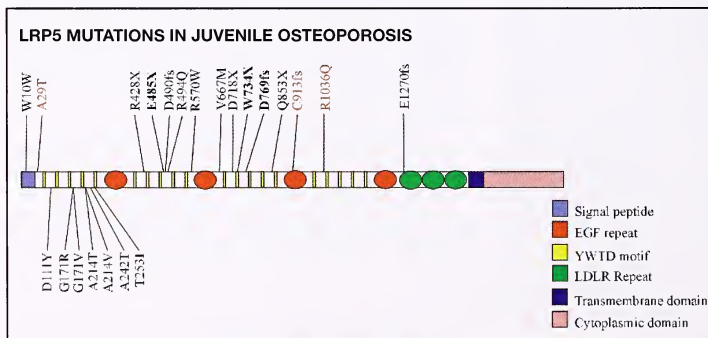
decreased bone mineralization.

Hartikka et al found heterozygous LOF mutations in *LRP5* in 3 out of 20 children and adolescents with primary osteoporosis, defined as isolated osteoporosis without stigmata of other illnesses and manifested by fractures with low impact trauma beginning in early childhood. Two missense mutations (Ala29Thr, Arg1036Gln) and one frame shift mutation (Cys913fs) were detected. Examination of family members revealed osteoporosis and similar mutations in a parent and/or a sibling, indicating autosomal dominant transmission of this trait attributable to haploinsufficiency of *LRP5*.

Hartikka H, Mäkitie O, Männikkö M, et al. Heterozygous mutations in the LDL receptor-related protein 5 (*LRP5*) are associated with primary osteoporosis in children. *J Bone Miner Res*. 2005;20:783-789.

**Editor's Comment:** Osteopenia and osteoporosis in children and adolescents are most commonly secondary to chronic illnesses, nutritional deprivation,

limited mobility, excessive exposure to glucocorticoids, or deficiencies in growth, sex, and/or thyroid hormones. Osteogenesis imperfecta (OI) is due to heterozygous LOF mutations in the genes encoding components of type I collagen (*COL1A1*, *COL1A2*). In addition to osseous fragility, patients with OI often have blue sclerae, joint laxity, and dental abnormalities. None of the patients studied by Hartikka et al had a mutation in either of these genes. Juvenile idiopathic osteoporosis



Schematic representation of the LRP5 protein and its domain structure. Mutations of individuals with OPGG are indicated above the protein structure; heterozygous mutations are marked in bold. Mutations of individuals with high bone mass phenotype, situated in the first YWTD/EDF domain, are indicated below the protein. Mutations of individuals with primary osteoporosis (this study) are marked on red. Reprinted with permission. Hartikka H, et al. *J Bone Miner Res*. 2005;20:783-789. Copyright © 2005. American Society for Bone Mineral Research. All rights reserved.



develops 2 to 3 years before puberty and is manifested by the acute onset of bone pain due to long bone fracture(s) or vertebral collapse. Heterozygous gain-of-function (GOF) mutations in *LRP5* impair binding to DKK and lead to increased bone mass, a trait transmitted in an autosomal dominant manner.<sup>1</sup> Although initially considered a benign variant, later reports associated this trait with intracranial hypertension, cranial nerve palsies, and extensive maxillary and mandibular exostoses.<sup>2</sup> The phenotype of the subject with homozygous GOF

mutations in *LRP5* has not been described, but might be anticipated to be a lethal form of osteopetrosis.

Allen W. Root, MD

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## Aromatase Inhibitor and Growth in the Pubertal Male with GHD

Mauras and colleagues conducted a 12-month pilot study of 20 adolescent males with clinical and biochemical evidence of growth hormone deficiency (GHD) who were treated with GH (mean dose ~0.3/mg/kg/wk) for at least 6 months (range: 6 months–9 years) prior to the study. The investigation sought to determine whether treatment over a period of 12 months with the aromatase inhibitor anastrozole can achieve sustained suppression of estrogen production and delay epiphyseal fusion in adolescent males with GHD. Physical examination, genital Tanner staging, bone age, DEXA scan, and an early morning blood sample were obtained at baseline and throughout the duration of the study. Ten boys were maintained on GH only and 10 were started on anastrozole (1 mg orally daily) in addition to GH.

Results showed a 60% drop in estradiol

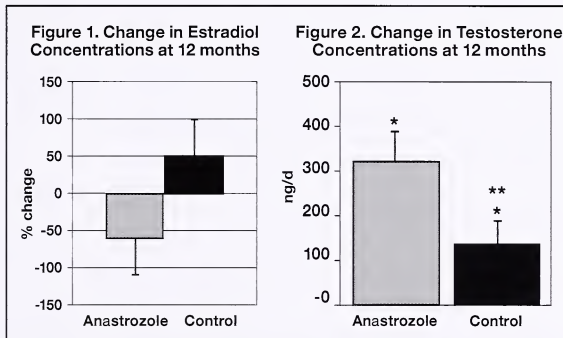


Figure 1. Percentage change in plasma estradiol concentrations.

Figure 2. Absolute change from baseline in serum testosterone concentration. \* refers to the difference within each group at 12 mo vs baseline; \*\* refers to the difference between groups: \*  $p = 0.001$ ; \*\*  $p = 0.03$ .

Adapted from Mauras N, et al. *J Pediatr Endocrinol Metab.* 2004;17:1597–1606. Copyright © 2004. JPEM.

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concentrations in the anastrozole group and a 50% increase in concentrations in the control group (GH only; Figure 1). The reciprocal increase in testosterone and free testosterone concentrations in the anastrozole group was substantially greater than the rise in testosterone during spontaneous puberty in the control group (Figure 2). IGF-I and IGFBP-3 did not change significantly in the anastrozole group, whereas IGF-I rose significantly at 12 months in the control group. There were no significant differences between the anastrozole and control groups with regard to lipid concentrations, body composition, or bone density, nor any differences in growth velocity rates, height SD scores, bone age advancement, or predicted adult height.

The authors concluded that compared to GH-deficient boys treated with only GH, 12-month treatment with an aromatase inhibitor in combination with GH results in a significant and sustained suppression of circulating estrogen concentrations and reciprocal increases in testosterone concentrations. Anastrozole treatment was not associated with detectable detrimental effects on body composition, tempo of puberty or bone mineralization, and was well tolerated and safe over the period studied. The lack of effect of anastrozole on growth velocity, bone age advancement, or predicted

adult height was interpreted by the investigators to be due to the limited duration of use (ie, 12 months).

Mauras N, Welch S, Rini A, Klein KO. An open label 12-month pilot trial on the effects of the aromatase inhibitor anastrozole in growth hormone (GH)-treated GH deficient adolescent boys. *J Pediatr Endocrinol Metab.* 2004;17:1597-1606.

**Editor's Comment:** It is reasonable to predict that there will be more studies examining the growth-promoting benefits of aromatase inhibitors. They offer the promise of prolonged growth without the metabolic and psychological drawbacks of arresting pubertal development. This controlled pilot study opens the way to a larger and longer duration study of the synergistic benefits of GH and anastrozole on adult height. Because of the role that sex hormones play in brain development and function,<sup>1</sup> it would be prudent to include neuropsychological endpoints in any study that alters the typical ratios observed between testosterone and estradiol in adolescent males.

David E. Sandberg, PhD

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## COMT Polymorphism in Early Puberty

Estrogens are initially metabolized by a P450 hydroxylase to less biologically active catechol-estrogens and further degraded by catecholamine-O-methyltransferase (COMT) to inactive products. COMT transfers a methyl group from S-adenosylmethionine to the catechol-estrogen. Within the structure of COMT (OMIM 116790, chromosome 22q11) is a functional polymorphism that results in the substitution of methionine for valine in codon 158 (val158met). The COMT product containing val158 is 3 to 4 fold more biologically active than is that with met158.

Hypothesizing that the COMT val158met polymorphism effect on estrogen catabolism might be reflected in the biologic activity of endogenous estrogen, the investigators examined the relationship between linear growth, bone mineralization, and sexual development in prepubertal and early pubertal, 10-12 year old girls and the high (H) and low (L) polymorphic variants of COMT (COMT<sup>HH</sup> and COMT<sup>LL</sup>, n=43 and n=85, respectively). Although total serum estradiol concentrations were similar in COMT<sup>HH</sup> and COMT<sup>LL</sup> girls, levels of free estradiol as well as IGF-I were higher in COMT<sup>LL</sup> subjects. The authors reported that at the time of study COMT<sup>LL</sup> subjects were 5.4 cm taller than were COMT<sup>HH</sup> girls, had greater lean body mass, and appeared to progress further into puberty at a more rapid rate than did COMT<sup>HH</sup> girls. Total bone mineral content (BMC) by DEXA was 12.7% greater in COMT<sup>LL</sup> than in COMT<sup>HH</sup> girls due primarily to increased bone size; thus, volumetric bone mineral density was

similar among groups. Cortical BMC and cortical cross-sectional area by peripheral quantitative computerized tomography (pQCT) were highest in COMT<sup>LL</sup> subjects due primarily to increased periosteal circumference. There was no relationship between COMT variant and trabecular volume or mineralization. Serum free estradiol values were related to these varied aspects of growth and mineral metabolism and thus indirectly to the COMT polymorphic variants. The authors concluded that the val158met COMT polymorphism exerted significant effects on growth, pubertal development, and bone mineralization in pre- and early adolescent girls, primarily by increasing serum concentrations of free estradiol by altering the rate of catabolism of endogenous estrogens.

Eriksson A-L, Suuriniemi M, Mahonen A, Cheng S, Ohlsson C. The COMT val158met polymorphism is associated with early pubertal development, height and cortical bone mass in girls. *Pediatric Res* 58:71-77,2005.

**Editor's Comment:** COMT may now be added to the growing list of recognized genes that influence the rates of growth and sexual development and bone mineralization; this report emphasized the extensive genetic variability in these processes. Although COMT<sup>LL</sup> girls were 5 cm taller than COMT<sup>HH</sup> subjects at 10-12 years of age, the effect of the COMT val158 met polymorphism on adult height is likely to be less impressive as the COMT<sup>LL</sup> subjects will probably

complete their pubertal development and achieve their adult height at an earlier age than the COMT<sup>+/+</sup> children. It would be of interest to learn the bone ages of the study subjects and later their ages of menarche and their adult

heights. Similar studies relating COMT genotype and growth, pubertal maturation, and bone mineralization would also be of interest.

Allen W. Root, MD

## Novel Deletions Downstream of *SHOX* Cause Léri-Weill Dyschondrosteosis

Léri-Weill dyschondrosteosis (LWD, MIM 127300) is a dominantly inherited bone dysplasia characterized by short stature, mesomelic limb shortening, and Madelung deformity of the forearm. Heterozygous deletions of the short stature homeobox-containing gene (*SHOX*) occur in LWD, and homozygosity for such deletions has been found in the more severe Langer mesomelic dysplasia (MIM 249700). *SHOX* resides in the pseudoautosomal region 1 (PAR1) of the short arm of the X and Y chromosomes. Its product is involved in cell cycle and growth regulation; and loss of *SHOX* function has also been implicated in Turner syndrome and in some cases of idiopathic short stature.

Detection of *SHOX* defects in only about 60% of LWD patients has raised the possibility that some of the remaining patients could have mutations in regulatory elements that lie upstream or downstream of the *SHOX* locus. Indeed, Benito-Sanz and colleagues have shown this to be the case.

The authors screened 80 LWD patients in whom mutation in the *SHOX* coding sequence had been excluded. Novel deletions in PAR1 downstream of *SHOX* were detected in 12 patients, 8 of whom came from families showing dominant inheritance of LWD. Deletion mapping revealed that the deletions were ~30–205 kb downstream of *SHOX* and varied in size from <81 to ~501 kb. Fine mapping disclosed a minimal commonly deleted region of 29 kb. The 5' end of the deletion was similar in 5 families, suggesting the possibility of a hotspot for a deletion breakpoint.

Large-scale deletions were detected in 4 families, raising the possibility of a chromosomal rearrangement. However, inversion or translocation of the deleted region to an autosome was excluded by fluorescent in situ hybridization analysis, showing that the deletions were contiguous in all 4 cases.

Two explanations were considered. The first was the presence of a nearby second gene in PAR1 that influences skeletal growth much like *SHOX*. The authors found no evidence for a second gene. The second hypothesis, which was favored by the authors, was that a positive regulatory element for *SHOX* resides in the deleted region; deletion of this region would be expected to produce loss of *SHOX* expression. They noted other examples of long-distance gene regulators, especially involving transcription factors during development.

Benito-Sanz S, Thomas NS, Huber C, et al. A novel class of pseudoautosomal region 1 deletions downstream of *SHOX* is associated with Léri-Weill dyschondrosteosis. *Am J Hum Genet.* 2005;77:533–544.

**Editor's Comments:** *These results help to explain at least some of the 40% of patients in LWD in whom mutations in the SHOX coding sequence are not detected. They underscore a mechanism that is probably under-appreciated: disturbance of long-range gene regulation. It will be interesting to see how common this mechanism will be found to explain similar situations for other genetic diseases.*

William A. Horton, MD

## Circadian Rhythms in Obesity and Metabolic Syndrome

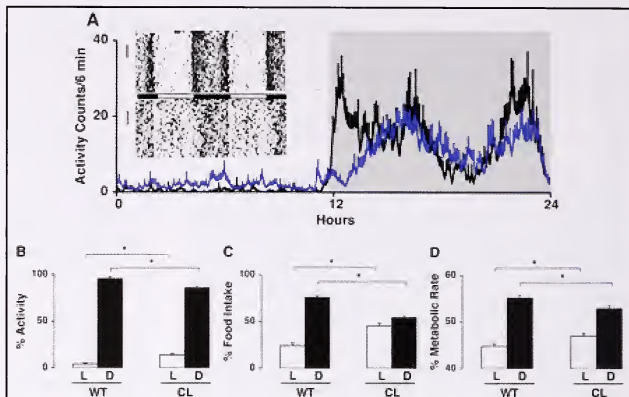
Circadian rhythms are governed by a series of regulatory oscillators expressed in the suprachiasmatic nucleus (SCN), and elsewhere in the CNS as well as in most peripheral tissues, that oscillate with an approximate 24-hour periodicity usually entrained to the light-dark cycle.<sup>1</sup> In mice, *Clock* (Circadian Locomotor Output Cycles Kaput - OMIM 601851) encodes an 855 aa transcription factor involved in this process. Overexpression of *Clock* shortens period length. Expression of an A to T nucleotide transversion in a splice donor site that leads to exon skipping and deletion of 51 aa results in 1-hour lengthening of locomotor activity in the heterozygous state and 3- to 4-hour increase in periodicity and dampening of the amplitude of circadian rhythms, leading to loss of periodicity (arrhythmia) in the homozygous

animal maintained in constant darkness. Stimulated by the observation that reduced forms of the nicotinamide adenine dinucleotide (NAD) cofactors enhance, and oxidized forms inhibit, DNA binding of the *Clock* transcript, Turek et al investigated the relationship between circadian rhythmicity and intermediary metabolism in homozygous *Clock* mutant mice (*C<sup>-/-</sup>*) maintained on a 12-hour light-dark cycle. They demonstrated that relative to wild-type (WT) mice, the *C<sup>-/-</sup>* mice had decreased locomotor activity during darkness. Also, the *C<sup>-/-</sup>* animals ate rather evenly through the 24-hour period, whereas the WT mouse ate 3-fold more during darkness than during light. In addition, the *C<sup>-/-</sup>* mice expended 10% less energy per 24 hours than did the WT animals. *C<sup>-/-</sup>* animals were heavier than WT animals by 6 weeks

of age; between 6–16 weeks of age,  $C^{-/-}$  mice ate greater amounts of food and gained more weight than did WT mice, whether ingesting a normal or high-fat diet. At 7 to 8 months of age,  $C^{-/-}$  animals had higher concentrations of leptin, glucose, cholesterol, and triglycerides than did WT mice, but they had similar levels of insulin. Histologically, there were hypertrophy of adipocytes and excessive glycogen and lipid within liver cells (steatosis) in  $C^{-/-}$  animals. In the mediobasal hypothalamus, the diurnal patterns of expression (mRNA levels) of orexin and ghrelin (orexigenic agents) and of CART (cocaine- and amphetamine-regulated transcript—an anorexigenic agent) were decreased in  $C^{-/-}$  mice relative to WT animals. The authors concluded that *Clock* and the circadian rhythms it controls have regulatory effects on energy intake and expenditure and fuel metabolism. When altered, the resultant abnormalities lead to a syndrome of obesity, hyperglycemia, and hyperlipidemia that mimics the metabolic syndrome and that might be mediated through hypothalamic pathways that regulate appetite and energy utilization.

Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian *Clock* mutant mice. *Science*. 2005;308:1043–1045.

**Editor's Comment:** Circadian rhythms are present not only in neurons within the SCN but also in single cells in most peripheral tissues and utilize the same regulatory mechanisms found in the SCN.<sup>2</sup> Thus, it is likely that the SCN synchronizes overall diurnal rhythms, while local oscillators regulate tissue-specific circadian function. It is unclear whether the metabolic effects of the described loss-of-function mutation in *Clock* are exerted



Altered diurnal rhythms in locomotor activity, feeding, and metabolic rate in *Clock* mutant mice. (A) Activity counts over the 24-hour cycle during light (unshaded) and dark (shaded) periods (B) Diurnal rhythm of locomotor activity for mice in (A). Total activity over the 24-hour period was similar between wild-type (WT) and *Clock* mutant (CL) genotypes. (C) Diurnal rhythm of food intake. Results shown are average food intake during light and dark periods as a percentage of total food intake ( $P < 0.001$ ). (D) Diurnal rhythm of metabolic rate. Results shown are average metabolic rates during the light and dark periods as a percentage of total metabolic rate. All results shown are expressed as group means  $\pm$  SEM.

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through the SCN or in peripheral tissues, but the results of loss of diurnal variability on lipid and carbohydrate metabolism are striking. In volunteer human males, sleep deprivation lowered leptin and increased ghrelin values leading to increase in hunger and appetite.<sup>3</sup> Future studies evaluating the role of the sleep-wake cycle on intermediary metabolism and the genesis of the metabolic syndrome in man are clearly warranted.

Allen W. Root, MD

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## Adult Height in Turner Syndrome

Although it has long been recognized that growth hormone (GH) treatment increases the adult height of those with Turner syndrome, this Canadian study is the first randomized controlled trial carried through to adulthood. Girls with Turner syndrome, aged 7–13 years, were randomly assigned to either receive GH treatment (0.30 mg/kg/wk by subcutaneous injection 6 times per week;  $n = 76$ ) or to be a part of an untreated control group (C) that did not receive GH treatment

( $n = 78$ ). Sex hormone replacement was used to induce puberty in both cohorts at age 13 years if onset did not occur spontaneously. Growth hormone treatment lasted on average 5.7 years. Protocol completion required an annualized height velocity of  $<2$  cm/yr and a bone age of 14 years or greater. There were 104 patients (61 GH, 43 C) that completed the protocol (50 withdrew). At protocol completion, mean heights were  $147.5 \pm 6.1$  cm (GH) and  $141.0 \pm 5.4$  cm (C) ( $P < 0.001$ ). Girls who



started GH at an earlier age showed a greater increase in adult height (+0.22 SD, 95% CI 0.10–0.33 SD, or +1.5 cm/yr for each year of earlier GH initiation [ $P<0.001$ ]), although this age effect was highly variable between patients. Two follow-up visits further verified the adult height and assessed safety. For those available at least 1 year after protocol completion ( $n = 59$ ; 40 GH, 19 C), mean heights were  $149.0 \pm 6.4$  (GH) and  $142.2 \pm 6.6$  cm (C) ( $P<0.001$ ). The estimated height gain attributable to GH was +7.2 cm at protocol completion (CI = 6.0–8.4), and +7.3 cm (CI = 5.4–9.2) at follow-up (at least 1 year after protocol completion).

This report was accompanied by an editorial by Carel, in which he chronicled the history of GH treatment for short stature in Turner syndrome. Carel estimated that height gains across studies ranged from “minimal” to 17 cm for high-dose GH treatment. He applauded the Canadian researchers for adopting a powerful research design and carefully following participants. Although he noted that GH treatment unquestionably increased adult height in women with Turner syndrome, he posed a number of provocative questions regarding the cost-effectiveness and safety of GH in this population.

Canadian Growth Hormone Advisory Committee. Impact of growth hormone supplementation on adult height in Turner syndrome: Results of the Canadian randomized controlled trial. *J Clin Endocrinol Metab*. 2005;90:3360–3366.

Carel JC. Editorial: Growth hormone in Turner syndrome: Twenty years after, what can we tell our patients? *J Clin Endocrinol Metab*. 2005;90:3793–3794.

**Editor's Comment:** Although some readers may view the study's findings as old news, the accompanying editorial highlights details of its importance. By contrasting these findings with those from a recently published report of a French population-based cohort of GH-treated patients,<sup>1</sup>

several important questions/observations arise, as succinctly summarized in the Carel editorial. First, insofar as adult height gained (2.7–11.7 cm for those initiating treatment between 7–13 years) varies substantially across patients, most importantly attributable to age at treatment initiation, Carel asks whether GH should be used if only a minor effect is anticipated. Second, Carel claims that adult height gained with GH is not a validated proxy measure for “quality of life” which he identifies as the primary rationale for treatment. In his study, 88% of young adult participants favorably rated their GH treatment. However, when asked to estimate the minimal height gain they thought would make GH treatment worthwhile, the figure was above 8 cm in 64% of cases. Thus, based on adult heights achieved in the French cohort, two-thirds of patients treated with GH in the Canadian trial and many other studies would not consider treatment “worthwhile.” Third, findings from this study cannot be extrapolated to adult height outcomes that might be achieved should GH treatment be initiated earlier than 7 years or using higher doses. Finally, Carel focuses on the safety profile of GH treatment. He acknowledges that the overall safety record is good, but cites 2 studies that linked GH therapy with a heightened risk of otitis media. Furthermore, the presence of hearing difficulties in adulthood was found to be a robust predictor of a more negative quality of life in Turner syndrome patients. GH treatment and the associated theoretical risk for cancer was also noted, and careful monitoring of IGF-I levels and long-term follow-up studies were recommended.

David E. Sandberg, PhD

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1. Carel JC, Ecosse E, Bastie-Sigeac I, et al. *J Clin Endocrinol Metab*. 2005;90:1992–1997.

## Effectiveness of Hydrocortisone and Cortisone Acetate for the Treatment of CAH

Although cortisone acetate (CA) is used worldwide as corticosteroid substitution therapy in congenital adrenal hyperplasia (CAH), its effectiveness is uncertain since CA must be converted to cortisol to be biologically active. Its biologic activity depends on the activation by 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) reductase. Inada et al reported that hydrocortisone (HC)

is more effective than CA for the treatment of CAH. The authors compared the effect of CA with that of HC in 10 patients (aged 4–35 years) with 21-hydroxylase deficiency (21-OHD). Of the 10 patients, 8 were salt losers who required fludrocortisone in addition to glucocorticoids. HC was administered to all subjects instead of CA; the initial dose was 80% of the previous CA dose,

since the overall bioactivity of oral CA has been reported to be 80% of that of HC. The dose of HC was subsequently changed in accordance with the circulating levels of serum 17-hydroxyprogesterone (17-OHP) and/or plasma adrenocorticotropin (ACTH). Doses of fludrocortisone were

Serum levels of Cortisol, Cortisone, and Cortisol/Cortisone Normal Subjects by Age<sup>1</sup>

| Age                             |        | Cortisol<br>(ng/ml) | Cortisone<br>(ng/ml) | Cortisol / Cortisone |
|---------------------------------|--------|---------------------|----------------------|----------------------|
| <2 months                       | n = 58 | 29.2 $\pm$ 32.6     | 39.4 $\pm$ 21.1      | 0.9 $\pm$ 1.0        |
| $\geq$ 2 months, <2 years       | n = 30 | 53.7 $\pm$ 30.5*    | 36.3 $\pm$ 18.5      | 1.7 $\pm$ 1.0*       |
| $\geq$ 2 years, $\leq$ 20 years | n = 30 | 71.2 $\pm$ 39.1*    | 24.3 $\pm$ 12.3      | 3.3 $\pm$ 1.7*       |

Data are mean  $\pm$  SD. \* $P<0.01$  compared with <2 months.



not changed. Target concentrations were below 10 ng/ml for 17-OHP and below 50 pg/ml for plasma ACTH. The mean observation period after the drug changes was 10 months. Mean concentrations of serum 17-OHP decreased from 48.6 ng/ml to 10.1 ng/ml, as did those of plasma ACTH from 198.0 pg/ml to 35.1 pg/ml. The average drug requirement for CA was 33.9 mg/m<sup>2</sup>, while it was 20.3 mg/m<sup>2</sup> for HC when disease control was stable. The relationship can be expressed as an equation,  $HC = 0.58 \times CA$ ; the coefficient was substantially lower than the conventionally reported dose ratio of 0.8. The authors concluded that CA is inferior to HC as the substitution therapy in patients with CAH.

Inada H, Imamura T, Nakajima R, Yamano T. Poor response to substitution therapy with cortisone acetate in patients with congenital adrenal hyperplasia. *Clin Pediatr Endocrinol*. 2004;13:11–15.

**Editor's Comment:** CA may be used as the glucocorticoid component of substitution therapy for CAH. However, the paper by Inada and a previous paper by Jinno<sup>1</sup> indicate that oral administration of CA was inappropriate as glucocorticoid replacement therapy in patients with 21-OHD. The Jinno group compared the effect of CA with that of HC in 7 neonates with 21-OHD. From the time of diagnosis, CA was administered to 4 subjects, while HC was given to the other 3 subjects. The serum cortisol (F), cortisone (E), and 17-OHP in these 7 neonates with 21-OHD were compared with 118 normal subjects. In the normal subjects, serum E concentrations were greater than F during the first 2 months after birth, whereas F concentrations exceeded E after 2 months of age (Table). Infants with 21-OHD who received high CA doses had extremely low serum F concentrations, while 17-OHP concentrations were high until about 2 months of age. Thereafter, the serum F exceeded E, and 17-OHP became fully suppressed even though infants received moderate doses of CA. In contrast, HC administration successfully normalized serum 17-OHP in the neonatal period. With temporary switching from HC to CA, serum F concentrations immediately decreased and 17-OHP concentrations increased. Thus, conversion of E to F may be limited during early infancy, adversely affecting the treatment with CA. Jinno and colleagues also noted that CA was

inappropriate as a glucocorticoid replacement during early infancy in patients with 21-OHD.

To this author's knowledge, no comparative studies of CA and HC treatment during the neonatal period or infancy have been published. In the Jinno et al study, serum E concentration exceeded that of F in normal subjects until the age of 2 months. Conversion of E to F by CA may be difficult, as production of E is greater than that of F in the adrenal cortex from the fetal period to approximately 2 months of age. The predominant E production may reflect age-related morphologic findings of the neonatal adrenal. The human fetus extensively converted F to E (an oxidation reaction), but was unable to convert E to F (a reduction reaction).

At term, in normal infants, each adrenal gland weighs 4 to 5 g, more than 80% of which consists of an inner, hyperemic fetal zone. In this zone, conversion of F to E overshadows conversion of E to F. One-half of the adrenal weight is lost by 1 month of age, and by the age of 1 year the average gland weighs only about 1 g. This postnatal involution of the adrenal cortex involves gradual remodeling of fetal zone cells into the zona fasciculata during the first weeks and months of life. As the fetal zone is associated with a predominance of E, its involution was associated with the age-related changes of serum concentrations of E and F shown in normal subjects.<sup>1</sup>

Activity of 11 $\beta$ -HSD is high in human tissues, especially the inner fetal zone of the adrenal cortex. Results suggest the occurrence of a physiologic inability to respond to treatment with CA during early infancy in patients with 21-OHD, because oxidation by 11 $\beta$ -HSD predominates in the residual fetal cortex.<sup>1</sup> In contrast to cortisone, HC possesses an 11 $\beta$ -hydroxyl group and does not require activation by the enzyme 11 $\beta$ -HSD.

HC should be the drug of choice for substitution therapy in children with CAH. The Japanese Society of Pediatric Endocrinology recommends HC for the maintenance therapy of CAH.

Yoshikazu Nishi, MD

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## Growth on Stimulant Medication

Stimulant medication for the treatment of attention deficit hyperactivity disorder (ADHD) has long been suspected to have an adverse effect on linear growth. The first studies concerning this were published in the 1970s and since that time, there have been numerous other studies, which rather than clarifying this relationship, seem to have added to the controversy. Poulton reviewed 29 cohort studies published through September 2004 of children treated with methylphenidate or dexamphetamine.

Twenty-two of the studies involved children, 6 involved adolescents or adults close to their adult height, and 1 study included both children and adults. Of the 29 studies, 9 gave results consistent with reduction in height growth while on stimulants and 12 had negative findings. There was a slight difference in the median medication dose (31.4 mg vs 23.9 mg) in those studies which showed significant growth attenuation. Various methods were used to describe height, but the most frequently used

method was height deficit, meaning that the child was a certain amount shorter than he would have been had he continued to grow at a previous rate. Some studies used height z-scores.

The most sensitive studies were the longitudinal studies analyzing periodic observations taken before and after the initial period of treatment. Half of these studies (8 of 16) showed an attenuation of growth on the stimulants by at least 1 method, most reliably a change in height z-scores. The most scientifically rigorous study was one in which children with ADHD were randomly assigned to different treatment groups. This study showed a height deficit of 0.9 cm/yr in the first 14 months and 1.04 cm/yr from 14–24 months in children who received pharmacological treatment. Eight of the longitudinal studies used normative data as the controls, three of which showed an attenuation of height.

The studies of late adolescent and adult heights were mostly cross-sectional, and none showed any significant difference between those treated and the controls. The author stated that many of the studies were of poor quality. However, those of better quality demonstrated a significant association between treatment and attenuated height growth. The conclusion was that despite the large number of studies, most of those failed to detect any adverse affect on growth due to stimulant medication. Many did not stand up to any rigorous analysis. They further stated that it is reasonable to anticipate a reduction in height velocity when children are placed on stimulant medication, but that further studies should be performed in order to better understand the significance of this reduction.

Poulton A. Growth on stimulant medication; clarifying the confusion: a review. *Arch Dis Child*. 2005;90:801–806.

**First Editor's Comment:** *This paper is a welcome analysis of a large number of studies involving stimulant medications and the measurement of height in children with ADHD. Pediatric endocrinologists are often faced with the question of whether or not stimulant medication will adversely affect growth, and it is very difficult to*

*reference opinions with well-conducted longitudinal trials. Thus, one is left with the conclusion that the results are uncertain. Poulton has shown that at least in those studies that were more rigorously performed, there did seem to be a significant height deficit in these children. However, he also points out that children often do not remain on stimulant medications for the duration of their linear growth. Thus, an overall effect on final height is difficult to discern. This review will hopefully encourage investigators to perform the kinds of studies needed to answer this question conclusively. Such studies need to be randomized, control trials with varying doses of stimulant medications. With so many children currently receiving these medications, such a trial seems feasible.*

William L. Clarke, MD

**Second Editor's Comment:** *The efficacy of ADHD treatment and the growth of patients was also studied by the MTA Cooperative Group at the National Institutes of Mental Health<sup>1</sup> and reviewed in GGH.<sup>2</sup> It was clearly documented that there were behavioral benefits in treating ADHD patients, but there was decreased growth (–1.9 cm in height suppression in 24 months). As well, there were weight changes (–2.5 kg in the first 14 months and –1.22 kg in the following 20 months of treatment). These changes were more prominent in patients who adhered to the medication regimen. However, there were many who stopped taking the medication and thus, the effects were less marked. Suboptimal nutrition may play a role in the reduced growth and weight gain due to the effects of these medications. Thus, when these medications cannot be interrupted, physicians should attempt to overcome the decreased dietary intake and correct any nutrient deficits.*

Fima Lifshitz, MD

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## Cardiovascular Effects of Adolescent Growth Hormone Deficiency

The metabolic effects of growth hormone (GH) led to FDA-approval of rhGH therapy for GH deficiency (GHD) in adults, even though they have no prospect of height benefits. These effects include improvements in body composition, serum lipid levels, and cardiac function, among others. Lanes and colleagues sought to determine whether cardiovascular function is already altered in adolescents with GHD. These authors compared 10 adolescents with GHD on GH treatment (0.03 mg/kg/d for a mean of  $3.8 \pm 1.1$  yr), 12 adolescents with untreated GHD (4 of whom had previously

received  $1.6 \pm 0.2$  yr of treatment but had been off GH treatment for  $3.4 \pm 1.2$  yr due to financial reasons) and 14 healthy adolescent controls. The 3 groups were similar in chronologic age, bone age, height (but not height z-score), BMI, pubertal distribution (65–70% Tanner stages 2–4; remainder prepubertal), blood pressure, and pulse. GHD was defined by abnormally low serum IGF-I and IGFBP-3 concentrations plus failure on clonidine/L-DOPA stimulation testing (peak GH concentrations were  $3.2 \pm 2.4$  and  $3.0 \pm 2.3$   $\mu$ g/L with a range of 0.9–5.6  $\mu$ g/L).

A pediatric cardiologist and his technician, blinded to the GH status of the adolescents, performed echocardiography, carotid sonography, and measurement of endothelium-dependent vasodilation. For this last measurement, Doppler ultrasonography was used to quantify right brachial artery blood flow and brachial artery diameter before and 45 to 60 seconds after release of 5 minutes of 300 mm Hg applied by a standard sphygmomanometer cuff to the forearm (to induce hyperemia). They also measured, during echocardiography, the epicardial adipose tissue on the right ventricle, which was described in 2003 as a correlate with MRI measurement of abdominal visceral fat, clinical parameters of metabolic syndrome, and hence, cardiovascular risk in adults.<sup>1</sup>

Left ventricular mass was significantly lower in the untreated and treated GHD groups than the normal controls, although left ventricular posterior wall and interventricular septal thicknesses were both similar across groups. Left ventricular ejection fraction (%) was also similar, but the controls had significantly larger end systolic and end diastolic volumes than the 2 GHD groups. Carotid artery intima-media thickness did not differ, but the hyperemia-induced increases in brachial artery diameter and blood flow were both related to GH status; vasodilation was lower in the untreated GHD group than in the treated and control groups, and blood flow was greatest in the treated GHD group. Epicardial adipose tissue, which correlated positively with BMI in all 3 groups, was significantly greater in the untreated GHD adolescents than the other groups. Thus, GHD has been associated with decreased cardiac size, increased large-artery stiffness (IGF-I has a direct releasing effect on nitric oxide, an endothelial relaxing factor), and increased epicardial adipose tissue (a correlate of cardiovascular risk factors in adults).

Lanes R, Soros A, Flores K, Gunczler P, Carrillo E, Bandel J. Endothelial function, carotid artery intima-media thickness, epicardial adipose tissue, and left ventricular mass and function in growth hormone-deficient adolescents: Apparent effects of growth hormone treatment on these parameters. *J Clin Endocrinol Metab.* 2005;90:3978–3982.

**Editor's Comment:** Quite extensive data have been accumulating on the cardiovascular effects of GH and GHD. I refer the reader to references 2 and 3 for reviews of GH effects and reference 4 for review of IGF-I effects on cardiovascular system. Growth hormone replacement therapy for GHD in adults is too new to allow analysis of the ultimate question; that is, if rhGH can significantly ameliorate the increased cardiovascular mortality seen in adults with GHD. The interim markers are encouraging; however, most of the work has examined adults.<sup>5</sup> Lanes and colleagues alert us that potentially detrimental cardiovascular changes can be seen in patients with GHD as early as adolescence. Thus, cardiovascular health joins body composition issues (muscle mass and bone mineralization) as factors to consider in optimizing GH treatment during the transition period, the time between cessation of linear growth and attainment of full adult body maturity.<sup>6</sup>

Adda Grimberg, MD

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## GROWTH AND MINERALS: ZINC

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### INTRODUCTION

Zinc (Zn) is well known to be essential for somatic growth of children. Zinc has a close relationship with the endocrine system; it sustains normal growth, secondary sex characteristics, reproductive function and thyroid function. Therefore, Zn deficiency causes not only growth retardation, but also delayed sexual maturation, hypogonadism, and thyroid dysfunction. In this paper, the effects of Zn on childhood growth are presented.

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### From The Editor's Desk

Those of you who have followed this column may be aware of the trials and tribulations of the recent past, as *GGH* faced an uncertain future. But, we are back on track and are delighted to advise you that *GGH* will forge ahead.

This issue brings to an end the era of the long-term sponsorship of *GGH* by Genentech, Inc. They generously supported this educational vehicle since its inception in 1985. On behalf of our readers and Editorial Board I extend our thanks for what they did for the journal. Their support allowed *GGH* to become established and develop into a highly sought-after journal. *Growth, Genetics & Hormones* is read by most pediatric endocrinologists worldwide and other specialists interested in the field of growth.

I am happy to report that we will enjoy an unrestricted educational grant from our new sponsor, INSMED (Glen Allen, Virginia). Thanks to them, we will continue publishing *GGH* as we have done for two decades and you will continue to receive *GGH* on a complementary basis.

While searching for the means to continue *GGH*, I was very motivated by countless colleagues who wrote of the high value they placed on *GGH*; many were willing to pay for a subscription to the journal if needed. I thank all of you who encouraged me, and in this way helped with the task of eliciting funding to serve the educational goals of our colleagues.

The Editorial Board has pledged their time and effort to review the latest advances in the field and grace us with their insightful comments. I am looking forward to a new era of *GGH* and to continue bringing you the most updated reviews and lead articles of interest to the readership. We will continue to enhance the impact of *GGH* and strive to ensure that readers continue to enjoy and treasure it.

Please keep me posted of your needs and recommendations for continuous enhancements. There are multiple journals and other means to stay informed, but none like this journal. Join me in extending your thanks and appreciation to our past sponsor and a heartfelt welcome and thanks to our new sponsor, INSMED, for making this possible.

Respectfully,  
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## THE ROLE OF ZN ON THE HOMEOSTATIC MECHANISMS THAT AFFECT GROWTH AND GROWTH HORMONE

Zinc ion ( $\text{Zn}^{2+}$ ) is present in high concentrations in the somatotrophs in the anterior pituitary of rats, chiefly localized in the growth hormone (GH) secretory granules, and to a smaller extent in the Golgi apparatus. Particle induced X-ray emission (PIXE) measurements reveal that the content of Zn in the anterior pituitary is significantly different between male and female rats ( $100.5 \pm 7.0$  vs  $74.2 \pm 3.6$  [SD] ng/mg dry weight,<sup>1</sup> respectively). On the other hand, in human subjects, the anterior pituitary of women contains more Zn than that of men, but the concentration of Zn in young males is higher than that of young females.<sup>2</sup> However, the reason for the sex difference of Zn content of the pituitary gland is not clear.

Growth hormone is synthesized and secreted into storage granules before its release from the anterior pituitary. Zinc induces GH dimerization; two Zn ions associate per dimer of GH in a cooperative fashion. The  $\text{Zn}^{2+}$ -GH dimer is more stable than monomeric GH and the formation of the dimeric complex is considered to be important for storage of GH in secretory granules.<sup>3</sup> However, the function of Zn in the release of GH from the somatotrophs is not known.

The mechanism by which Zn deficiency causes growth disturbance is considered controversial. Zinc is required for the activity of more than 200 enzymes (Zn metalloenzymes) in which Zn is located at the active site, including DNA polymerase, RNA polymerase, and thymidine kinase. In general, Zn serves catalytic, co-catalytic, and/or structural functions in metalloenzymes containing this ion. Because these enzymes are important for nucleic acid and protein synthesis and cell division, Zn is considered to be essential for growth. Furthermore, several hundred Zn-containing nucleoproteins are probably involved in the gene expression of various proteins.<sup>4</sup> The molecular mechanisms by which Zn controls the expression of the insulin-like growth factor (IGF)-I and the growth hormone receptor/growth hormone binding protein (GHR/GHBP) genes remain unsettled.<sup>5</sup>

Zn seems to play a role in the intracellular transduction pathways of several hormones and might activate protein kinase C which could play a role in the transduction of the GH signal.<sup>6</sup> Zn is an essential component of the "Zn-finger" structures which function as the DNA-binding domains of transcription factors. Zinc-finger is a structure in which an atom of Zn is tetrahedrally coordinated to spatially conserved cysteines and histidines; the Zn atom is absolutely required for binding to DNA.<sup>7</sup> The presence of Zn in these proteins is essential for site-specific binding to DNA and gene expression.

Zn serves as a strut that stabilizes folding of the domain into a finger loop, which is then capable of site-specific binding to double-stranded DNA.

The Zn-finger loop proteins provide one of the fundamental mechanisms for regulating gene expression of many proteins. It is estimated that there may be approximately 200 to 300 Zn-finger nucleoproteins involved in gene expression. Whether or not Zn deficiency affects these nucleoproteins and gene expression remains to be demonstrated.<sup>4</sup> Nuclear receptors of several hormones—including steroid hormones and thyroid hormones—contain Zn-finger structures. Therefore, Zn deficiency might cause alterations of these hormonal actions through the dysfunction of Zn-finger proteins.

The presence of a large amount of Zn in bone tissue suggests that this ion also plays an important role in the development of the skeletal system.<sup>8</sup> Zinc has a stimulatory effect on bone formation and mineralization,<sup>9</sup> whereas retardation of bone growth is a common finding in various conditions associated with Zn deficiency. Zn is required for the action of alkaline phosphatase (ALP) activity, this enzyme is mainly produced by osteoblasts whose major function is to provide calcium deposition in bone diaphysis. Zinc increases the half-life of ALP activity in human osteoblast-like cells.<sup>10</sup>

The administration of both Zn or vitamin  $\text{D}_3$  produced a significant increase in bone ALP activity and DNA content, and the effect of vitamin  $\text{D}_3$  was synergistically enhanced by the simultaneous treatment with Zn.<sup>11</sup> The receptors for 1,25-dihydroxyvitamin  $\text{D}_3$  were shown to have two Zn-finger structures at the site of interaction with DNA.<sup>12</sup> One possible function of Zn is to potentiate the interaction of the 1,25-dihydroxyvitamin  $\text{D}_3$ -receptor complex with DNA.

Zinc directly activates aminoacyl-tRNA synthetase in osteoblastic cells, and it stimulates cellular protein synthesis. Moreover, Zn inhibits osteoclastic bone resorption by suppressing osteoclast-like cell formation from marrow cells. Zinc may act on the process of bone-resorbing factors induced by protein kinase C activation; these are involved in  $\text{Ca}^{2+}$  signaling in osteoclastic cells.<sup>9</sup>

## OPTIMAL AND SUBOPTIMAL ZN NUTRITURE

It has been estimated that the body of the infant newborn contains approximately 60 mg of Zn based on a concentration of 20  $\mu\text{g/g}$  of tissue.<sup>13</sup> During growth and maturation, Zn concentration of the human body increases to approximately 30  $\mu\text{g/g}$ . The adult total body Zn content ranges from about 1.5 g in women to 2.5 g in men.<sup>14</sup> Thus Zn nutrient intake is essential and is particularly important in rapidly growing children, adolescents, as well as pregnant and lactating women.

RDA of Zn in the United States<sup>15</sup>

| Age   | Zn mg/day |
|---|-----------|
| normal infants from birth to 12 months of age | 5         |
| children 1 to 10 years of age                 | 10        |
| males older than 11 years of age              | 15        |
| females older than 11 years of age            | 12        |
| pregnant women                                | 15        |
| lactating women                               |           |
| first 6 months after delivery                 | 19        |
| second 6 months after delivery                | 16        |

The recommended dietary allowances (RDA) of Zn in the United States are listed (Table). The RDA is neither the minimal requirement nor necessarily the optimal level of intake. Rather, the RDA is a safe and adequate level, incorporating margins of safety intended to be sufficiently generous to encompass the presumed variability in requirements among individuals, reflecting the state of knowledge concerning a nutrient, its bioavailability, and variations among the population.<sup>15</sup>

Zinc nutriture has been a subject of worldwide concern as a public health problem. The mean and median intakes of Zn reported in 171 studies summarized by the International Atomic Energy Agency ranged from 4.2 to 19 mg/day; the 10<sup>th</sup>, 50<sup>th</sup>, and 90<sup>th</sup> percentiles of intake were 7, 10, and 14 mg/day, respectively.<sup>16</sup> Zinc intake varies with the mode and type of feeding. Zinc intake of breast-fed infants ranged from 1.9 mg/day at 1 month of age to 2.7 mg/day at 6 months, and those of bottle-fed infants were 3.6 and 4.6 mg/day at 1 and 6 months, respectively.<sup>17</sup> However, Zn in human milk is absorbed more efficiently than that in bovine milk. Absorption of Zn was 41 ± 9 % (SD) from human milk, 28 ± 15% from cow's milk, 31 ± 7% from humanized cow's milk formula, 22 ± 11% from cereal-cow's milk formula, and 14 ± 4% from soy formula.<sup>18</sup>

Total dietary Zn intake is greatly influenced by food choices. Animal products provide abundant amount of Zn and cereals supply the primary plant source. However Zn intake is correlated with protein intake and is markedly influenced by the protein source. Diets consisting primarily of eggs, milk, poultry, and fish have lower Zn:protein ratios than those composed of shellfish, beef, and other red meats. Similar variations occur in vegetarian diets. Diets with rich Zn:protein ratios are provided by liberal quantities of legumes, whole grains, nuts, and cheese, whereas those with low ratios are contained primarily fruits and vegetables.<sup>19</sup>

Zinc absorption is a function of the solubility of Zn compounds at the absorption site and the body status or need. Zinc bioavailability is defined as the fraction of Zn intake that is retained and used for normal physiologic

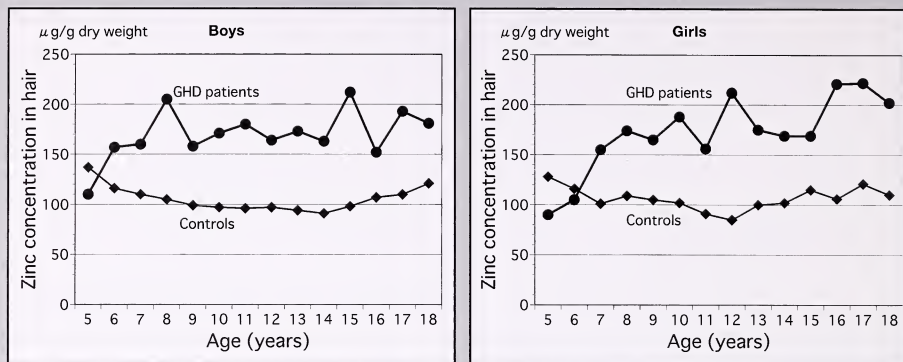
functions. Meats, liver, eggs, and seafood are considered good bioavailable sources of Zn because of the relative absence of compounds that inhibit its absorption, as well as the presence of certain amino acids that improve Zn solubility.<sup>19</sup> For example, the absolute amount of Zn absorbed was about 80% higher when a high meat diet (280 g meat/day) was consumed than with a low meat diet (42 g meat/day).<sup>20</sup> On the other hand, whole-grain cereal products and plant proteins, such as soy protein, contain Zn in a less available form. The phytic acid content of plant foods accounts for, at least in part, to the lower availability of Zn from these foods. Dietary fiber is considered to have little or no effect on Zn availability.<sup>19</sup>

## EFFECTS OF ZN DEFICIENCY AND MARGINAL ZN DEFICIENCY ON GROWTH AND GROWTH HORMONE

It is well known that Zn deficiency causes growth retardation in children and adolescents. Patients with growth retardation caused by Zn deficiency were first described by Prasad et al<sup>21</sup> in 1963. These patients presented with short stature and hypogonadism; their diets were lacking in protein and were rich in phytate and fiber. They were shown to have Zn deficiency by decreased Zn concentrations in plasma, erythrocytes, and hair. Furthermore, <sup>65</sup>Zn studies revealed that plasma Zn turnover was greater, the 24-hour exchangeable Zn pool was smaller, and the excretion of <sup>65</sup>Zn in stool and urine was less in the growth-retarded subjects than in the controls.<sup>21</sup> The growth velocity was increased and was greater in those who received supplemental Zn than those receiving only an adequate animal protein diet.<sup>4</sup> Since then, many cases of marginal or moderate growth impairment in children with Zn deficiency as a consequence of an inadequate Zn nutriture have been reported from various regions of the world.<sup>22,23</sup>

Zinc deficiency is also known to affect GH metabolism and the concentration of GH also influences or is associated with changes in the concentrations of Zn in blood, urine, and other tissues.<sup>8</sup> In patients with GH deficiency (GHD) the mean plasma Zn concentration was within normal limits before treatment, but was significantly reduced after 4 to 12 months of GH administration. The urinary excretion of Zn was significantly higher than that of controls before treatment and was decreased after GH therapy.<sup>24</sup> The average Zn concentration in hair of GHD patients given GH therapy was about 1.7 times higher than that of the controls (Figure), and the hair Zn concentrations of newly diagnosed GHD patients significantly increased after GH administration.<sup>25</sup>

On the other hand, in patients with acromegaly there was a negative correlation between plasma Zn and serum GH levels, and a positive correlation between urinary Zn excretion and serum GH levels. After hypophysectomy, Zn was observed to increase in plasma and decrease in urine.<sup>24</sup> These findings may reflect a negative Zn balance

**Mean zinc concentrations in hair of GHD patients and the controls 5-18 years of age.<sup>25</sup>**

and chronic mild Zn deficiency in some GHD patients on long-term GH therapy and in untreated patients with acromegaly. The data suggest that an increased Zn requirement exists during catch-up growth or overgrowth accelerated by GH, and that GH might promote intestinal absorption of Zn and/or promote Zn uptake of hair root cells. It may also be speculated that Zn may be a limiting factor in growth-regulating mechanisms by modulating both GH release and GH action.<sup>8</sup>

Zinc deficiency may adversely affect GH production and/or secretion.<sup>26</sup> IGF-I synthesis may also be impaired by Zn deficiency since exogenous GH fails to raise IGF-I levels in Zn-deficient rats.<sup>27</sup> Low IGF-I levels in Zn-deprived rats were closely associated with a decreased hepatic IGF-I gene expression and with a diminution of liver GH receptors and circulating GHBP. The decreased hepatic GH receptors and/or GHBP concentrations might be responsible for the decline of circulating IGF-I in Zn-deficient animals.<sup>28</sup>

The incorporation of labeled thymidine into DNA is also impaired by Zn deficiency. This effect has been detected within a few days of the institution of a Zn-deficient diet in experimental animals, suggesting that DNA biosynthesis<sup>4</sup> is compromised due to an adverse effect of Zn restriction on the activity of deoxythymidine kinase.<sup>29</sup>

There have been a few reports concerning the relationship between Zn deficiency and GH secretory insufficiency in humans. We described a 13-year-old Japanese patient with short stature who had partial GH deficiency due to chronic mild Zn deficiency.<sup>26</sup> This patient's diet was low in animal protein and consisted primarily of rice and vegetables (he disliked meats, fish, eggs, and dairy products) and plasma Zn level and GH responses to pharmacological stimulation tests were low. After 3 months of oral Zn supplementation, the patient's growth velocity improved

without GH replacement therapy, and the plasma Zn levels and GH responses to stimulation tests normalized.

On the other hand, Siklar et al<sup>30</sup> investigated the Zn nutriture of prepubertal GHD patients given GH treatment in Turkey. They measured erythrocyte Zn levels and reported that about one-half of them were Zn deficient. Growth velocity during GH treatment was higher in children with normal erythrocyte Zn levels than those with low erythrocyte Zn concentrations. They also showed that oral Zn supplementation improved the growth velocity of GHD children with Zn deficiency, but not of those without Zn deficiency. These data indicate that Zn status should be evaluated before GH provocative tests and during GH treatment.

## MATERNAL ZN NUTRITURE AND PREGNANCY OUTCOME

It has been well known that Zn deficiency during pregnancy may be associated with increased maternal morbidity, prolonged gestation, inefficient labor, atonic uterine bleeding, and increased risks to the fetus.<sup>4</sup> Maternal Zn deficiency may also cause intrauterine growth retardation (IUGR) and low-birth-weight (LBW) infants.<sup>31-33</sup> The Zn levels of polymorphonuclear and mononuclear white cells in postpartum women at 24 to 48 hours after delivery were lower in women giving birth to small-for-gestational-age (SGA) infants than those giving birth to appropriate-for-gestational-age (AGA) infants, irrespective of smoking habits.<sup>31</sup> A significant correlation existed between maternal plasma Zn concentrations measured at mid-pregnancy and an infant's birth weight. The maternal weight at 3 months of gestation and plasma Zn concentrations in the second trimester formed the best predictor model of birth weight.<sup>32</sup> It was also reported that the prevalence of LBW infants was significantly higher (8 times) among women with serum Zn concentrations in the lowest quartile in early pregnancy, independent of other



risk factors.<sup>33</sup> However, there have been other studies that showed no association between maternal Zn nutriture and pregnancy outcome.<sup>34,35</sup> It is also known that plasma Zn concentrations are not reliable indicators of the Zn status and are not useful in estimating marginal Zn deficits.<sup>36</sup>

The effects of Zn supplementation on pregnancy outcome are not clear.<sup>37-40</sup> The incidence of LBW is very high in many developing countries where Zn deficiencies are prevalent. For example, an estimated 40% to 50% of all live births in Bangladesh were classified as LBW, 70% to 80% of which were the result of IUGR.<sup>40</sup> Effective interventions aimed at preventing LBW are particularly important to reduce childhood malnutrition and improve infant health. In developing countries maternal Zn supplementation has been suggested as one possible nutritional intervention during pregnancy to improve pregnancy outcomes.<sup>41</sup> Studies of Zn supplementation during pregnancy have been positive and resulted in reduced incidence of IUGR.<sup>38,39</sup> In a randomized, double-blind, placebo-controlled trial in 580 African-American women, Zn supplementation (25 mg/day) during pregnancy was associated with an increase in birth weight (+126 g) as compared with infants of women who received placebo.<sup>39</sup>

However, the results of Zn-supplementation trials in pregnant women aimed to improve pregnancy outcome are not consistent.<sup>40</sup> A double-blind, prospective study carried out in the United Kingdom found no differences in gestational age, birth weight, neonatal abnormalities, and complications of labor and delivery between mothers given a Zn supplement and those given a placebo.<sup>37</sup> It is now speculated that Zn supplementation during pregnancy might be beneficial only in populations that are Zn deficient and at high risk for poor fetal growth.<sup>40</sup>

## PREVALENCE OF ZN DEFICITS IN HEALTH AND DISEASE STATES

The population groups at risk of Zn deficiency are those who consume low Zn-quality diets. Such diets are rich in phytate and usually contain other ligands that prevent the intestinal absorption of Zn.<sup>42</sup> On a global scale, protein energy malnutrition is the most common cause of poor growth and short stature, and it appears that Zn deficiency is also prevalent in such populations.<sup>4</sup> Stunted growth linked to Zn deficiency was found throughout childhood, and depending on the country, 5% to 30% of children were suffering from moderate Zn deficiency, resulting in for small-for-age height.<sup>43</sup> However, in recent experimental studies in rats, suboptimal nutrition restricted growth primarily when energy was not ingested in sufficient quantities, whereas suboptimal intake of Zn with an appropriate intake of calories did not stunt growth.<sup>44</sup>

Several studies indicated that marginal Zn deficiency might also be prevalent in infants and children in developed countries. Michaelsen et al<sup>45</sup> investigated Zn

intake and status in healthy term infants from birth to 12 months of age in Denmark, and found suboptimal Zn status in many subjects during late infancy. They also reported that serum Zn levels at 9 months of age were positively correlated with growth velocity during the period from 6 to 9 months of age. We studied Zn status in short Japanese children with normal GH secretion using the body Zn clearance test to detect marginal Zn deficiency, and found that about 60% of the short children had such a problem. The reason for the high incidence of marginal Zn deficiency in Japanese short children may be due to the recent dietary preference for precooked meals, snacks and convenience foods.<sup>46</sup>

Disorders of the gastrointestinal tract are frequently complicated with Zn deficiency. Breakdown of the integrity of the gastrointestinal tract reduces the normal absorption of dietary Zn and disrupts the enteropancreatic circulation of the ion.<sup>19</sup> There is evidence that patients with Crohn's disease, sprue, or short bowel syndrome may develop Zn deficiency. Several investigators have reported low serum Zn concentrations present in 30% to 70% of patients with Crohn's disease,<sup>47-49</sup> and it is not unusual to find depressed urinary Zn excretion.<sup>50</sup> It has been reported that about 20% to 30% of children with Crohn's disease have severe linear growth retardation, mainly due to malabsorption and malnutrition.<sup>51</sup> On the other hand, it has been reported that about 30% to 70% of children with Crohn's disease have reduced serum Zn levels. Brignola et al<sup>52</sup> evaluated the effect of oral Zn supplementation on serum Zn levels in patients with Crohn's disease with hypozincemia and concluded that administration of very high doses of Zn (200 mg/day ZnSO<sub>4</sub>) for 3 months increased serum Zn levels, but that moderate doses (60 mg/day) did not. We studied Zn status in 30 patients with chronic inflammatory bowel disease (CIBD) and found that 11 subjects had hypozincemia. In addition, those with moderate and severe clinical disease activity had a decreased rise of serum Zn concentration after oral Zn administration. Urinary excretion of Zn after oral load was also remarkably low in all CIBD patients. The abnormalities of Zn metabolism were more frequent among the CIBD patients with growth abnormalities, although they were also found in normal height patients.<sup>51</sup>

## GROWTH ENHANCEMENT CAPABILITIES OF ZN IN "HEALTHY" INFANTS AND CHILDREN

There have been several reports indicating positive effects of oral Zn supplementation on growth of SGA and/or LBW infants fed artificial formulas.<sup>45,53,54</sup> In a longitudinal, double-blind, randomized clinical trial in preterm infants in Spain, those fed standard milk formula supplemented with Zn for 6 months had greater linear growth velocity corrected for postnatal age than those without Zn supplementation. Zinc supplementation significantly increased serum and erythrocyte Zn levels and serum ALP activity,<sup>53</sup> but no differences were induced in serum IGF-I and IGFBP-3.



IGF-I and IGFBP-3 are of course essential for linear growth in children from childhood to adolescence, but might not be as important for neonates and young infants. There was also a positive effect of Zn supplementation on linear growth in SGA infants fed artificial formula, but not in those fed exclusively breast-milk.<sup>54</sup> This may be attributed to the lower bioavailability of Zn contained in formula compared to the Zn in human milk, placing formula fed infants at a higher risk of Zn deficiency. Therefore, the effect of Zn supplementation on artificially fed infants would be more evident.<sup>53</sup> Mild Zn deficiency in SGA and LBW infants, especially those fed artificial formula, could be a public health problem even in developed countries.

There are several studies that assessed the effects of Zn supplementation on children's growth.<sup>36,46,55,56</sup> Nakamura et al<sup>56</sup> conducted an age-matched control study and showed that oral Zn supplementation was effective in improving the growth rate of short children with marginal Zn deficiency. They also reported that oral Zn supplementation induced increases of serum IGF-I, osteocalcin, and ALP activity.

The effects of oral Zn supplementation were evaluated in short Japanese children with normal GH secretion assessed for Zn status with a body Zn clearance test.<sup>46</sup> The results indicated that oral Zn supplementation was effective on height gain in short boys with marginal Zn deficiency, but not in girls. There was a significant correlation between the body Zn clearance values and the increase in the growth velocity after oral Zn supplementation in boys, indicating that the degree of Zn deficiency was important. Although the reasons for the difference in the effects of oral Zn supplementation on growth velocity between both sexes are not clear, other studies showed similar differences.<sup>55</sup> oral Zn supplementation improved growth velocity in boys with idiopathic short stature, but had no effect in girls. On the other hand, a relatively large scale randomized, double-blind, placebo-controlled study showed no positive effect of Zn supplementation on height gain of preschool children.<sup>56</sup>

The results of many other studies are also inconsistent. Brown et al<sup>57</sup> completed a meta-analysis of randomized controlled intervention trials to assess the effect of Zn supplementation on the physical growth of prepubertal children. After evaluating 33 reports, they found that 26 studies showed positive effects of Zn supplementation on children's linear growth and 7 studies did not. They concluded that interventions to improve children's Zn nutrition should be considered in populations at risk of Zn deficiency, especially where there are high rates of underweight or stunted growth.

#### **ASSESSMENT OF ZN DEFICIENCY AND MARGINAL ZN DEFICIENCY**

Unfortunately there is no simple, accurate way, to determine the Zn status of individuals, and this is the

major factor that handicaps the interpretation of the data of most studies and of individual patients. There have been various kinds of laboratory biomarkers proposed to detect definite and/or marginal Zn deficiency. However, these measurements do not accurately reflect nutritionally available Zn pool sizes.<sup>19</sup>

Although plasma/serum Zn concentration has been widely used to assess the nutritional status, Zn levels may respond to metabolic conditions unrelated to Zn status and are insensitive to changes in dietary Zn.<sup>58</sup> The insensitivity of plasma Zn to reductions in dietary Zn intake reflects the tremendous capacity of the organism to conserve tissue Zn by reductions in Zn excretion and/or reductions in the rate of growth. A reduction in plasma Zn concentration does not occur until the capacity to reestablish homeostasis by reducing excretion and/or growth has been exceeded. Plasma Zn represents about 2% of a labile, or nutritionally available, total-body Zn pool that exchanges with isotopic Zn tracers in 24 hours.<sup>58</sup> Because plasma Zn is the source of this ion for all tissues, plasma concentrations are maintained longer than other components of the body Zn pool.<sup>19</sup>

Plasma Zn kinetics or turnover tends to increase with Zn depletion. Thus, the rate of Zn turnover in the plasma compartment or in the total labile pool of the body might indicate the Zn status of an individual. Miller et al<sup>59</sup> estimated the size of the combined pools of Zn with which plasma Zn exchanged using isotopic Zn. The exchangeable Zn pool size was determined from the amount of isotope introduced into the plasma and the coefficient of the simple exponential decay function fitting enrichment data between day 3 and 9 after isotope administration. They reported that the exchangeable Zn pool size correlated with habitual dietary Zn intake. This excellent assay to detect marginal Zn deficiency may be of little practical use in the clinical situation because of the necessity for isotope administration.

Nakamura et al<sup>56</sup> recommended a body Zn clearance test which needs no isotope. This is a kind of a Zn kinetic study; serum Zn levels are measured just before and at 30, 60, 90, 120 minutes after intravenous administration of Zn, the serum Zn decay curve is obtained, and the biological half-life and elimination constant of serum Zn are calculated. The resultant "body Zn clearance" value becomes a sensitive indicator of marginal Zn deficiency.

Other static measurements of Zn status hold little promise. Erythrocyte Zn is mildly affected by Zn deficiency and may not be a sensitive index. The response of leukocyte Zn to changes in Zn status is not consistent among laboratories, and the assay is laborious.<sup>19</sup> Hair Zn levels may be depressed in mild Zn deficiency. However, it is affected by the rate of hair growth and shows seasonal variations.<sup>60</sup> Urinary excretion rates of Zn are diminished

in severe deficiency states, but this measurement is not sensitive and is confounded by many clinical disorders that increase urinary Zn losses.<sup>19</sup>

## SUMMARY AND SPECULATION

Zinc, although present in minute quantities in humans, is an essential nutrient and plays an important role as a component of many enzyme systems regulating cell growth, including DNA and protein synthesis, energy metabolism, regulation of gene transcription, hormone levels, and growth factor metabolism.

Nutritional Zn deficiency is still a worldwide public health problem. In developing countries, protein energy malnutrition is the most common cause of poor growth and short stature of children, and Zn deficiency is prevalent in such populations. Zn deficiency in pregnant women is also a serious problem, since it might cause IUGR and LBW infants. Since the incidence of LBW is very high in many developing countries, Zn supplementation in pregnant women should be considered extensively in such regions.

Marginal to moderate Zn deficiency is not uncommon even in developed countries. Zn deficiency should be considered as one of etiologic factors in some children with unexplained short stature. Oral Zn supplementation may be considered as the growth-promoting therapy for children with short stature once marginal Zn deficiency is established. However, the interrelationships among Zn, growth, gonadal function, and GH-IGF-I axis appear to be complex and deserve further investigation.

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## ABSTRACTS FROM THE LITERATURE

### Summary Highlights: ESPE and LWPES Joint Meeting

Summarized here are some highlights of the joint meeting of ESPE and LWPES in Lyon, France, September, 2005.

#### CONGENITAL HYPERINSULINISM

The molecular basis of congenital hyperinsulinism in infancy (CHI) was reviewed by de Lonlay et al (S7-30) from Paris. CHI is characterized by severe dysregulation of insulin secretion that causes profound hypoglycemia. It is associated with either focal or diffuse pathology of the endocrine pancreas. These pancreatic anatomical forms of pathology require major differences in the treatment of CHI.

Mutations in genes encoding the beta-cell sulfonylurea receptor (SUR1) and the inward-rectifying potassium-channel (Kir6.2) have been identified in CHI. These genes encode subunits of KATP channels which couple glucose metabolism to insulin release. Hypoglycemia is related to homozygosity of a paternally inherited mutation of one of these genes that results in diffuse hyperplastic pancreatic pathology. The CHI is more complex in the *diffuse* form which present as a heterogeneous disorder involving several genes and various inheritances. Forms occurring in the CHI, resistant to medical treatment, are mostly due to mutations of the KAPT channel with a recessive inheritance and often require total pancreatectomy. Forms occurring after the first month of life are mostly sensitive to medical treatment and may be related to *de novo* or dominantly inherited mutations. Nonetheless about half of the patients in the later group do not carry these mutations.

*Focal lesions* in CHI represent areas of adenomatosis related to the loss of the maternal allele in the 11p15 region. It is mostly sporadic. This somatic molecular event disrupts the balanced expression of imprinted genes involved in the control of cell growth and lead to pancreatic tumor development. This clinical form of CHI is potentially curable by limited pancreatic resection. Until recently rather invasive techniques, with direct pancreatic catheterization using transhepatic portal venous sampling, and arterial calcium-stimulated venous sampling were used to aid the surgeon with the localization and the extent of the pancreatectomy. In localizing pancreatic focal lesions Otonkoski (S7-31) from Helsinki successfully used the PET technique to detect neuroendocrine tumors using (18F)-DOPA uptake by the hyperplastic islet cells. Very promising results were also obtained by several other centers, including Blankenstein et al (P3-1260) from Berlin and Stanley et al (S7-32 and P3-1262) from Philadelphia. All patients with positive PET technique localization of a focal lesion had the

tumor removed while preserving the healthy portion of the pancreas and the hypoglycemia ceased. Stanley et al described their experience in 217 cases with neonatal hypoglycemia over 6 years. They confirmed the accuracy of the PET scan technique performed before surgery and recommended that candidates for surgery be referred to specialized centers.

#### GROWTH HORMONE TREATMENT

Simon et al (OR3-75) from Paris reported on the early recombinant human growth hormone (rhGH) treatment started one year after initiation of glucocorticoid therapy of children with juvenile rheumatoid arthritis. This randomized-controlled study was carried out over 3 years in prepubertal children receiving prednisone ( $0.6 \pm 0.4$  mg/kg/day) and rhGH (0.46 mg/kg/week). Growth was maintained at a normal rate for chronological age and lean body mass was improved. However, there was no significant effect on fat mass or bone mineralization. Although increased insulin resistance was expected with rhGH therapy, glucose intolerance was mild and transient, occurring only in pubertal patients. It was concluded that rhGH treatment was safe and prevented growth retardation in these patients. However, higher steroid doses may limit the beneficial effects of rhGH. Thus rhGH treatment may prove to be more significant when given early as prevention of growth retardation. Further studies that follow patients until final height is achieved and focus on muscle mass and long-term function are in progress.

Mauras et al (P1-149) from Jacksonville reported on the limited efficacy of rhGH treatment during the so-called transition period in a well defined cohort: children with GH deficiency (GHD). Subjects had been treated early and reached normal final height, metabolic control, muscle strength, and bone mineral density (BMD). The authors delved with the question of the timing of rhGH treatment: namely the continuation or the temporary discontinuation throughout late adolescence to adulthood, until the persistence of GHD could be re evaluated. A phase III double-blind, randomized 2-year trial was performed. Subjects were classified in 3 groups: persistently GHD randomized to either continued rhGH treatment or to placebo injections, and GH sufficient on retesting considered controls and given no treatment. After 2 years metabolic measures, cardiac function, BMD, and quality of life were comparable in all 3 groups. It was concluded that GHD adolescents in good metabolic control at time of epiphyseal fusion may safely discontinue rhGH therapy for at least 2 years.



If such an attitude is chosen, a careful follow-up is needed to determine if and when rhGH is warranted. Such an approach may help manage the so-called transition period before adulthood in previously well treated GHD patients. (Refer to abstract on page 14.)

### IGF-I

Camacho-Hubner, Savage, and Underwood (S10-40) from London and Chapel Hill updated their experience with rh insulin-like growth factor (IGF)-I alone or combined with rhIGF binding protein (BP)-3 in patients with GH insensitivity (GHI) due to GH receptor defects, to growth attenuating antibodies to GH, or to extreme insulin resistance due to genetic defects. The doses of rhIGF-I ranged from 60 to 120 mg/kg/day. Height improved by 1.2 to 1.5 SD over 2 years of therapy; the best responses occurred when the treatment was started at a young age. However long-term responses varied among treated children and were not as well sustained as the responses elicited with rhGH therapy in GHD children. Adverse events were: coarsening of facial features, hypoglycemia in younger children, lymphoid hyperplasia, and pseudotumor cerebri. The combined rhIGF-I and IGFBP-3 (0.5 to 2 mg/kg/day) given as a single injection, resulted in a prolonged half-life of IGF-I, allowing once daily injection with appropriate tolerance and good results. The only treatment available for patients with severe genetic insulin resistance and genetic IGF defects is rhIGF-I. A multicenter open labelled phase III study is in progress in children with GHI.

### GENETICS OF GH RECEPTOR

A recent issue of interest focuses on genetic factors possibly influencing the growth response to rhGH therapy. A provocative, well documented study of GH

gene polymorphism and variations in growth in GH treated children by Bougneres et al (S3-20) from Paris investigated the potential role of the 2 forms of the GH receptor (GHR), the full length (fl) or the exon-3 deleted (d3) receptor, on the response to rhGH treatment. In transfection experiments a 30% increase in the transduction in GH signalling had been demonstrated in d3 homo or heterodimers of the GHR. This suggested that there could be a potential genetic factor influencing the response to rhGH. An advantage for the d3-allele carriers was shown in children with small for gestational age or idiopathic short stature (ISS) who showed 1.7 to 2 times greater growth acceleration as compared with those who did not have this GHR. These results were confirmed in a cohort with complete GHD treated for 3 years by Thomas-Teinturier (P1-152) from Paris.

Two other studies were at variance with these data. Blum et al (OR3-71) from Lilly's Genesis Program studied a large cohort of GHD children treated with rhGH ( $0.2 \pm 0.06$  mg/kg/week). This group evaluated the first year of rhGH treatment response according to the exon-3 genotype and reported a small but consistent (although not significant) increase in growth parameters in the d3 groups. Ito et al (P1-150) from Japan evaluated Japanese children with partial GHD and could not demonstrate a significantly increased growth during the first rhGH treatment year in patients with 3d allele.

However, the initial finding by Bougneres remains an important and provocative paper that will generate future studies to better understand the large individual differences in growth response to rhGH. The role of exon-3 genotype must be confirmed with strong methodological approaches selecting groups according to etiology of short stature, rhGH dosage, duration of treatment, and ethnicity before it can be applied as a genomic tool for therapy.

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**CONGENITAL ADRENAL HYPERPLASIA**

The presence of testicular adrenal rest tumors in congenital adrenal hyperplasia (CAH) patients is known to cause Leydig cell failure and impaired spermatogenesis. These rest tumors are often unresponsive to intensified corticoid therapy. Bachelot et al (OR 14-142) from Paris reported treatment of 3 adult patients with mitotane, an adrenolytic agent, for 2 to 3 years and obtained a reduction in the testicular rest tumor volume with an improved sperm count. This may represent a potent tool to improve fertility of some poorly controlled CAH patients.

**OVARIAN FAILURE**

The causes of premature ovarian failure (POF) are rarely identified in adults. However Conway (S9-38)

from London approached this issue with data related to optimization of the substitutive estrogenic therapy in adolescents. Age at onset of this treatment was critical for adult carotid intima media thickness, predictive of vascular complications, and was inversely correlated with the estrogen dosage. Appropriate uterine thickness for future pregnancy was obtained if treatment was not delayed. Finally, better results in assisted conception with donated oocytes were also obtained in women with early ovarian failure who received treatment before the age of 14. These data may also be relevant to patients with gonadal dysgenesis and those with ovarian failure secondary to cancer therapy in pediatric practice.

Raphaël Rappaport, MD

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**Measured versus Reported Parental Height**

Cizmecioglu and colleagues interviewed 200 parents (100 males, 100 females), mean age 37.8 years and ascertained their reported height. Their actual height was then measured by a single observer using a Harpenden stadiometer. On average, males overestimated their height, while females reported their height relatively accurately. However, there was a wide spread of discrepancies for both sexes. Overall there was a small positive correlation between age and the difference between reported and measured height. Of interest, subjects who had been measured previously were less accurate at reporting their height than those who guessed their height. The mean difference in reported versus measured height was 1.09 cm for men (range -3.3 to 5.2) and -0.09 for females (range -6.2 to 6.4). The authors pointed out that there was considerable individual variation among both sexes in over or under estimating their exact height and state that their data reinforces the need for accurate height measurement and recording of both mother and father at the earliest possible opportunity.

**Editor's Comment:** *This is a very short paper which represents some interesting and very important information. It is a relatively common practice in pediatric endocrine clinics to calculate the mid-parental height as a target height for the child being evaluated. Clearly it is important that this target height is calculated correctly. It is not uncommon for parents to state that they are unaware of their precise height or to report their height with obvious discrepancy from observation. In addition it is not uncommon for children to come the clinic with either one or more non-biological parents, or for information regarding the "no longer present" parent's height to be estimated with little precision. The recommendations of the authors of this study should be taken seriously: parental height should be measured at the earliest possible time and become part of the child's permanent medical record. Such information could be exceedingly helpful in guiding the evaluation and treatment of children with growth failure at a later date. At the very least, pediatricians and pediatric endocrinologists should be encouraged to actually measure parents who accompany their child for evaluation of growth failure.*

Cizmecioglu F, Doherty A, Paterson WF, Young D, Donaldson MD. Measured versus reported parental height. Arch Dis Child. 2005;90:941-942.

William L. Clarke, MD

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**Apnea in Prader-Willi Syndrome Patients on Growth Hormone Therapy**

Case reports of sudden fatalities, primarily respiratory, in children with Prader-Willi Syndrome (PWS) receiving growth hormone (GH) therapy caused alarm and prompted a voluntary label change to include a new warning. Benefits of GH treatment in these patients include improved linear growth, increased muscle mass and amelioration of hypotonia, and decreased total body fat. Sleep-disordered breathing is common in PWS, both obstructive (from pharyngeal narrowing, respiratory muscle hypotonia, and later compounded

by obesity) and central (hypothalamic dysfunction with abnormal arousal and response to hypercarbia which can be further blunted by obesity).

Miller and colleagues performed a prospective study of the respiratory effects of 6 weeks of GH treatment in 25 patients with genetically confirmed PWS. All patients underwent standard overnight polysomnography (PS) at baseline (either GH-naïve or voluntarily withdrawn from GH treatment for 3 months) and after 6 weeks of GH (0.24 mg/kg/wk for children and 0.0006 mg/kg/day for adults,

based on ideal body weight); two of the patients were also retested after 6 months. Subjects ranged in age from 5 months to 39 years. All had sleep-disordered breathing during the baseline PS, with both obstructive and central apneic events. After 6 weeks of treatment, 19 of the patients (76%) had improvement of the apnea/hypoxia index (AHI); the frequency of central events decreased by a median of 1.7 events/hr, while the frequency of obstructive events did not change significantly. However, 6 patients (24%) had worsening of obstructive sleep apnea/hypopnea, related to upper respiratory tract infections (URIs) and tonsillar hypertrophy. Two of these patients had high insulin-like growth factor (IGF)-I levels for bone age (z scores of +1 and +2; the others had IGF-I z scores of 0). After GH dose reduction and normalization of IGF-I level, one patient had an improved AHI on repeat PS while the other had increased AHI and a URI at the time of the repeat study. Body-mass index was not related to PS results.

The authors concluded that PS should be performed in all PWS patients at baseline, after 6 weeks of treatment with GH, and with otorhinolaryngologic evaluation whenever symptoms of sleep apnea or snoring develop. Adenotonsillectomy and titrating GH dose to achieve an IGF-I z score of 0 were also recommended as needed. Finally, they supported the warning of GH manufacturers contraindicating GH use in PWS patients with CRI or lung infections.

Miller J, Silverstein J, Shuster J, Driscoll DJ, Wagner M. Short-term effects of growth hormone on sleep abnormalities in Prader-Willi Syndrome. *J Clin Endocrinol Metab*. 2006;91:413–417.

**First Editor's Comment:** *I applaud the authors for performing a prospective study to directly address the question of GH effects on respiratory function in PWS patients, and I agree with the proposed pathophysiologic mechanisms. However, the finding of sudden death*

*in individuals with hypothalamic dysfunction and the recurrent theme of exacerbation by intercurrent infections make me wonder about central adrenal insufficiency, which was not mentioned. Indeed, a PubMed search of adrenal insufficiency and PWS produced only one paper.<sup>1</sup> In this retrospective series report of 8 children and 2 adults with unexpected death or critical illness, 3 of the children had below-average sized adrenal glands on autopsy; childhood illnesses in general under the age of 2 years were associated with high fever and rapid demise or near-demise. Increased mortality among individuals with GH deficiency (GHD) despite GH treatment has been attributed to under-diagnosed and under-treated central adrenal insufficiency, and recent papers highlighted the increased risk for central adrenal insufficiency even in patients with idiopathic GHD or familial isolated GHD.<sup>2,3</sup> Thus, in addition to the recommendations by Miller et al, I would encourage monitoring of adrenal function in PWS patients.*

Adda Grimberg, MD

**Second Editor's Comment:** *Excellent points made by the authors of the paper and the editorial comment of Dr. Grimberg. I urge caution and continuous monitoring of PWS patients throughout their life, not just after initiating GH therapy, and particularly when ill.*

Fima Lifshitz, MD

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## Suppression of Aging

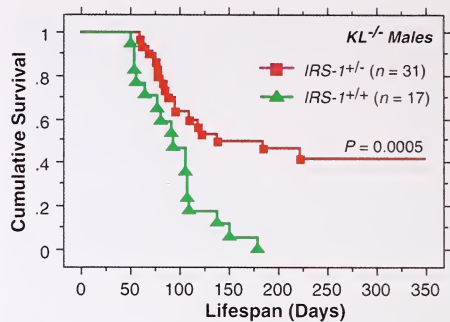
A spontaneous homozygous loss-of-function mutation in *KLOTHO* (KL) gene (OMIM 604824, chromosome 13q12) was initially described in a strain of mice with accelerated aging and premature death.<sup>1</sup> Its human homolog was later identified. *KL* encodes a transmembrane protein expressed in renal distal convoluted tubules and neural choroid plexus. Kurosu et al developed 2 strains of transgenic mice that overexpressed *Kl* under the control of the promoter of human elongation factor 1 $\alpha$ . Both male and female animals overexpressing *Kl* lived 20% to 30% longer than did wild-type (WT) control mice. They did so without restricting caloric intake or impeding somatic growth; however, fecundity was reduced in like-breeding pairs. Mice overexpressing *Kl* were euglycemic, but males had higher serum insulin concentrations than did WT controls, and both genders had attenuated hypoglycemic responses to exogenous insulin and/or

insulin-like growth factor (IGF)-I. The serum concentration of the extracellular domain of Klotho was twice as high in transgenic as in WT mice. Intraperitoneal administration of Klotho protein increased blood glucose concentrations and depressed the hypoglycemic effect of co-injected insulin. *In vitro* in cultured cells, Klotho peptide did not inhibit binding of insulin or IGF-I to their specific receptors, but specifically suppressed autophosphorylation of these receptors and impaired insulin-stimulated glucose uptake. Furthermore, Klotho down-regulated intracellular signaling transmitted through insulin receptor substrate (IRS)-1 and -2 and phosphoinositide 3-kinase p85. In *Kl*<sup>+/+</sup> mice who die prematurely, life could be substantially prolonged and signs of aging halted (ie, arteriosclerosis, renal calcification, testicular atrophy) by decreasing the generation of IRS-1. The authors concluded that Klotho was a secreted protein (ie, a hormone) that extends life.

and suppressed aging by antagonizing the cellular effects of insulin and IGF-I.

Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science*. 2005;309:1829–1833.

**Editor's Comment:** Klotho may be the long sought after elixir from the "fountain of youth." KLOTHO is named after the mythological Greek Fate who spun the "thread of life." By alternative RNA splicing, KL generates 2 transcripts: a 1012 amino acid protein with extracellular, transmembrane, and intracellular domains and a 549 amino acid peptide, the amino terminal sequence of the extracellular domain that is secreted and is the predominant form produced. In man, single-nucleotide polymorphisms in KL have been associated with altered life span and risk for atherosclerosis and osteoporosis.<sup>2</sup> That increased generation of Klotho extended life span without impairing growth emphasizes the distinctive difference between the effects of this gene and that related to caloric deprivation, another experimental mechanism to prolong life. Although both processes act by impeding insulin and IGF-I action, Klotho apparently enhances their production but antagonizes their function, while caloric deprivation depresses their production and impairs growth and fertility. These studies reinforce the concept that decreased secretion of growth hormone, insulin, and IGF-I extends life and suppresses aging,<sup>3</sup> a concept that is the opposite of that voiced by many lay "anti-aging authorities." Although excess Klotho decreased fecundity between like-breeding pairs of mice, the effect of this protein on the fertility of a mouse with a high level of Klotho when mated with a WT animal remains to be explored. Conceptually, there appears to



Rescue of aging-like phenotypes in *KL*<sup>-/-</sup> mice by genetic intervention in insulin and IGF-1 signaling.  
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be a "trade-off" between life span and reproduction. It will be of great interest to measure serum concentrations of Klotho at various stages of life and in various hormonal and metabolic disorders, particularly those involving energy utilization, as well as to determine its physiologic (and potentially therapeutic and anti-aging) effects in humans of all ages.

Allen W. Root, MD

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## Motivations for GH/GnRHa Treatment and Psychosocial Functioning

Visser-van Balen et al reported (in the first paper) on the psychological consequences of combined growth hormone (GH)/gonadotropin-releasing hormone agonist (GnGHa) treatment in a multicenter, randomized-controlled study conducted in early pubertal youths (ages 11 to 13 years; Tanner breast stage 2 or 3 for girls, Tanner genital stage 2 or 3 for boys) with a diagnosis of either idiopathic short stature (ISS; 17 girls, 9 boys) or born small for gestational age (SGA; 8 girls, 4 boys). The authors explained the unusual predominance of girls as reflective of the combination of SS and relatively early puberty is more common in girls than boys. Participants had a height SDS below -2, or between -1 and -2 with a predicted adult height SDS below -2. In the second paper, the authors examined patients' and parents' motivations in choosing to participate in this study.

Adolescents in the treatment group were administered GH (4 IU [1.33mg]/m<sup>2</sup> BSA, SQ, daily) and GnRHa (3.75 mg, IM depot, every 4 weeks). At baseline, 1, 2, and 3 years

after beginning treatment, adolescents and their parents (mostly mothers) in both groups completed questionnaires to assess the psychosocial functioning of the adolescents by completing a standardized assessment evaluating adolescents' health-related development, current height-related stressors, and parental concerns about their child's future behavioral and emotional functioning; perceived current and expected adult height; global intelligence; perceived competence, psychological distress, and personality characteristics.

At baseline, a minority of parents (28%) reported their child experienced teasing or juvenilization by peers; however, a higher proportion (44.5%) anticipated their child would face challenges in the labor market as an adult (39% of boys, 48% of girls) and 39% expected their child to have lower prospects of finding a spouse (77% of boys, 17% of girls, *p*<0.01). Parent reports of behavioral and emotional functioning suggested a statistically significant excess of problems. In contrast, adolescents'



self-reports of emotional distress and self-concept did not systematically differ from normative values. Differences in psychosocial variables at baseline were not detected between the treatment and control groups, ISS and SGA subgroups, or children whose parents reported stature-related psychosocial stressors. With regard to motivation to participate, patients were categorized into 4 subgroups based on the presences of height-related psychosocial stressors, parental worries about their child's current behavior and about future prospects, and patients' self-reported problems in psychosocial functioning.

During treatment, parent reports of current stigmatization and worries over future challenges did not change, and did not differ between the treatment and control groups. The same was not true for perceptions of the child's behavioral and emotional functioning. In contrast, self-perceived scholastic and athletic competence in the treatment group significantly decreased over time (ie, became more negative), while that of the adolescents in the control group increased (moderate effect size). Trait anxiety decreased for adolescents in the control group, but remained at approximately the same level for adolescents in the treatment group. The authors noted that, despite these statistically significant effects, there was considerable overlap of scores between the 2 groups and one apparent outlier in the treatment group.

As noted above, parents perceived an excess of psychological adjustment problems in their children, however this difference was not matched by the children's self-reports. As such, the investigators concluded that it is primarily the parents' perceptions of problems (current or anticipated) that drive the process in search of a medical intervention. The adolescents wanted to gain height, but their underlying motivation remains unclear.

Visser-van Balen H, Geenen R, Moerbeek M, J et al. Psychosocial functioning of adolescents with idiopathic short stature or persistent short stature born small for gestational age during three years of combined growth hormone and gonadotropin-releasing hormone agonist treatment. *Horm Res.* 2005;64:77–87.

Visser-van Balen H, Geenen R, Kamp GA, Huisman J, Wit JM, Sinnema G. Motives for choosing growth-enhancing hormone treatment in adolescents with idiopathic short stature: a questionnaire and structured interview study. *BMC Pediatr.* 2005;5:15.

**Editor's Comment:** *My initial reading of this study left me somewhat confused: why would researchers look for effects of combined GH/GnRHa treatment on psychological outcomes when the long-term benefits of GnRHa on adult height had not yet been realized? In fact, the addition of GnRHa could have slowed growth. The answer to this puzzle is that this study was not about psychological effects of changes in height, but rather was examining the influence of arrested pubertal development on adolescents' psychosocial adaptation. An implicit assumption justifying GnRHa as an adjunct to GH treatment is that the benefits of taller adult height outweigh the potential psychosocial liabilities of delayed*

*or arrested pubertal development. The findings of a more negative self-concept in the treatment group give reason for pause. There are many reasons for viewing these findings as tentative, not the least of which is the high rate of missing data by the third year of treatment, confounding interpretation of the findings.*

*It was not so long ago that delayed puberty (in males, at least) was considered a significant threat to the individual's psychosocial development.<sup>1,2</sup> Perhaps the time has come to consider a head-to-head comparison of the short- and long-term psychological benefits of on-time puberty versus taller adult stature.*

*Few studies directly examine parents' motivations in seeking care for their child,<sup>3,4</sup> and no studies of the children themselves. This study begins the process of filling an important gap in knowledge. It has long been known that there is limited concordance in the reports of parents and their children when a description of the child's psychosocial adaptation is in question;<sup>5</sup> a clear limitation in employing "parent-proxy only" assessments. Another methodological cautionary note derives from the likelihood of overestimating the incidence of emotional/behavioral problems when comparing clinical samples to population norms. The authors correctly pointed out that norms for a commonly used behavior problem checklist, the Child Behavior Checklist, are biased towards mental health and not representative of the general population.<sup>6</sup>*

*This study documented that future (even more than current) worries about the short child, are on the minds of parents when they seek treatment for their child. To the extent that this finding is replicated in independent and larger studies, it suggests that parents' decisions may hinge predominantly upon the negative stereotypes of foreclosed life options for adults with short stature. The empirical basis for these stereotypes are shaky.<sup>7,8</sup> Accordingly, it is the clinician's responsibility to check for, and to correct these faulty assumptions when present, lest they engender self-fulfilling prophecies.*

David E. Sandberg, PhD

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## Efficacy of Growth Hormone During Transition from Adolescence to Adulthood in Patients with Growth Hormone Deficiency

Mauras and colleagues conducted a multicenter, double-blind, placebo-controlled 2-year follow-up study of 58 subjects (mean age  $15.8 \pm 1.8$  years; 33 males) who were treated for GH-deficiency as children and who, upon retesting at near adult height, were still GH-deficient (GHD). The study consisted of 3 phases: a basal phase, a washout phase, and an assessment phase. Twenty-five subjects were enrolled in the GH group (15 males, 10 females), 15 in the placebo group (9 males, 6 females), and 18 in the GH-sufficient control group (8 males; 7 females) of which 3 were excluded from analysis because they had evidence of multiple anterior pituitary hormone deficiencies. Forty-two subjects completed the study period that included baseline assessment and follow-up assessments at 2, 4, 8, 12, 16, 20, and 24 months: 21 patients in the GH group, 11 in the placebo group, and 10 in the control group (assessed only at 12 and 24 months). The primary objective of the study was to establish the efficacy of GH treatment with regards to body composition and bone mineral density changes, as well as the safety of a transition dose ( $20 \mu\text{g/kg/d}$ ) of GH as replacement therapy in subjects with GHD during the transition from adolescence to adulthood. Secondary objectives included exploring the effects of GH treatment on plasma lipids, insulin-like growth factor (IGF)-I concentration, carbohydrate metabolism, cardiac function, exercise tolerance, and quality of life (QoL).

The results, in general, failed to reveal a significantly beneficial effect of GH on measures of either body composition or bone mass over the 2-year study compared with the placebo group. There were also no measurable improvements in functional measures of muscle strength. Cardiovascular assessment revealed normal cardiac function and exercise tolerance in the study subjects at baseline and throughout the study. The lipid profile did not change during GH therapy, and measures of carbohydrate metabolism showed only mild increases in measures of insulin resistance. QoL measures were unchanged during the 24-month trial. The authors concluded that GHD adolescents who are in good metabolic status at the time of discontinuation of GH treatment may be able to discontinue GH for at least 2 years without any deleterious effects, and that replacement treatment in adulthood needs to be individualized.

(GH) during transition of GH-deficient patients from adolescence to adulthood: A phase III multicenter, double-blind, randomized two-year trial. *J Clin Endocrinol Metab*. 2005;90:3946–3955.

**First Editor's Comment:** *The investigators provided several plausible explanations for the finding that treatment of GHD adolescents in transition to adulthood did not elicit metabolic or QoL benefits, including the younger age of these research participants than those in studies showing benefits, the brief length of time off of GH, a possibly over-liberal threshold for diagnosing persistent GHD ( $<5 \mu\text{g/liter}$ ), and sample attrition.*

*It is noteworthy that the QoL scores of the GHD participants were indistinguishable from those of the general population while on GH and prior to the washout phase of the study. Unfortunately, one can not surmise what the level of functioning was before initiating treatment years earlier. Without such baseline data, it would be erroneous to conclude that GH treatment in childhood and adolescence had any effects on QoL.*

*Finally, if the results of this well-designed study can be replicated, then this would come as good news to patients, families, and clinicians. No one, least of all the adolescent patient, looks forward to continuing daily injections beyond the period of active linear growth. Most GHD patients will end this initial phase at some point during adolescence, a phase of development notoriously difficult from the perspective of adherence to medical regimens.<sup>1</sup> Knowing that no physical or psychological harm will come to patients by introducing a hiatus in treatment for at least 2 years provides the opportunity to re-educate the now increasingly mature patient about the changing hormonal requirements to support optimal health (physical and QoL).*

David E. Sandberg, PhD

**Second Editor's Comment:** *This work was presented at the ESPE – LWPES Joint Meeting and reviewed on page 8 of this issue of GGH.*

Fima Lifshitz, MD

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1. La Greca AM, Bearman KJ. In: Roberts MC, ed. *Handbook of Pediatric Psychology*. New York: Guilford Press; 2003:119–140.

Mauras N, Pescovitz OH, Allada V, Messig M, Wajnrajch MP, Lippe B for the Transition Study Group. Limited efficacy of growth hormone

## Pituitary GH-secretory Cells

Bonnefont and colleagues answered a long-standing question: if growth hormone (GH)-secreting cells are heterogeneously distributed and scattered throughout

the anterior pituitary, as shown by histology, how do they physiologically mount GH pulsatile release that is frequently a thousand-fold in magnitude, especially since

their GH pulses are much smaller when studied *in vitro*?

Using GH-GFP transgenic mice and custom-made computer software, these investigators were able to identify and localize the 3-D position of the labeled somatotrophs within the pituitary gland. Examination of fixed pituitaries from adult male mice revealed a connected 3-D, multi-cellular system comprised of numerous intercrossing strands of single GH cells with larger cell clusters at the intersections. This GH multi-cellular assembly withstood dispersion by a high-pressure *in vivo* perfusion procedure, and was shown to be linked by focal adherens junctions containing  $\beta$ -catenin.

The system was shown to be both functional and plastic. Comparing the volume-to-surface ratios of the GH cell clusters within the lateral and median pituitary zones, the ratios were similar in prepubertal animals. However, GH cell clusters increased in the lateral zones from puberty to adulthood, and then returned to prepubertal geometries in the oldest mice. Cell clustering was prevented by prepubertal castration of male mice, without a significant change in GH cell density in the lateral zones; organizational geometry was the important factor for the pubertal increase in growth. Multi-cellular calcium recordings of GH-EGFP cells in acute pituitary slices were measured as a marker of cell-cell connectivity in hormone release. No large-scale cell connectivity was observed during spontaneous electrical activity. This increased in the lateral pituitary zones following GH-releasing hormone (GHRH) stimulation, leading to temporally precise, synchronized, recurrent calcium spikes that correlated with the frequency of small GH pulses reported in other studies; enzymatic dispersion of the GH cells prevented GHRH-stimulated calcium spike synchronization. GHRH also increased calcium spiking in the median pituitary zone by changing the cell connectivity into small islets of more highly functionally connected GH cells at some points in the system interspersed with functionally less connected GH cells.

The authors concluded that, "GH cells function as a geometry-driven network of cells, connected to each other by adherens junctions." It logically follows that disruption

of network architecture constitutes a novel mechanism for impaired GH release in pathological conditions, an issue the authors are pursuing in follow-up experiments.

Bonnefont X, Lacampagne A, Sanchez-Hormigo A, et al. Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci*. 2005;102:16880–16885.

**Editor's Comment:** A 3-D approach to functional analysis of the GH cell network provided novel and interesting insights into its physiology that were heretofore unobtainable. Because it is noninvasive and provides sensitive, real-time data of cellular and molecular events within their biological context,<sup>1</sup> *in vivo* bioluminescent imaging has recently emerged as a powerful new approach to elucidate physiologic and pathophysiologic mechanisms. It can be used grossly, such as monitoring rejection of transplanted tissues<sup>2,3</sup> or growth of cancer metastases.<sup>4</sup> It can also be used to study protein-protein interactions,<sup>5</sup> transcription,<sup>6</sup> and gene silencing.<sup>7</sup> Bioluminescent or fluorescent imaging holds great promise as a means of drug testing, both for therapeutic efficacy<sup>8</sup> and potential effects on normal tissues,<sup>9</sup> as well as in *in vivo* evaluation of gene therapy strategies.<sup>10</sup>

Adda Grimberg, MD

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## Genomic Alterations in Human Embryonic Stem Cells

The potential use of human embryonic stem cells (hESC) is an exciting but controversial area in medicine today. In concept, hESC cells might be able to repair and/or regenerate damaged tissues and replace injured cells. It is often assumed that after harvesting, these cells are genetically stable, even though they must be expanded substantially through repeated cell division to generate enough cells for current experiments and for possible future therapeutic uses. However, like all dividing cells, it is probable that cultured hESC undergo a low level of spontaneous mutation, which in some cases could adversely affect their therapeutic potential. Maitra et al examined this issue by comparing several parameters

of genomic stability in 9 hESC lines that were available as both early and late passage cells, ie, early and late passage paired cell lines. Cells normally stop dividing when they reach high density in culture, but they will start dividing again if diluted. Passage refers to this dilution process; it is a crude measure of the number of times cells have divided, ie, late passage cells have divided many more times than early passage cells.

The authors used 3 assays to search for alterations of cellular DNA: nuclear DNA copy number, mitochondrial DNA sequence, and gene promoter methylation. In the first case, initial Affymetrix high-density array analysis of approximately 115 000 single nucleotide polymorphisms

(SNPs) distributed across the genome showed no significant differences between the early and late passage hESC. However, further analysis revealed copy number alterations in late but not early passage cells from 4 of the 9 paired cell lines. The alterations ranged from large genomic regions of amplification or deletions, such as amplification of the entire chromosome 17q arm, to discrete changes such as a 2-Mb amplification that included the MYC oncogene. These changes were verified by *in situ* hybridization (FISH) or quantitative genomic PCR.

Next, they screened the mitochondrial genome, which is often mutated in cancer, again using array technology. Sequence alterations were detected in 2 of the late passage hESC cell lines that were not observed in early passage cells from the same cell lines.

Promoter methylation, an epigenetic phenomenon observed in almost all cancers, was assessed in a panel of 14 genes known to be differentially methylated in cancer cells. Differential methylation of 3 genes was detected in late passage cells. For one gene, RASSF1 – a putative tumor suppressor gene, increased methylation was found in late but not early passage cells from 7 of the 9 paired hESC lines.

In conclusion, the authors suggest that most but not all

hESC lines maintained in cell culture acquire clonal DNA alterations over time. Many of these alterations are similar to what has been observed in cancer, such as loss of tumor suppressor genes or amplification of oncogenes. These alterations may provide a growth advantage that allows the cells that harbor them to dominate late passages. The authors acknowledge that much more work is needed to better define the nature of these alterations and their functional consequences. However, they argue that their findings underscore the need to periodically monitor hESC lines before they are used in *in vivo* applications and that some late-passage hESC may be unusable for therapeutic purposes due to genomic alterations over time.

Maitra A, Arking DE, Shivapurkar N, et al. Genomic alterations in cultured human embryonic stem cells. *Nat Genet.* 37:1099–1103.

**Editor's Comment:** *It is clear that hESC have great potential in regenerative medicine. However, this paper illustrates that the field is still relatively young with many troublesome issues, such as long-term genomic fidelity, must be resolved before it can be applied clinically.*

William A. Horton, MD

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## GROWTH HORMONE AND MORTALITY IN PRADER-WILLI SYNDROME

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### INTRODUCTION

Prader-Willi syndrome (PWS) is a unique condition associated with lack of normal expression of paternal alleles in a highly imprinted region of chromosome 15q11-13. Involvement of regions encoding small nucleolar RNA (snoRNA) clusters HBI152 and, perhaps more crucially, HBI185, have been identified as particularly associated with phenotype expression.<sup>1,2</sup> Minimum birth incidence has been recently estimated at ~1 in 20,000 to 30,000 and population prevalence at ~1 in 50,000 to 80,000.<sup>3-5</sup> Fewer than 1% of cases are due to inherited mutations.

Affected individuals suffer from excessive body fat independent of weight, marked deficits in muscle mass and function, growth failure with adult short stature, osteoporosis, scoliosis, hypogonadism, acromicria, neurodevelopmental delay, hyperphagia, and cognitive defects.<sup>6</sup> A variable deficiency of induced growth hormone (GH) secretion and more consistently-observed low insulin-like growth factor (IGF)-I levels are characteristic and may play a role in pathophysiology.<sup>7</sup>

In 2000, after nearly 15 years of favorable clinical experience, recombinant human (rh)GH became the first and, to-date, only pharmaceutical agent specifically approved for treatment of PWS. The FDA labeling states: "...for long-term treatment of pediatric patients who have growth failure due to PWS" and European

### From The Editor's Desk

Yes, *GGH* has a new look to acknowledge a new era with our new sponsor, INSMED. Also, with this change we welcome 2 new distinguished colleagues to the editorial board, Dr. Roberto Lanes from Caracas and Dr. Martin Savage from London. Biographical sketches highlighting their wonderful credentials are available on the journal's website. The above mentioned changes have brought about a renewal of the journal's scope and mission that we hope will be appreciated and relished by the readers.

In this issue the lead article deals with an important current dilemma—the treatment of individuals with PWS with rhGH. Dr. Phil Lee was invited to review the mortality risks to these patients and his paper clearly presents the current status and issues. Although the data are not sufficient to fully determine patients' risks, with or without this therapy, the review was necessary. The lead article facilitates an understanding of the facts as they now stand and therefore it aids in formulating the clinical choices that need to be made when treating PWS. There clearly is a need to gather additional scientific information for a precise risk analysis in PWS that will lead to well substantiated recommendations.

The abstracts and editorial comments are also very timely, all dealing with a variety of subjects that affect patients and help elucidate important pathophysiological mechanisms i.e., growth in Noonan syndrome and the role of Obestatin, a new hormone that opposes Ghrelin, among other important contributions. Altogether this issue constitutes another very successful issue of *GGH*; enjoy it and thank our new sponsor for their commitment to continuous medical education.

Sincerely,  
Fima Lifshitz, MD  
Editor-in-Chief



labeling states “for improvement of growth and body composition” (Genotropin®/Genotonorm®, Pfizer, New York, NY); similar approvals have been obtained worldwide for other rhGH manufacturers. Numerous beneficial effects of rhGH, including improvements in linear growth, physical appearance, functional muscle mass, and infant neurodevelopment have been observed in children with PWS.<sup>7-10</sup> Treatment of adult PWS patients with rhGH is under investigation.<sup>11</sup>

In the nearly 20 years since the first reported use of rhGH in PWS, remarkably few adverse effects have been reported.<sup>8,9</sup> One case of intracranial hypertension<sup>12</sup> and a few cases of asymptomatic fluid retention<sup>10</sup> have been reported. Exacerbation of hyperglycemia and type 2 diabetes mellitus has been reported, usually with preceding risk factors.<sup>8</sup> Malignancy has not been reported with rhGH, although an increased risk for malignancy has been suggested for PWS without rhGH based on individual reports of various types of cancer<sup>6</sup> Neuromuscular scoliosis, a common progressive condition in PWS, is not worsened by rhGH.<sup>6,10</sup>

Two cases of death were reported in 2002 in children with PWS receiving rhGH.<sup>13,14</sup> On January 23, 2003, the Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society issued a statement (revised May 20, 2003) including 5 additional cases ([www.lwpes.org](http://www.lwpes.org)). On April 30, 2003, Pharmacia (now, Pfizer) applied a warning label to its rhGH, Genotropin, followed by a letter to health care professionals dated May 30, 2003 (approved by the FDA on October 31, 2003) stating that “Growth hormone is contraindicated in patients with Prader-Willi syndrome who are severely obese or have severe respiratory impairment.” In 2004, other manufacturers were required to add this warning to their rhGH products.

While the application of this warning was prudent in many respects, it has also led to considerable concern and confusion regarding the safety of rhGH in PWS. As a member of the Prader-Willi Syndrome Association USA (PWSA) Scientific Advisory Board, this author has been made aware of several cases in which rhGH has been denied or withdrawn by the treating physician because of this warning. Since denial of rhGH may be detrimental for children with PWS, it seems equally prudent to review the evidence for and against an association of rhGH with mortality in PWS.

## MORTALITY DURING rhGH THERAPY

Following the initial reports of death during rhGH, intensive investigations for additional cases were conducted by rhGH manufacturers. As of February 2003, a total of 7 cases of death during rhGH treatment had been identified; 3 of these cases were previously registered in the Kabi International Growth Study (KIGS).

At that time, a total of 675 rhGH-treated PWS cases were registered in KIGS (personal communication, Pfizer, October 3, 2003), giving an overall mortality ratio of 0.4%. Of the remaining cases in KIGS, 16% had only one recorded clinic visit while 84% (n=565) had received rhGH for a mean period of 2.4 years.

As of May 01, 2006, a total of 18 pediatric and 2 adult deaths have been identified in individuals with PWS treated with rhGH<sup>13-19</sup> (additional information from Dr. M. Wajnrajch, Pfizer and Dr. B. Lippe, Genentech). Considering the degree of attention given to this issue, it may be assumed that these cases represent a fairly comprehensive survey of deaths within the rhGH-treated PWS population throughout most of the industrialized world. (A database project conducted by PWSA may contain a few additional cases, as discussed in the next section.)

The 2 adult cases included a 33-year-old male who had been off rhGH for 6 weeks prior to his demise and a 48-year-old male who was known to be noncompliant with rhGH therapy. One pediatric case was a victim of bathtub drowning and another had been off rhGH for 11 months prior to death. These cases do not appear to be relevant to the current concerns. One case occurred in a 3-year-old who was known to be noncompliant with rhGH therapy; this case is included in the following analyses since therapy is not confirmed to have been discontinued for a significant period prior to death (case #8 in the Table).

As detailed in the Table, the 17 remaining cases were all pediatric, 0.7 to 15.8 years of age ( $7.0 \pm 4.3$  yr) (mean  $\pm$  SD), including 13 males. Duration of rhGH ranged from 2 weeks to 2.5 years ( $0.57 \pm 0.66$  yr). Eight of 11 cases were known to be significantly overweight. The cases include 5 previously registered in postmarketing surveillance databases (KIGS-3, Genentech National Collaborative Growth Study [NCGS]-2), 1 case reported to Genentech (GEN), 4 investigated via a regulatory process known as Pharmacovigilance (PV), and 7 from the published literature and other sources. Therefore, approximately 60% of cases were detected via postmarketing surveillance databases or other reports to rhGH manufacturers, while 40% were not.

For the 16 cases for which data are available, the rhGH dose ranged from 0.10 to 0.33 mg/kg/wk ( $0.18 \pm 0.06$  mg/kg/week, mean  $\pm$  SD). Twelve of 16 cases were receiving less than the labeled dose (0.24 mg/kg/week), 2 were at this level and 2 were above this level. The Figure depicts the doses for these cases and published doses from treatment series.

For the 9 cases in which a possible contributory factor is listed, respiratory illness was listed in all cases. Eight of 17 cases were characterized by “sudden” death. Six of these had respiratory impairment preceding rhGH therapy, one

Table. Deaths During GH Treatment

| Case          | Year Reference*  | Age(yr) Sex   | Country     | Duration rhGH (yr)                                    | Dose rhGH (mg/kg/wk)           | Weight***  | Cause of Death****   | Comments  |
|---------------|------------------|---------------|-------------|---|--------------------------------|--|--|---|
| 1             | 1996 (KIGS)      | 15.8 M        | Japan       | 0.58  | 0.10                           | BMI=46.6   | acute pneumonia, respiratory failure; no autopsy                                       |   |
| 2             | 1999 (NCGS)      | 6 M           | USA         | 0.50  | 0.23                           | BMI=23.7   | died in hospital no autopsy  | cardiomegaly, on carbamazepine and O <sub>2</sub>   |
| 3             | 2001 (PV)        | 3 M           | USA         | 0.25  | 0.5 mg qd** (-0.15)            | >200% IBW  | found dead in bed; autopsy: ? pneumonitis  | asthma-on albuterol;  |
| 4             | 2001 (KIGS)      | 8-9 M         | Spain       | 0.04 (2 wks)  | 0.15                           | BMI=38.5   | acute bronchitis, respiratory failure  | history of OSA, nocturnal hypoventilation   |
| 5             | 2001 [14]        | 0.7 M         | Switzerland | 0.20  | 0.18                           | WT 0.63SD  | aspiration pneumonia; autopsy: bronchopneumonia  | died 2 days after aspirating milk   |
| 6             | 2001 [13]        | 6.5 M         | Switzerland | 0.50  | 0.26                           | 145% IBW   | found dead in bed  | history of snoring, OSA, large tonsils  |
| 7             | 2002 (KIGS)      | 4.7 M         | USA         | 0.25  | 0.24                           | BMI=31.3   | aspiration pneumonia, sleep apnea; no autopsy  | history of OSA  |
| 8             | 2003 (PV)        | 3 F           | USA         | 2.50  | 0.5 mg qd** ~0.18 noncompliant | BMI= 17.6 at 2 yr (-75 <sup>th</sup> percentile) | pneumonia  | history of aspiration pneumonia   |
| 9             | 2003 (PV)        | 13 M          | UK          | 0.42  | no data                        | overweight                                       | sudden death, unexplained  |   |
| 10            | 2003 (PV)        | 14.6 M        | UK          | 1.50  | 0.11                           | BMI=42.0   | viral respiratory infection, respiratory and right heart failure                       |   |
| 11            | 2003 [19] (NCGS) | 4.5 M         | Canada      | 0.17  | 0.17                           | 259% IBW   | died during sleep; autopsy: pneumonia, left ventricular hypertrophy, subdural hematoma | history of progressive snoring, headaches   |
| 12            | 2003 (GEN)       | 10 M          | USA         | ~0.13   | 0.15                           | BMI= 51.6  | abrupt deterioration   | history of albuterol use  |
| 13            | 2005 [16]        | 4.7 F         | Austria     | ~0.13   | 0.24                           | BMI=19.5   | abrupt deterioration, ? cardiorespiratory arrest at home                               | previous adenoidectomy; no apnea on PS; nocturnal NCPAP   |
| 14            | 2005 [16]        | 9.3           | Austria     | 1.0, stopped 1.3, restart 0.5; total treatment=1.5 yr | 0.28, restart 0.14             | BMI 30.2, 27.3 after 1st rhGH, 38.5 at restart   | minor respiratory infection, sudden death at home                                      | Progressive deterioration after stopping first course of rhGH; PS-hypoventilation & apnea, noncompliant with CPAP |
| 15            | 2005 [17]        | 3.9 F         | Italy       | 0.30  | 0.33                           | 130% IBW   | sudden death, morning  | adenoid hypertrophy, snoring, apnea preceding therapy   |
| 16            | 2005 [17]        | 6.3 M         | Italy       | 0.20  | 0.20                           | 144% IBW   | sudden death, morning apnea  | TA hypertrophy, respiratory impairment preceding rhGH, worsened during treatment                                  |
| 17            | 2005 [18]        | 3.9           | Greece      | 0.58  | ~0.10                          | Severe obesity                                   | sudden death   |   |
| Mean $\pm$ SD |                  | 7.0 $\pm$ 4.3 |             | 0.57 $\pm$ 0.66                                       | 0.18 $\pm$ 0.06                |  |  |   |

Notes: OSA=obstructive sleep apnea, PS=polysomnography, TA=tonsillo-adenoidectomy, NCPAP=nasal CPAP

\*Year of death, if known, or publication. Source: Kabi International Growth Study (KIGS), National Cooperative Growth Study (NCGS), Pharmacovigilance (PV, Pfizer), 1 case reported to Genentech (GEN)

\*\*Weight at time of death or rhGH dose per kg were not available for these 2 cases. For the purposes of analysis, the weights in both cases were assumed to be 20 kg, giving approximate rhGH doses of 0.18 mg/kg/wk in each case.

\*\*\*IBW= percent ideal body weight. All BMI calculations are >97<sup>th</sup> percentile for age and sex, except as indicated.

\*\*\*\*Causes of death as reported to database are usually based on clinical reports. Autopsy findings are indicated if available.

For case 14, total treatment duration of 1.5 yr and the dose at time of death (0.14) were used for the analyses in the text.

case was reported to have worsened while on rhGH (case 15), while most of the others had no known change during therapy. Inadequate details were available for several cases, and one individual was said to have been improving on rhGH without known respiratory problems (case 17). Twelve cases were known to be morbidly overweight (>200% IBW or BMI >95<sup>th</sup> percentile for age and sex), while 5 cases (5, 6, 8, 15, and 16) were apparently within normal weight guidelines for height. Autopsy data was notably lacking for most cases, and the available data do not reveal unexpected findings. Large tonsils were noted for case 6, but this was not thought by the authors to be contributory to death.<sup>13</sup> Adrenal abnormalities have not been noted in the autopsied cases.

## MORTALITY WITHOUT rhGH THERAPY

Premature mortality and sudden death in PWS are not new concerns; these predate rhGH treatment of PWS by many years.<sup>20</sup> In 1981, Laurance<sup>21</sup> reported a series of 33 patients, of whom 24 were alive at 15 to 41 years of age, and 9 of whom had died before age 23 years. The deaths were attributed to cardiorespiratory failure.

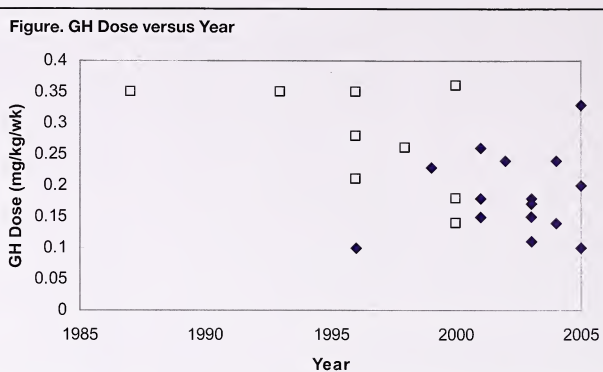
A retrospective clinical review of 36 individuals with PWS found 10 deaths (20–49 years old) over a 10-year period.<sup>22</sup> Respiratory or cardiorespiratory illness was identified as causative in 40% of cases. In 2003, 6 deaths (20–43 years old) were tabulated in a follow-up study of 37 non-rhGH treated individuals with PWS who had been entered into

the Australian Child Development Study in 1989.<sup>23</sup> The calculated death rate was >4-fold higher than in a control group of 547 individuals with intellectual disability drawn from the same prospective study. A respiratory component was noted for 3 of the 4 cases for which cause-of-death was identified.

In an international summary of 27 deaths in PWS,<sup>24</sup> approximately half were related to respiratory or cardiorespiratory disease, including 9 of the 13 cases in those less than 5 years of age. Small adrenals were not observed in autopsied cases, but were noted in 3 of 4 autopsied cases in a separate review of 10 cases.<sup>25</sup> It should be noted that no functional adrenal abnormalities have been identified in PWS patients on standard biochemical

Support-group survey studies are limited by substantial bias, including survival and younger age. However, by virtue of their size and geographic representation, these efforts may provide valuable information regarding the characteristics of the PWS population. Nagai et al<sup>26</sup> examined the records of 494 individuals with PWS registered in 2 regional PWS support groups (2 months to 48 years of age, 46% females, 54% male). Thirteen deaths were identified (2.6% of the group, 6 female, 7 male), none had received rhGH; 7 (58%) were under 2 years of age, with deaths attributed to possible aspiration or SIDS (n=2), upper airway infection with diarrhea (n=1), cardiomyopathy with known respiratory disorder (n=1), and diarrhea (n=3). None of the infant deaths were associated with obesity. Other deaths included a 14-year-old and 20-year-old with bathtub drownings. The 4 adult deaths (23, 26, 28 and 34 years old) were attributed to cellulitis, pulmonary embolism, renal, and heart failures, respectively.

An ongoing survey of the PWSA membership (approximately 3000) has thus far revealed 190 deaths since 1977 (74% dated since 2000 [courtesy of J Heinemann, PWSA]). Mean age at death was 28 years (2 months to 63 years, 33% <21 years, 12.5% <5 years). The 14 cases reported to have received rhGH included 4 who were off therapy for several months or years, 3 with gastric perforation—a known cause of mortality in PWS,<sup>27</sup> (3 without information, and 1 each: motor vehicle accident, sepsis, severe asthma attack, and possible poor intubation (pre-existing respiratory problems)). None of the cases appear to be related to rhGH, although complete analyses for some cases are pending.



**Empty squares:** rhGH doses reported in published series demonstrating rhGH efficacy.<sup>7,9</sup>  
**Closed diamonds:** Individual cases of death while on rhGH, plotted by dose at time of death and year reported (see Table).

Population-based morbidity and mortality data for PWS are not available except from regional cross-sectional surveys. Recent regional surveys in England<sup>2</sup> and Belgium<sup>3</sup> indicate high morbidity and mortality rates; survival past the 6th decade of life has been rarely documented. In the English study,<sup>5</sup> 50% of individuals with PWS reported recurrent respiratory disease, and lifetime mortality rate was roughly estimated at 3%/yr (approximately 3 times higher than the general population). Except for these 2 population-based studies, no conclusions can be reached regarding mortality rates, and within these studies the data are insufficient to construct survival curves.

### IS MORTALITY INCREASED WITH rhGH THERAPY?

Given the similarity in causes of death between the rhGH-treated and untreated cases and the apparently high underlying mortality rate at all ages in the untreated population, a logical question is whether rhGH is having a positive, negative, or neutral effect on mortality risk in PWS. The answer to this question is complicated by the lack of sufficient population-based data to construct survival curves or risk ratios. In addition, little is known about the effects of age, sex, and accompanying morbidities on mortality; information that would be crucial for estimating the additional effect of rhGH treatment.

Moreover, most clinicians are not personally familiar with the natural history of PWS, have not cared for PWS patients as a series, and are unlikely to systematically follow patients who are not rhGH-treated. There are few centralized PWS care facilities from which experiential information can be collected. Since deaths without rhGH do not engender the same level of interest as those occurring during therapy and clinical experience is limited, the casual reader or incidental PWS practitioner may have the impression that the latter represent a new and unusual series of events.



Given the lack of rigorous statistical data for epidemiologic analyses, logical models of disease causation can provide an alternate framework for consideration. For instance, at least 3 of the Evans criteria<sup>28</sup> (paraphrased for the current discussion), originally formulated for infectious diseases but often applied to other cause-and-effect associations, appear to be in doubt:

1. The prevalence of death should be significantly higher in those treated with rhGH than in those not treated:

As noted above, as of February 2003, mortality occurred in 0.4% of 675 rhGH-treated PWS cases registered in KIGS. Although this information has not been recently updated, using a conservative estimate that 1000 PWS patients have received rhGH for more than 1 year over the past 15 years and 20 deaths occurred during therapy, the death rate would be <0.2%/year. This compares to the 2.6% mortality ratio,<sup>26</sup> and 2.8% and 3%/year mortality rates<sup>5,22</sup> estimated for untreated PWS individuals. Therefore, although the available data are not perfect, these suggest that mortality may not be higher in those treated with rhGH.

2. There must be a certain strength of association, eg, duration, dose-response relationship:

Higher doses do not result in increased mortality; 70% of cases were receiving rhGH doses below the labeled recommendation of 0.24 mg/kg/wk, while the published literature indicates that major PWS treatment centers are using the labeled or higher doses (Figure). In addition, higher doses were not associated with shorter duration of therapy prior to death.

Continued exposure to rhGH apparently does not continue or increase the risk of mortality. The 17 cases (Table) received rhGH for an average of 6 months (median 4 months, 2 weeks to 2.5 years). As of February 2003, the average treatment duration for 565 PWS patients in KIGS was 2.4 years, and we can assume that the numbers receiving therapy for extended periods has increased since then. However, there is no evidence for increasing numbers of deaths during longer-term therapy. In fact, the paucity of reported deaths after 1 year of rhGH provides suggestive evidence for rhGH-related reduction of the high underlying mortality rate in PWS.

Arguments have been made that the apparent early clustering of deaths represents a time-limited risk of rhGH therapy. For instance, there could be a dual effect of higher mortality in the initial phase of rhGH treatment and lower mortality thereafter, although a mechanism for the initial-phase effect has not been elucidated.

It is also possible that these 17 cases represent continuation of the natural history of the condition. At standard doses, positive effects of rhGH on respiratory parameters are particularly evident after 6 to 12 months of therapy; thereby providing a window during

the early phase of treatment during which natural history may take precedence. The situation may be as suggested in the first report: "The boy reported here...thus died before the effects of rhGH could manifest themselves."<sup>13</sup>

3. A coherent association should exist between rhGH treatment and death; the cause-and-effect interpretation should not conflict with the known pathology of the disease:

For the 17 cases (Table), the most commonly identified disease at time of death was respiratory failure, which is also the most commonly identified mortality association in the non-rhGH treated PWS population. There is no evidence that rhGH worsens risk for respiratory-related morbidity. In fact, rhGH has been shown to improve pulmonary function and respiratory control in PWS.<sup>29-31</sup> Since excess GH levels are associated with respiratory complications in acromegaly, it has been postulated that rhGH could cause similar problems in PWS. However, such complications in acromegaly are complex and thought to be due to a combination of soft-tissue and bone remodeling,<sup>32</sup> changes which have not been observed in rhGH-treated pediatric populations. In addition, one might expect acromegalic airway changes at higher rhGH doses and with longer duration of therapy.

## POLYSOMNOGRAPHY AND rhGH THERAPY

The involvement of respiratory compromise in the initial 7 cases of death during rhGH therapy prompted the manufacturer in April 2003 to expand the warning label on Genotropin to include: (1) severe respiratory impairment as a contraindication to therapy, (2) worsening "upper airway obstruction," including snoring, as an indication for interruption of therapy, and (3) evaluation and monitoring for sleep apnea. No statistical data in support of this sternly-worded warning label and no specific methods for assessment were presented. The result was clinical practice and liability concerns amongst clinicians accompanied by alarm amongst parents of children with PWS, primarily concerned that an approved and beneficial treatment would be withheld from their child. Many clinicians interpreted this label to mean that all children with PWS should have polysomnography and that rhGH should be withheld upon receipt of abnormal results. This was despite the fact that no relationships between polysomnographic results and morbidity or mortality in children with PWS had been identified, a population in which 0% to 100% occurrence of obstructive sleep apnea had been reported in various series.<sup>33</sup>

After careful consideration of all available data and viewpoints, the Clinical Advisory Board of PWSA issued reasoned recommendations for sleep studies and other testing in 2003 ([www.pwsausa.org](http://www.pwsausa.org)).<sup>6</sup> As stated: "At this time



there is no evidence of a causative link between growth hormone and the respiratory problems seen in PWS.<sup>29</sup> Several studies have shown improvements in breathing and pulmonary function in children with PWS after 6 to 12 months of rhGH.<sup>6,8,9,29-31</sup> Over a much shorter rhGH treatment period of 6 weeks in a mixed group of children and adults with PWS, 19 of 25 (76%) had improved polysomnographic parameters.<sup>34</sup> A non-treatment control group was not studied and test/re-test reproducibility was not reported. Nonetheless, this latter study indicates that rhGH efficacy might be observed even over a short term. Also, in this latter study, IGF-I levels were noted to be high in 2 subjects with worsened parameters, leading the authors to postulate a role for rhGH/IGF. However, the other 4 subjects with deteriorating measures had normal IGF-I levels, and 2 subjects in the improved group had high IGF-I levels.

As of this writing, there is no evidence linking results of polysomnography with morbidity or mortality in PWS, regardless of rhGH therapy. Whether an abnormal polysomnogram itself defines morbidity is a matter for debate that is beyond the limits of this manuscript. This author concurs with recommendations that polysomnography be reserved for individuals with clinical evidence of sleep-disordered breathing or excessive daytime sleepiness, and should be preferably performed as part of a clinical research program in other cases.<sup>6,33</sup> Similar guidelines may be logically applied to pulmonary function testing.

### CONCLUDING PERSPECTIVES

If left untreated, PWS can be a devastating condition, with affected individuals suffering considerable physical handicap, largely related to severe lifelong hypotonia. The efficacy of rhGH, particularly in children with PWS, has provided a new outlook on life that goes beyond obvious improvements in height and somatic appearance. Against these recognized benefits are concerns that rhGH may increase mortality in the initial phase of therapy. Although conclusive data supporting or refuting this concern may or may not be available in the near future, the bulk of information reviewed above may serve as an argument against the validity of this concern.

Based on the available information, rhGH may be considered in children with PWS with prudent consideration of the following points:

1. Many deaths in infants with PWS, regardless of rhGH therapy, have been related to possible aspiration of feedings. Therefore, reflux precautions should be stringently followed until the child is ambulatory.
2. Many deaths in older children and adults with PWS, regardless of rhGH therapy, have been associated with obesity; albeit without direct demonstration of cause/effect in most cases. In addition, rhGH can exacerbate the insulin resistance associated with being overweight. Therefore, proper attention should be given to weight control.
3. Several tub-drowning deaths have occurred in individuals with PWS, regardless of rhGH treatment. Caretakers should be warned not to leave individuals with PWS unattended in a bathtub or pool.
4. Most of the reported deaths during rhGH treatment occurred with doses at or below the labeled recommendation of 0.24 mg/kg/week. Therefore, there is no apparent reason to limit the rhGH dose in relation to preventing morbidity or mortality.
5. All but one of the reported deaths during rhGH therapy occurred within the first 18 months of treatment; 82% within the first year. Therefore, clinical follow-up should be especially attentive during the first 12 to 18 months of rhGH. For patients who are not receiving rhGH, this heightened level of attention should be continual given the high inherent mortality rate.
6. There is currently no medical reason for rhGH to be conditional on the results of polysomnography or pulmonary function testing. Such testing should be considered only if clinically indicated and/or within the guidelines of a clinical research protocol.

Unfortunately, a current lack of population-based data regarding mortality and rhGH therapy in PWS prevents conclusive analyses, such as survival curves and hazard ratios, which are required to define therapeutic risk. However, assessment of current available information argues against a cause and effect relationship between rhGH treatment and mortality. Coordinated multicenter studies of treated and untreated populations are needed to bring closure to this issue. Meanwhile, in keeping with the principles of *primum non nocere* and the Doctrine of Double Effect,<sup>34</sup> each clinician involved with decisions regarding rhGH therapy of PWS should maintain an awareness of current knowledge regarding therapeutic efficacy, natural history and adverse effects to insure optimal care of individual patients.

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## ABSTRACTS FROM THE LITERATURE

### GH Resistance in Noonan Syndrome: From Cause to Clinical Outcome

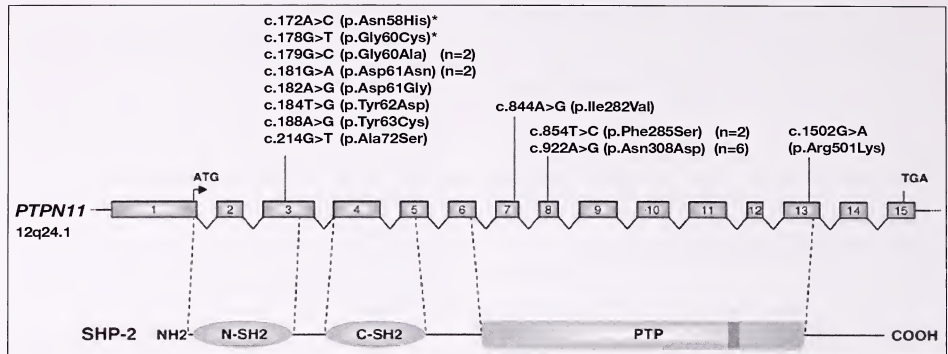
Proportionate short stature (SS) occurs in more than 70% of individuals with Noonan syndrome (NS), an autosomal dominant disorder found in 1:1000 to 1:2500 live births. NS is also characterized by typical facial dysmorphisms and cardiac defects, especially pulmonic stenosis and hypertrophic cardiomyopathy. Although prior growth hormone (GH) studies in these patients have shown mixed results (some normal, some abnormal, some suggesting neurosecretory deficiency), in general classic GH deficiency is a rare finding.

A causative gene for NS was identified in 2001: *PTPN11* (protein tyrosine phosphatase, nonreceptor type 11), which encodes Src homology region 2-domain phosphatase-2 (SHP-2). About half of individuals with NS harbor heterozygous missense mutations of SHP-2, the majority of which involve the amino SH2 (N-SH2) or the protein tyrosine phosphatase (PTP) domains (exons 3, 8, and 13). Both N-SH2 and PTP normally interact, keeping the ubiquitously expressed, cytosolic SHP-2 in a closed, inactive conformation. SHP-2 is activated upon binding of N-SH2 to a phosphotyrosine residue, such as those on activated receptors for GH, cytokines and other growth factors. By chronically stabilizing the SHP-2 open, and hence active, conformation, the missense mutations of NS would be expected to cause gain of function of this negative regulator of receptor signaling. SHP-2 can

not only dampen signaling through dephosphorylation of the receptor itself, it can also dampen downstream signals like dephosphorylating STAT5. Thus, SHP-2 mutations would be expected to cause GH resistance in patients with NS. Three recent papers studied this proposed hypothesis.

#### Mild GH Resistance

Binder and colleagues recruited all 29 children who presented to their center during the past 5 years with SS and at least 3 typical anomalies of NS or pulmonic stenosis. Blood lymphocyte DNA was extracted for PCR amplification and sequencing; 11 different missense mutations of *PTPN11* were found in 16 children from 14 unrelated families (55% of patients). Of these 11 mutations, 8 occurred in exons 3, 8 or 13. Comparing the mutation-positive (mut<sup>+</sup>) vs mutation-negative (mut<sup>-</sup>) subgroups, the former were found to have a higher incidence of pulmonic stenosis (81% vs 15%) and septal defects (63% vs 15%), and younger mean age at presentation ( $5.1 \pm 2.7$  vs  $10.3 \pm 5.2$  years). Minor anomalies and height ( $-3.15 \pm 0.92$  vs  $-3.01 \pm 1.35$  SD) did not differ significantly, and all children were approximately 1 SD shorter in height than the mean for NS. While the higher spontaneous overnight and arginine-stimulated GH levels did not reach statistical significance, insulin-like growth factor (IGF)-I ( $-2.03 \pm 0.69$  vs  $-1.13 \pm 0.89$  SD) and IGF binding protein (BP)-3



Distribution of *PTPN11* missense mutations identified in 20 of the 35 NS patients. Mutations that have never been described are marked by an asterisk. The number of patients carrying the same mutation is indicated in parentheses.

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( $-0.92 \pm 1.26$  vs  $0.40 \pm 1.08$  SD) were significantly lower in the *mut*<sup>+</sup> group.

A subgroup of 11 prepubertal children received recombinant human (rh) GH for one year. Mean change in height SDS in the 8 *mut*<sup>+</sup> children ( $+0.66$  SD) was significantly lower than that in the 3 *mut*<sup>-</sup> children ( $+1.26$  SD). However, the *mut*<sup>-</sup> children received a lower mean rhGH dose ( $0.042$  mg/kg/d vs  $0.05$  mg/kg/d).

Binder G, Neuer K, Ranke MB, Wittekindt NE. *PTPN11* mutations are associated with mild growth hormone resistance in individuals with NS. J Clin Endocrinol Metab. 2005;90:5377–5381.

### Response to 3 Years of rhGH Treatment

Ferreira and colleagues retrospectively analyzed the 14 children (10 male) followed at their Endocrinology Unit for NS; all had presented with SS (mean height  $-3.5 \pm 0.9$  SD) and were treated with ( $0.033$ – $0.05$  mg/kg/d) after a 6-month observation of baseline growth velocity. Eight of the children had been treated for 3 years, 4 for 2 years, and 2 for at least 1 year at the time of analysis. At the start of treatment, mean age was 12.3 years, bone age  $9.8 \pm 2.7$  years, and 10 were prepubertal. Seven children initiated puberty during treatment, and one received concomitant gonadotropin releasing hormone (GnRH) analog therapy. Treatment with rhGH was discontinued during the second year in one patient for increasing ventricular wall thickness; this patient had mild left ventricular hypertrophy before starting rhGH, and cardiac function continued to worsen afterwards despite cessation of rhGH.

Gene sequencing revealed 5 different, *de novo* heterozygous *PTPN11* missense mutations in 7 (50%) patients, 3 of whom were also seen among the children in the above Binder paper. At the start of treatment, the 7 *mut*<sup>+</sup> and 7 *mut*<sup>-</sup> patients did not differ in their GH secretory capacity (all had normal peak responses to clonidine stimulation; mean  $13.1 \pm 7.1$  ng/mL), nor

in their low IGF-I levels ( $-2.0 \pm 1.4$  SD). However, the rhGH-stimulated increment in IGF-I was significantly smaller in the *mut*<sup>+</sup> patients, as was the improvement in growth velocity, such that by the end of the third year of treatment, the *mut*<sup>+</sup> group had a significantly smaller gain in height SDS ( $+0.8 \pm 0.4$  vs  $+1.7 \pm 0.1$  SD;  $p < 0.01$ ). Bone age advancement did not differ between the 2 groups.

Ferreira LV, Souza SA, Arnhold LJ, Mendonca BB, Jorge AA. *PTPN11* (protein tyrosine phosphatase, nonreceptor type 11) mutations and response to growth hormone therapy in children with NS. J Clin Endocrinol Metab. 2005;90:5156–5160.

### Prospective Study of 2 years of rhGH Treatment

Limal and colleagues prospectively recruited 35 patients (19 boys) with NS and growth retardation (height  $< -2$  SD), excluding those with severe congenital heart malformations and/or hypertrophic cardiomyopathy. The 25 prepubertal children at study start (mean age  $10.4 \pm 3.1$  yr) were given rhGH  $0.30$  mg/kg/wk while the 10 pubertal children (mean age  $14.7 \pm 1.7$  yr) were given rhGH  $0.46$  mg/kg/wk to compensate for their late treatment start.

Sequence analysis revealed 12 different heterozygous *PTPN11* missense mutations in 20 children (57%) (Figure), 10 of which were previously reported; all but one occurred in exons 3, 8 or 13. The *mut*<sup>+</sup> subgroup had a higher frequency of small-for-gestational age (SGA [32%]) than the *mut*<sup>-</sup> (13%), though birth weight and head circumference were normal in all. At age 6 years, the *mut*<sup>+</sup> group was significantly shorter, as was their mean target height. Starting at age  $10.4 \pm 3.1$  years, 2 years of rhGH resulted in less catch-up growth among the prepubertal *mut*<sup>+</sup> children than the prepubertal *mut*<sup>-</sup> children; their end heights were  $-3.1 \pm 1.4$  SD (vs  $-2.0 \pm 0.9$  SD;  $p < 0.05$ ) and deficit from target heights were  $-2.5 \pm 0.9$  SD (vs  $-1.1 \pm 0.7$  SD;  $p < 0.01$ ).



At initiation, peak GH level following pharmacologic stimulation was  $15.4 \pm 6.5$  ng/mL (5–34.3) in all 35 children, though 5 of the *mut*<sup>+</sup> had peaks of 5 ng/mL to 10 ng/mL. Of the 19 patients studied (11 *mut*<sup>+</sup> and 8 *mut*<sup>-</sup>), all had normal IGFBP-3, but they had IGF-I at or below the lower limit of normal, and acid-labile subunit (ALS) levels were extremely low in all 10 patients (5 *mut*<sup>-</sup>) tested at rhGH initiation. There was no difference between the 2 genetic subgroups in rhGH-stimulated increases in IGFBP-3 and IGF-I.

Limal JM, Parfait B, Cabrol S, et al. NS: Relationship between genotype, growth and growth factors. *J Clin Endocrinol Metab*. 2006;91:300–306.

**Editor's Comment:** These 3 related papers offer intriguing glimpses into a possible mechanism of growth failure in NS. There are clearly additional mechanisms involved, since *mut*<sup>-</sup> patients frequently also have SS. Nonetheless, as a group, these papers suggest new directions.

### Mechanism

In the idiopathic SS age of non-GH deficient growth failure, the quest has been on for molecular causes of post-receptor GH resistance. The search for individuals who harbor mutations in the signaling cascade directly downstream of the GH receptor has yielded fruitful results: novel GH receptor mutation that impairs GH receptor/STAT5 signaling but maintains normal STAT3 signaling,<sup>1</sup> mutations of STAT5b itself,<sup>2</sup> IGF-I gene partial deletion,<sup>3</sup> single copy number of the IGF-I gene,<sup>4</sup> and IGF-I receptor mutation.<sup>5</sup>

Yet these papers on NS serve as a reminder that a signaling cascade can be turned off (or down) not just by mutations from within, but also by mutations affecting molecules from without; gain of function mutations of

negative regulators of a cascade, such as SHP-2, can serve to augment the normal checks and balances and overly suppress the signaling cascade. This is not the first time that such possibility was shown. In 2001, 8 years after the FDA approved rhGH treatment for SS associated with chronic renal insufficiency, the molecular mechanism underlying the GH resistance was discovered. Comparing rats status-post partial renal ablation (chronic renal failure) and sham-operated, pair-fed rats (controls), Schaefer and colleagues found the former to have blunted hepatic induction of IGF-I expression by GH treatment despite unchanged GH receptor protein levels and GH binding to microsomal and plasma membranes.<sup>6</sup> Normal protein levels of JAK2, STAT5, STAT3, and STAT1 completed the cascade. Instead, these authors<sup>6</sup> found a 75% reduction in GH-induced tyrosine phosphorylation of JAK2, STAT5, and STAT3, due to over-expression of SOCS (suppressor of cytokine signaling)-2 and -3. The SOCS proteins normally function as a cellular internal feedback loop; they are induced by GH and in turn, inhibit GH-stimulated GH receptor/JAK2 complex activation to turn down the GH sensitivity of the cell.

The over-expression of SOCS in chronic uremia and the gain of function mutations of SHP-2 in NS may be just the beginning. Further search may reveal additional conditions involving augmented negative regulators, as well as loss of positive stimulators and enhancers, of the GH receptor/JAK/STAT signaling cascade. Thus, the quest for non-GH deficient causes of growth failure just got a whole lot broader.

### Clinical Implications

The increased GH resistance of PTPN11 *mut*<sup>+</sup> vs *mut*<sup>-</sup> patients reported in these papers suggests that a genotype-driven approach may be more effective

for ameliorating the SS associated with NS. Two treatment strategies may be plausible, and additional studies designed to test these approaches will be needed to determine their relative efficacies and safety. First, to overcome the increased GH resistance, rhGH therapy may require higher doses, and an approach titrating rhGH dose to achieve desired IGF-I levels rather than a standard weight-based dosing scheme, may be the best way to gauge clinical requirements of *mut*<sup>+</sup> vs *mut*<sup>-</sup> individuals. Thus, we may discover 2 different

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optimal dosing levels based on genetic subtype. On the other hand, we may discover that the degree of GH resistance in the *mut<sup>+</sup>* individuals is so great that cranking up the rhGH dose really cannot compensate effectively or may be associated with undesirable side effects. In this scenario (the second treatment strategy), treating with recombinant IGF-I and/or IGF-1/IGFBP-3 rather than rhGH, may be more appealing. These therapies have now become available and were recently approved by the FDA.

Adda Grimberg, MD

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## Signal Transduction and Cardio-Facial Syndromes

The cardio-facio-cutaneous (CFC) syndrome (OMIM 115150) presents with heart malformations, skin defects, and characteristic facies. It overlaps phenotypically with Noonan syndrome (NS) and Costello syndrome (CS). Gain-of-function mutations have been identified in the protein tyrosine phosphatase SHP-2 (PTPN11) in about half of patients with NS. Recently, mutations of one of the RAS proteins known as HRAS were identified in several patients with CS. Interestingly, several CS mutations had been previously identified as somatic oncogenic mutations in tumors. SHP-2 and HRAS are components of a well-known signaling cascade through which many receptor tyrosine kinases transmit signals to the nucleus. Illustrated in the figure, this pathway, which is often referred to as the RAS-MAP kinase pathway, is often associated with proliferative and growth signals in developing tissues and in cancer.

Based on the suggestion that NS and CS might reflect activation of this pathway, a group headed by Aoki speculated that CFC syndrome might be due to mutations in genes encoding other proteins in this cascade. They first sequenced the entire coding regions of 3 RAS genes (*HRAS*, *KRAS*, and *NRAS*) in genomic DNA from 43 individuals with CFC syndrome. Two *de novo* *KRAS* mutations were detected.

Next, they screened for mutations in the 3 isoforms of RAF (*CRAF*, *BRAF*, and *ARAF*), which is immediately downstream of RAS in the signaling cascade. Eight *BRAF* mutations were identified in 16 patients, 6 of which mapped to the kinase domain, where mutations had previously been found in tumors.

The investigators proposed that the mutations they had identified enhance MAP kinase signal activity and tested this notion by expressing the mutant genes and their normal control counterparts in reporter cells that would allow downstream signal output to be measured. They observed a significant increase in signal output for 1 of the 2 *KRAS* mutations and in 4 of the 8 *BRAF* mutations, supporting their contention and the idea that increase MAP kinase signaling is common to all of the disorders in this group.

They reasoned further that if all of the disorders share a common increase in RAS-MAP kinase signaling activity, then there may be mutational overlap as well. Accordingly,

they screened for *BRAF* and *KRAS* mutations in *PTPN11*-negative NS patients and for *PTPN11* mutations in CFC patients negative for mutations in *BRAF* or *KRAS*. No additional mutations were detected, suggesting that the 3 disorders are distinct entities.

In an accompanying editorial,<sup>1</sup> it is noted that a recent publication identified *BRAF* mutations in 18 of 23 individuals with CFC. This study also found mutations in *MAP2K1* and *MAP2K2*, which are downstream effectors of *BRAF* in the RAS-MAP kinase signal pathway. The editorial also points out that molecules in which mutations have been found typically participate in other signaling pathways in addition to the primary linear RAS-MAP kinase pathway, which probably explains why each syndrome has unique features.

Niihori T, Aoki Y, Narumi Y, et al. Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. *Nat Genet.* 2006;38:294–296.

**Editor's Comment:** MAP kinase signaling pathways are more complex than suggested in the figure, and there is extensive crosstalk between subpathways. Nevertheless, placing these syndromes into a group that results from enhanced RAS-MAP kinase signaling serves a useful purpose, especially as inhibitors of this pathway might potentially have therapeutic benefit for postnatal manifestations of these disorders, such as short stature.

In contrast to most cell types in which RAS-MAP kinase signaling is associated with cell proliferation and growth, such signals in growth plate chondrocytes, where they are generated downstream of *FGFR3*, inhibit both cell proliferation and growth. Thus, it is conceivable that achondroplasia, which is due to activating mutations of *FGFR3*, NS, CS, and CFC syndromes share a common pathogenetic mechanism that involves excessive output of the RAS-MAP kinase signaling cascade in growing bone.

William A. Horton, MD

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## Defective Enzyme Degradation in Johanson-Blizzard Syndrome

The Johanson-Blizzard syndrome (JBS [OMIM 243800]), names not unfamiliar to *GGH* readers, is an autosomal recessive disorder characterized by congenital exocrine pancreatic insufficiency, mental retardation, facial abnormalities, and various other malformations. As reported by Zenker et al, an international group has now identified the mutant gene. They started by undertaking a genome-wide linkage scan of 7 families with JBS. Analysis of the consanguineous families revealed a region of homozygosity of 7.5 cM on chromosome 15q14-21.1 containing no obvious candidate genes. By high-throughput DNA sequencing of genomic DNA from JBS patients, they eventually detected mutations in the gene *UBR1* in patients from 12 unrelated families. Most of the mutations produced premature translation stop codons and most likely loss-of-function for the gene product.

*UBR1* encodes an E3 ubiquitin ligase, which transfers ubiquitin moieties to proteins destined for degradation by cytoplasmic proteasomes. Long chains of ubiquitin serve as molecular signals that direct targeted molecules for degradation. *UBR1* was one of the first of many E3 ubiquitin ligases to be identified; each possesses specificity regarding which molecules it ubiquitinates. Interestingly, several E3 ubiquitin ligases have been implicated in genetic disease, ie, UBE3A in Angelman syndrome, parkin in recessive juvenile parkinsonism, and VHL in von Hippel-Lindau disease.

To explore how loss of *UBR1*—which would be expected to lead to failure to ubiquitinate proteins normally ubiquitinated by *UBR1*—causes JBS, the investigators focused on the exocrine pancreas, since it is the most consistently affected organ system in JBS. Examination of pancreatic tissue from 2 fetuses and a newborn infant with JBS showed loss of acinar tissue with inflammation that worsened with gestation, suggesting a gradual destruction resembling pancreatitis as the fetus approaches term.

They next turned to *UBR1* null mouse model. These mice were viable and fertile, but display reduced weight with a proportionate decrease in both muscle and adipose tissue. Their feces contained reduced amounts of chymotrypsin and elastase, indicating pancreatic exocrine insufficiency. They next documented that compared to controls, exocrine cells cultured as acini-like structures exhibited a marked reduced response to treatment with cholecystokinin, the physiologic secretagogue of the exocrine pancreas. The investigators speculated that levels of pancreatic exocrine proenzymes and/or their derivatives may normally need to be kept in check by proteolytic degradation in proteasomes, and that this fails to occur in JBS. Similarly, they suggested that accumulation of proteins normally targeted for degradation by *UBR1* may occur in other tissues and organs affected by JBS.

Zenker M, Mayerle J, Lerch MM, et al. Deficiency of *UBR1*, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). *Nat Genet.* 2005;37:1345–1350.

**Editor's Comment:** *This paper nicely documents not only the mutant gene but also the likely mechanism that accounts for the clinical features of JBS. It should be noted that the biology of ubiquitin has become much more complicated than originally suspected. For example, the number of ubiquitins added to a protein may determine its fate: many ubiquitins (polyubiquitination) usually target molecules to proteasomes, whereas addition of one or a few ubiquitins (monoubiquitination) often targets molecules to lysosomes. Monoubiquitination at multiple sites is responsible for lysosomal targeting and signal termination of many if not most activated receptor tyrosine kinases.*

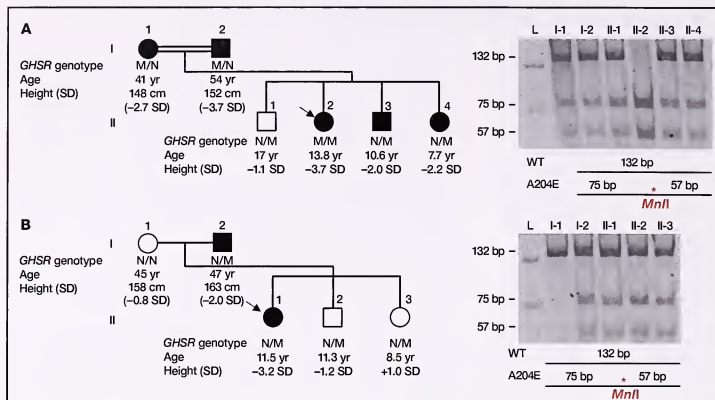
William A. Horton, MD

## Ghrelin Receptor Mutation: A Novel Pathogenic Mechanism of Growth Failure

In 1996, a leading article published in *Science*<sup>1</sup> described "a new receptor in the pituitary and hypothalamus that stimulates growth hormone release." It was cloned as the target of a family of synthetic molecules and named the growth hormone secretagogue receptor (GHSR). The endogenous ligand of this receptor is ghrelin, a hormone predominantly produced by the stomach, whose plasma levels fluctuate with food intake.<sup>2</sup> This hormone stimulates GH secretion and increases food intake and body weight. The G protein-coupled receptor (GPCR) displays a constitutive activity at almost 50% of its maximal capacity. The ligand-independent activity has remained unclear until the present study by Pantel and colleagues. This is the first report which identifies a GHSR missense mutation, Ala204Glu in the first exon,

that segregates with short stature (SS) within 2 unrelated families, one of which also had GH deficiency (GHD).

Initially, there was a systematic search among subjects with SS leading to the identification of the same nucleotide variation in 2 unrelated patients: one with idiopathic GHD (IGHD) was found to be heterozygous for the mutation, whereas the other with idiopathic SS (ISS) was homozygous. The families of the 2 probands were analyzed. Altogether, the data showed that all individuals with SS (n=7) carried at least one mutated allele. Conversely, 3 heterozygous individuals had a normal height. The finding is in keeping with a dominant mode of inheritance and incomplete penetrance (Figure). Idiopathic GHD, diagnosed by low response to standard stimulation tests, was found in 2 cases, one in each family



Inheritance of the A204E *GHSR* mutation in families 1 and 2. (A) Family 1. (B) Family 2. Circles and squares denote female and male family members, respectively. The SD to mean height for age is given below each symbol; height values are before GH treatment. Black symbols denote a short stature. The probands are indicated by arrows. The segregation of the *GHSR* A204E allele within both families was carried out by means of a specific restriction fragment length polymorphism (the A204E mutation creates an *Mnl*I site). Reprinted with permission: Pantel J, et al. *J Clin Invest*. 2006;116:760-768. Copyright © American Society for Clinical Investigation. 2006. All rights reserved.

and both heterozygous. The 3 patients who received GH treatment increased their growth velocity.

The mode of action of this mutation was carefully analyzed showing that it was a significantly impaired functional activity of the receptor:

- The A204E mutant was efficiently translated into a protein, but with only a small fraction properly expressed at the cell surface. This plasma membrane fraction displayed a normal activity for ghrelin.
- The mutation led to a loss of constitutive activity of the receptor, ie, ligand-independent activity. Some experiments using an *in vitro* transcription system also suggest the absence of negative dominant effect of the mutant over the wild-type.
- Ghrelin was able to stimulate *in vitro* the c-AMP response element (CRE) pathway through the mutant receptor in spite of its decreased cell-surface expression.

Pantel et al concluded that the involvement of the *GHSR* A204E mutation in SS transmitted over 2 generations was supported by the following evidence: all patients within the 2 families carried this mutation which in turn was absent in an appropriate control population; the mutation and the amino acid polarity predicts changes in molecular activity; and the findings point to a functional importance of the *GHSR* constitutational activity.

The authors speculated that given the documented pharmacological effects of ghrelin on GH release, the SS results from abnormal regulation of the GH axis. The heterogeneity of the findings related to GH and

insulin-like growth factor (IGF)-I in the 2 families may be related to the well-known limitations of the current methods of clinical investigation. This study, along with the mutated murine models, supports the (debated) view that ghrelin and GH/IGF-I interact in the control of growth.

Pantel J, Legendre M, Cabrol S, et al. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest*. 2006;116: 760-768.

**Editor's Comment:** This is an elegant and well-conducted study that introduces the concept of fine regulation of growth by a mutation of a receptor which until now

did not prove to be essential in achieving normal stature. In addition, it provides evidence for the *in vivo* importance of its ligand independent signalling as expressed by its constitutive activity. An interesting "commentary" paper is published in the same issue by Holst and Schwartz<sup>2</sup>; they refer to a previous German case of ISS and the same mutation<sup>1</sup> and also point out that obesity is an additional symptom that segregates with this mutation and a Phe279Leu mutation which shares the same effect on receptor constitutive activity. Holst and Schwartz suggest that selective loss of ghrelin receptor constitutive activity causes a syndrome of SS and obesity developing around puberty. How these molecular changes impair growth and GH secretion and furthermore, how they are involved in hunger control and obesity, remains in part speculative and challenging for future research.

It is interesting that clinical and genetic investigation essentially by systematic structure-function analysis opens new physiological concepts. It should be taken into account when performing population studies on the multifactorial control of growth and, more specifically, by GH and IGF secretion and activity. In addition, these studies open new possible mechanisms on the weight-to-height relationship in patients with SS. The authors briefly commented on the positive response to GH therapy in 3 patients. Eventually, their data, part of which are presented in the Table, would deserve an additional publication. More studies need to be performed in order to consider the potential development of pharmacological tools in relation to this uncommon and likely pathophysiology of SS and/or obesity.

Raphaël Rappaport, MD



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## Obestatin Opposes Ghrelin's Effects on Food Intake

Ghrelin, primarily a product of the oxyntic cells of the gastric fundus, is an orexigenic agent that was originally identified as the endogenous ligand of the growth hormone (GH) secretagogue receptor. Ghrelin is a 28 amino acid peptide with its serine-3 residue n-octanoylated; it is encoded by *GHRL* (OMIM 605353, chromosome 3p26-p25) and is derived from a 117 amino acid precursor peptide. Ghrelin reduces peripheral energy expenditure and enhances appetite by activating neurons that express Agouti-related peptide and neuropeptide Y that in turn inhibit expression of the anorexigenic neuromodulators that function through melanocortin and MC4R to depress appetite as well as to increase peripheral energy utilization.<sup>1</sup> At the carboxyl terminal of the 117 amino acid precursor, Zhang and colleagues identified an amidated 23 amino acid peptide that suppressed appetite in rats and named it "obestatin".<sup>2</sup> Its name was derived from the Latin "obedere," meaning "to devour."<sup>2</sup> This peptide decreased food intake whether administered peripherally or centrally, suppressed body weight gain, delayed gastric emptying and inhibited jejunal contractility. The investigators next identified the putative receptor for obestatin—the orphan G-protein-coupled receptor (GPR)-39—that functioned by enhancing adenylyl cyclase activity ( $G_{\alpha s}$ ). Although derived from the same precursor, the secretory patterns of ghrelin and obestatin differed significantly. In response to fasting, serum concentrations of immunoreactive ghrelin increased while those of obestatin did not change. The investigators concluded that the physiological role of obestatin in the regulation of energy consumption and use had yet to be determined.

Zhang JV, Ren P-G, Avsian-Kretschmer O, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*. 2005;310:996–999.

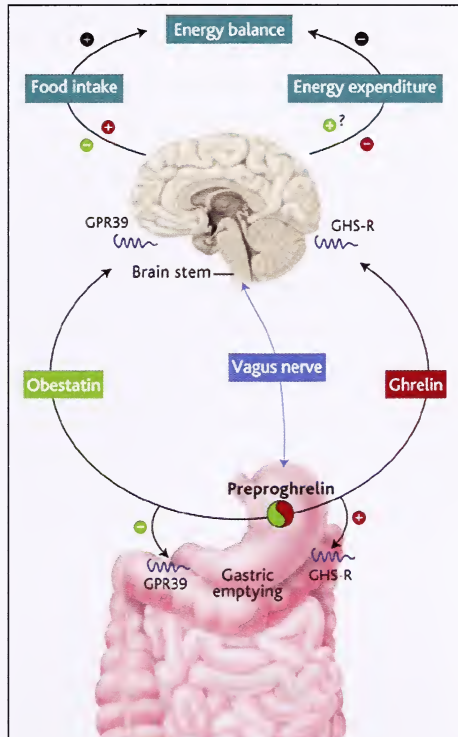
**Editor's Comment:** That one gene can encode more than one peptide product is well illustrated by POMC whose primary product proopiomelanocortin is the precursor peptide from which ACTH, MSH, and  $\beta$ -endorphin are derived. Similarly, *CALCA* encodes calcitonin and calcitonin gene-related peptide. However, it appears unique that one peptide gives rise to products that apparently antagonize each other's actions and yet are differentially secreted (or alternatively catabolized). One eagerly awaits elucidation of the regulation of obestatin secretion and its physiologic role. Although obestatin did not stimulate or suppress the secretion of growth hormone from cultured rat pituitary cells in vitro, its effects on ghrelin-stimulated growth hormone release were not examined. It would be of interest

if it had properties similar to those of somatostatin in this regard. Study of the effect of obestatin on ghrelin-mediated food intake would also be of immense interest.

Allen W. Root, MD

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- Nogueiras R, Tschöp M. *Science*. 2005;310:985–986.



## The Yin and Yang Personalities of Ghrelin and Obestatin.

Both hormones derive from the same precursor protein and are predominantly secreted by the stomach and released into the blood. Each acts on a different receptor (GPR39 and GHS-R, as shown) and has an opposite effect on food intake, body weight, and gastrointestinal motility.

Reprinted with permission. Nogueiras R, Tschöp M. *Science*. 2005;310:985. Copyright © AAAS, 2005. All rights reserved. PHOTO CREDIT: K. SUTLIFF/SCIENCE



## Endocrinological and Auxological Abnormalities in Children with Optic Nerve Hypoplasia: A Prospective Study

Ahmad and associates performed a prospective observational study of 47 children with optic nerve hypoplasia (ONH [deMorsier's syndrome]) who presented to the Pediatric Ophthalmology clinic at Children's Hospital Los Angeles. Subjects 3 years of age and under were enrolled in the study and were followed annually until 5 years of age for visual growth and neurodevelopment outcomes. Although 170 subjects have been enrolled, the data presented are for the first 47 subjects to have completed the study. All subjects had baseline endocrinological, electrophysiological, and neuroradiological findings. Growth hormone (GH) status was defined by insulin-like growth factor (IGF)-I and/or IGF binding protein (BP)-3 or subnormal GH responses to glucagon stimulation. Height (or length) and weight were measured at each visit.

Hormonal dysfunction was found in 71.7% of these children. A growth hormone axis abnormality was observed in 64.1%, hyperprolactinemia in 48.5%, hypothyroidism in 34.9%, adrenal insufficiency in 17.1%, and diabetes insipidus in 4.3%. There was no association between endocrine abnormalities and unilateral versus bilateral ONH. In addition, the absence of the septum pellucidum or other pituitary abnormalities was not associated with endocrinologic function. There was no statistically significant difference in the median start versus end height SDS, but there was a significant increase noted for the median weight SDS. In the cohort, 44.4% were >85<sup>th</sup> percentile for weight at the end of the study. There were 27 subjects who had both IGF-I and IGFBP-3 assessed. The data were dichotomized as "both normal" or "at least one abnormal hormone surrogate." Using this division, there was no significant difference in the median change in height, weight, or in body mass index (BMI) over time. Eight of the subjects received GH replacement. Of the 19 subjects not receiving GH therapy, 10 had one abnormal GH surrogate. Although

the change in height was statistically significant for those receiving GH therapy, those children who did not receive GH treatment continued to grow, with significant BMI increase.

The authors pointed out that there is an unclear understanding of the etiology of ONH. The current study which confirmed a high prevalence of endocrinopathy showed no association between endocrine abnormalities and unilateral versus bilateral ONH, although subjects with a pituitary abnormality on neuroimaging had an endocrinopathy. Seventy-one percent of those with a normal pituitary gland also had an endocrinopathy. The authors speculated that one of the possibilities for explaining the weight gain might be a decreased lipolytic activity resulting from the absence of GH, as suggested in patients with Prader-Willi syndrome.

Ahmad T, Garcia-Fillon P, Borchert M, Kaufman F, Burkett L, Geffner M. Endocrinological and auxological abnormalities in young children with optic nerve hypoplasia: a prospective study. *J Pediatr*. 2006;148:78–84.

**Editor's Comment:** *This is a very interesting observational study, which provides important information for pediatric endocrinologists, geneticists, and pediatricians who care for children with ONH. Importantly, it demonstrates that it is not sufficient to evaluate these children endocrinologically at only one point in time. In addition, it is not sufficient to assume that these children do not have GH deficiency because they continue to experience linear growth. Indeed, the authors have shown that many of these children continue to grow linearly and to gain excessive weight. The suggestion that these children may be candidates for GH treatment regardless of their GH surrogate status is appealing and deserves further investigation.*

William L. Clarke, MD

## Growth Hormone Receptor Exon-3 and Response to Growth Hormone Treatment

A polymorphism in the growth hormone receptor (GHR) gene, the presence or absence of exon-3, has recently been shown to influence the 1- and 2-year growth response to recombinant human growth hormone (rhGH) therapy in children without GH deficiency (GHD). To study the influence of GHR-exon-3 genotype on the short- and long-term response to rhGH therapy in children with GHD, Jorge et al genotyped and followed the first year growth velocity following rhGH treatment in 58 children (36 boys, 22 girls) who remained prepubertal and the adult height of 44 patients (included 27 patients analyzed for the first-year response) after  $7.5 \pm 3.0$  years of treatment.

Clinical and laboratory data at the start of treatment

were indistinguishable among patients carrying GHR-exon-3 genotypes. Patients carrying at least one exon-3 deleted GHR (GHRd3) allele had a significantly better growth velocity in the first year of treatment ( $12.3 \pm 2.6$  vs  $10.6 \pm 2.3$  cm/year,  $p < 0.05$ ) and achieved a taller adult height (final height SDS of  $-0.8 \pm 1.1$  vs  $-1.7 \pm 1.2$ ,  $p < 0.05$ ) when compared with patients homozygous for GHR full-length alleles (GHRfl). They conclude that patients with GHD who are homozygous for GHR exon 3fl were less responsive to short- and long-term rhGH therapy. Approximately half of the population is homozygous for GHRfl; thus, future studies adjusting rhGH therapy to genotype may improve outcome to therapy.

Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonça BB, Arnhold LJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab.* 2006;9:1076–1080.

**Editor's Comment:** Different variables can influence the growth velocity and the final height of children treated with rhGH, but so far there is no way of accurately predicting response to therapy. Duration of treatment, height SDS at the start of treatment, bone age delay, midparental height, and growth velocity during the first year of treatment, are some of the variables which could influence final height after therapy. However, as suggested by Jorge et al, these variables only partially explain the inter-individual variability response to rhGH treatment in children with GHD. The GHR gene is an obvious candidate to influence the response to rhGH. The GHR gene is located in the short arm of chromosome 5; two of the most common isoforms of GHR in humans are generated by retention of GHRfl or exclusion of GHRd3. The frequency of each allele in humans ranges from 68% to 75% for GHRfl and from 25% to 32% for GHRd3.

Patients reported in this paper with GHD who were homozygous for GHR exon 3fl were less responsive to short- and long-term rhGH therapy. However, Pilotta et al<sup>1</sup> recently evaluated 54 GHD children treated for at least one year with rhGH; they found no significant differences in growth velocities between groups of subjects defined by polymorphic genotypes, and concluded that the

most common polymorphisms, alone or in association, did not appear to affect the growth response to rhGH in GHD children. On the other hand, studies by Dos Santos et al<sup>2</sup> and Binder et al<sup>3</sup> support the theory that there is increased responsiveness to high dose rhGH in association with GHRd3 genotype in patients with Turner syndrome, small for gestational age (SGA), and idiopathic short stature; the magnitude of this effect may depend on the primary cause of the short stature. The Binder group demonstrated that girls with Turner syndrome who were homozygote for the GHRd3 variant showed the highest increment in height velocity and exceeded their growth prediction, whereas short children born SGA demonstrated only a mildly increased response to high-dose rhGH in the presence of the GHRd3 variant. Genotyping of the GHRd3 protein polymorphism may prove to be a tool for a more precise understanding of rhGH effects on growth and for the individualization of rhGH dosing in both GHD and non-GHD children; however, its effectiveness is still in doubt.

Roberto Lanes, MD

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## IGF-I and IQ in Middle Childhood

Gunnell et al examined the association between circulating levels of insulin-like growth factor (IGF)-I, its main binding protein, IGFBP-3, and subsequent measures of IQ. Data were obtained from the Avon Longitudinal Study of Parents and Children (ALSPC, n=13 617). The study consisted of 547 white singleton children (301 boys, 246 girls), with IGF-I and IGFBP-3 measurements obtained at a mean age of 8 years and IQ measured with the Wechsler Intelligence Scale for Children (WISC-III) at a mean age of 8.7 years. Speech and language were also measured by the Wechsler Objective Reading Dimensions (WORD; assessed at 8.7 years) and Wechsler Objective Language Dimensions (WOLD; assessed at 7.5 years) tests. Some children (n=407) had IGF-I levels measured at approximately 5 years of age in a previous study.

The mean IGF-I (ng/mL) level at age 8 years was 142.6 ( $\pm$  53.9) and 154.4 ( $\pm$  51.6) for boys and girls, respectively. For every 100 ng/mL increase in IGF-I, IQ increased by 3.18 points ( $p=.019$ ) for boys and girls combined. This relationship achieved statistical significance only for girls. A statistically significant association was not detected between IGFBP-3 or IGF-I/IGFBP-3 ratios and IQ. WISC-III subtests are classified as Verbal or Performance: associations between IGF-I and IQ were restricted to the

Verbal component. The IGF-I levels were not significantly associated with either WOLD or WORD test scores for the combined sample of boys and girls. A positive statistically significant association between IGF-I levels and WORD scores was detected for girls, but not for boys. Associations between IGF-I levels at age 5 and WISC-III scores were similar to those for IGF-I levels measured at age 7 to 8, applied to both the boys and girls, but were restricted to the Verbal IQ.

Follow-up analyses were performed statistically, controlling for potential confounding variables. Introducing birth weight (adjusted for gestation), breastfeeding, and BMI to the regression model strengthened the association between IGF-I and IQ; whereas controlling for maternal education and IGFBP-3 attenuated the association, as did adjustment for housing status and family socioeconomic status. The authors suggest that rather than confounding the associations of IGF-I levels with IQ, parental education and socioeconomic status may serve as markers of their offspring's intelligence. The authors concluded "Offspring IGF-I levels are likely to be associated with parental IGF-I levels, through shared genetic influences. This study provides some preliminary evidence that IGF-I is associated with brain development in childhood. Additional

longitudinal research is required to clarify the role of IGF-I in neurodevelopment. Because IGF-I levels are modifiable through diet and other environmental exposures, this may be one pathway through which the childhood environment may influence neurodevelopment."

Gunnell D, Miller LL, Rogers I, Holly JMP, the ALSPAC Study Team. Association of insulin-like growth factor I and insulin-like growth factor-binding protein-3 with intelligence quotient among 8- to 9-year-old children in the Avon Longitudinal Study of parents and children. *Pediatrics*. 2005;116:e681-e686.

**Editor's Comment:** *The prospect of an association between IGF-I, brain development, and intelligence is not new,<sup>1</sup> but remains intriguing. The importance of the Gunnell study lies in the cohort design of the ALSPAC, the quality of the psychological/cognitive assessments, and detailed characterization of important contextual variables in child development (eg, diet and socioeconomic status of the family). Evidence that growth factors (rather than psychosocial stress associated with short stature) may be responsible for educational and vocational outcomes suggests that stature and growth can be viewed as proxies for other biologic events rather than as a focus for its own sake.*

*Findings from a controlled study by Kranzler and colleagues<sup>2</sup> on the intellectual ability of children with growth hormone receptor deficiency (GHRD) (and accompanying severe IGF-I deficiency) are difficult to reconcile with the Gunnell report. Kranzler compared the intellectual ability of 18 school-age Ecuadorian GHRD probands with that of 42 relatives and 28 controls. The intellectual ability of those with GHRD was not significantly different from their relatives, and was*

*comparable to controls. Furthermore, homozygosity or heterozygosity for the mutation in the GHR gene common to Ecuadorian patients was unrelated to intelligence. The authors concluded that GH-induced IGF-I production is not required for normal brain growth in utero or for postnatal intellectual development.*

*It may be overly simplistic to question, but if circulating values of IGF-I are positively related to intellectual function, then would GH-mediated increases in IGF-I result in higher performance? Indirect supportive evidence comes from a study of the effects on IQ scores of GH administered to children born small for gestational age.<sup>3</sup> Growth hormone treatment was associated with significant increases in relative height along with improved IQ. Because it can be assumed that GH treatment raised IGF-I levels, then perhaps IGF-I effects on the central nervous system mediated the effects of GH on intellectual ability. Interestingly, it was only the Performance IQ that showed improvement with GH treatment; the opposite pattern was observed in the Gunnell study. Clearly, all these findings require replication, and hopefully future investigations will be guided by a priori predictions regarding the effects of growth factors on brain development and function in order to reduce the probability of Type I errors.*

David E. Sandberg, PhD

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## THE SOTOS SYNDROME - NSD1 HAPLOINSUFFICIENCY: CEREBRAL GIGANTISM UPDATE

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### INTRODUCTION

Cerebral gigantism (OMIM 117550) is characterized by excessive pre- and postnatal growth, a characteristic face, and developmental delay with a prevalence of ~1:14,000 births.<sup>1</sup> In more than 90% of patients, Sotos syndrome is due to haploinsufficiency of *NSD1* (Nuclear Receptor-Binding Site, Enhancer of Zeste, and Trithorax Domain Protein 1, chromosome 5q35, OMIM 606681).<sup>2</sup> Cerebral gigantism is thus a genomic

disorder—a pathologic state due to loss, gain, or disruption of a dosage-sensitive gene that results in a recognized phenotype.<sup>3</sup>

### CLINICAL CHARACTERISTICS

In patients with cerebral gigantism, rapid linear growth begins during gestation; at birth, length is more than +2 standard deviations (SD) above mean length for gestational age and gender in 85% of neonates, while birth weights are usually within the high normal range. Neonates with Sotos syndrome may also have prolonged jaundice, hypotonia, and feeding difficulties.<sup>2</sup> Linear growth remains rapid throughout infancy and childhood. Because skeletal maturation is also advanced, adult heights of patients with Sotos syndrome are usually near or slightly

### From The Editor's Desk

Dear Colleague:

This column usually highlights the content of the journal; in this issue I want to bring to your attention the e-reviews and editorial comments. This section was expanded to 10 reviews of current articles as more of our readers are taking advantage of this feature. The online reviews and comments are often slightly longer or contain more detail than the printed reviews. The article on sex assignment attitudes of pediatric urologists is interesting and worthy of your consideration. The new diagnostic imaging techniques in congenital hypothyroidism should become available in all medical centers. The issues of quality of life and mental health of adolescents seeking bariatric surgery need to be considered as we now deal with this problem on a frequent basis. Not least are the important data discussed in the other reviews dealing with clinical conditions such as CAH, Klinefelter's syndrome, and progeria, as well as reviews of experimental data on GHR, longevity and calorie restriction, hippocampal GH, and congenital contractures.

The lead article is a much-needed update on Sotos syndrome which was first described in 1964, at a time when heterozygous microdeletions in *NSD1* were not known, but are present in over 90% of these patients. Drs. Root and Diamond reviewed the genetic considerations of this condition and didactically clarified the mechanisms of the disease. The 8 reviews and comments complete this issue with an array of the most pertinent recent advances in the field.

Other pertinent additions to www.GGHjournal.com include new sections on clinical guidelines and clinical trials. These are most useful for those who want current recommendations or to apprise themselves of the clinical research trials ongoing in the field and other areas. The search capability and archives sections have been enhanced and a very important feature added—a CME offering is now available on the resources webpage.

Fima Lifshitz, MD



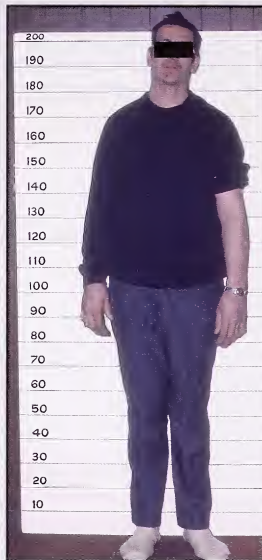
above +2 SD; however, adult stature usually exceeds target height by an average of 11 cm in males and 6 cm in females (Figure 1).

Occipito-frontal head circumference is also increased during infancy and childhood and remains above the 97<sup>th</sup> percentile in most adults with Sotos syndrome. However, in 10% of subjects with cerebral gigantism, height and head circumference remain within the normal ranges.<sup>4</sup> The “acromegalic-like” face of the patient with cerebral gigantism is characterized by a high, broad, and bossed forehead with sparse fronto-temporal hair, long and narrow face, down-slanting of the palpebral fissures, malar flushing, and a sharply pointed, prognathic mandible that becomes more evident over time.<sup>5,6</sup> The palate is highly arched; hands and feet are prominent; and scoliosis occurs frequently. Subjects with Sotos syndrome also have anomalies of the cardiovascular (patent ductus arteriosus, atrial septal defect), genitourinary (agenesis, duplication, vesicoureteral reflux, hypospadias, cryptorchidism), and central nervous systems (hypoplasia of the corpus callosum, ventricular dilatation, enlarged extracerebral fluid spaces); electroencephalographic abnormalities and seizures occur in some subjects. Most but not all patients with cerebral gigantism have mild to severe developmental delay, a problem that may ameliorate somewhat as the patient ages.<sup>7</sup> In addition, Sotos syndrome patients may develop aggressive behavior and may manifest psychoses in adulthood.<sup>8,9</sup> Neoplasms develop in 2% to 4% of children with cerebral gigantism, including acute lymphoblastic leukemia, T-cell lymphoma, Wilms tumor, sacrococcygeal teratoma, presacral ganglioneuroma, hepatocellular carcinoma, neuroblastoma, and ganglioglioma.<sup>10,11</sup>

## **PATHOGENESIS**

The mechanism(s) underlying the extreme growth of patients with Sotos syndrome is unknown. Growth hormone secretion is normal in patients with cerebral gigantism; serum concentrations of insulin-like growth factor (IGF)-I and acid labile subunit have been normal, a bit low, or somewhat elevated in various reports.<sup>12</sup> In some subjects, serum levels of IGF-II and IGF-binding proteins (IGFBP)-3 and -4 have been within the low normal range. The rate of IGFBP-3 proteolysis was accelerated in one report, suggesting that free IGF-I values might be increased in this disorder.<sup>12</sup> Prostate specific antigen (PSA) is one of several proteolytic

**Figure 1.**



A 22 year old male with cerebral gigantism.

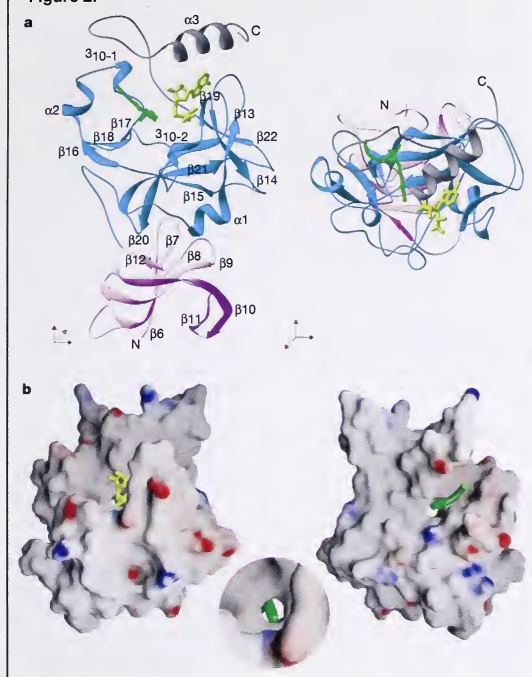
enzymes that degrade IGFBP-3.<sup>13,14</sup> Perhaps measurements of PSA levels in patients with cerebral gigantism would be of interest and contribute to our understanding of the pathogenesis of this disorder.

## **GENETICS**

Heterozygous microdeletions and loss-of-function mutations in *NSD1* resulting in haploinsufficiency of the gene product have been identified in more than 90% of patients with Sotos syndrome.<sup>2,4,5</sup> *NSD1* contains 23 exons and encodes a 2596 to 2696 amino acid, broadly expressed protein (brain, muscle, kidney, spleen, thymus, lymph node, lung) that functions as a co-regulator of transcription by interacting with nuclear transcription factors and as a histone methyltransferase.<sup>11</sup> Within the structure of *NSD1*, there is a SET domain, a conserved sequence of approximately 150 amino acids that remodels chromatin structure by histone methylation, thereby modulating gene transcription; *NSD1* specifically methylates lysine-36 of histone H3 and lysine-20 of histone-

H4.<sup>15</sup> By inserting its side chain into a cleft within the SET-containing protein, the selected histone H3/4-lysine accesses both the enzymatic site and its methyl donor S-adenosyl-L-methionine.<sup>16</sup> Figure 2 shows a structure of the SET/9 ternary complex. This structure is highly specific for the histone methyltransferase target. Methylation of one or the other histone H3/4-lysine residues usually, but not necessarily always, exerts an inhibitory (silencing) effect on the transcription of a targeted gene. Encoded within *NSD1* are several additional functional domains, including one SET-associated cysteine-rich domain, two nuclear receptor interactive domains (exon 2), two proline-tryptophan-tryptophan-proline (PWWP) domains (exons 3-4, 15-17), and 5 plant homeodomains (PHD) (exons 11-17, 22).<sup>5,6</sup> A PHD has a zinc-finger structure that permits interaction with chromatin, while the PWWP domains are involved in protein-protein interaction.

*NSD1* binds to transcription factors and co-factors where it may behave as either a co-activator or co-repressor, depending on which of its two nuclear interactive domains is involved.<sup>5,11</sup> By binding to the intact or carboxyl terminal region of the nuclear androgen receptor (AR) through its activating nuclear receptor interactive domain, *NSD1* acts as a co-regulatory factor that enhances AR transcriptional

**Figure 2.**

Structure of the SET 7/9 ternary complex. **a**, Two orthogonal views of the SET 7/9 ternary complex in ribbons representation. The N-terminal domain is colored pink, the SET domain is blue and the C-terminal segment is grey. The H3 peptide is indicated in green, with the side chain of methylated Lys 4 shown. The S-adenosyl-L-homocysteine (AdoHcy) cofactor is colored yellow. The secondary structure elements are labeled according to our earlier structure. Two small turns of the 310 helix are also labeled. **b**, Two views of the SET domain are shown in a surface representation colored according to electrostatic potential (the two views are related by a twofold rotation about a vertical axis). The left panel shows AdoHcy colored yellow; the right panel shows the H3 peptide colored green. The inset panel shows a close-up view of the lysine access channel containing the methyl lysine side chain as viewed from the S-adenosyl-L-methionine (AdoMet)-binding site.

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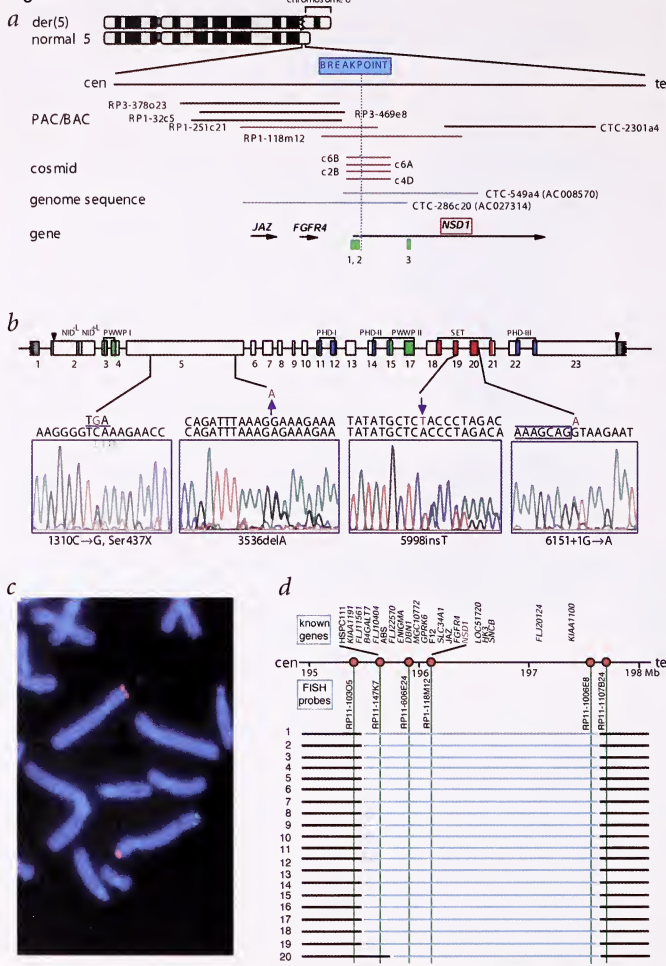
activity. *NSD1* also interacts with NIZP1, a zinc-finger DNA-binding repressor of transcription that directs the histone methyltransferase SET domain of *NSD1* to targeted gene promoters.<sup>17</sup> *NSD1* and other SET-domain containing proteins interact with factors that regulate cell growth and have been implicated in several human malignancies such as acute myeloid leukemia of childhood.<sup>18</sup> However, the mechanism(s) by which inactivating mutations of *NSD1* lead to the clinical manifestations of Sotos syndrome remains unclear at present. Since homozygous *NSD1*<sup>-/-</sup> mutant mouse embryos succumb very early in gestation, *NSD1* is also

crucial for early post-implantation mammalian fetal development.<sup>13</sup> However, the *NSD1*<sup>+/-</sup> mutant mouse is phenotypically normal.

Microdeletions of 1.9Mb-encompassing *NSD1* are the most common mutations identified in subjects with cerebral gigantism of Japanese ancestry<sup>19</sup> (Figure 3). Mechanistically important in the process that leads to microdeletions of *NSD1* in this population is non-allelic homologous recombination or unequal rearrangement of low-copy repeat sequences that flank the distal and proximal breakpoints that encompass *NSD1*.<sup>4,20,21</sup> Preferentially, microdeletions of *NSD1* are of paternal origin in Japanese patients with Sotos syndrome. Their fathers have a heterozygous inversion of nucleotides flanking *NSD1* on chromosome 5 that predisposes to unequal intrachromosomal recombination during meiosis that leads to microdeletions (or duplications) in their progeny.<sup>11,21,22</sup> In western populations, microdeletions of *NSD1* are variable in size, due to interchromosomal rearrangement, and far less frequent, accounting for less than 10% of the identified *NSD1* mutations in patients with Sotos syndrome. More than 100 intragenic inactivating splice site, frameshift (due to small insertions and deletions), nonsense, and missense *NSD1* mutations that account for more than 90% of the genetic abnormalities identified in non-Japanese patients with cerebral gigantism have been identified.<sup>5,23</sup> Missense mutations are clustered between exons 13 and 23 within conserved functional domains.<sup>24</sup>

There is a vague phenotype-genotype relationship in Sotos syndrome: thus, macrosomia, developmental delay, and minor anomalies are present in these patients with either intragenic mutations or gene microdeletions. However, patients with cerebral gigantism and microdeletions of *NSD1* tend to have rather severe developmental delay and major structural anomalies of the central nervous, cardiovascular, and genitourinary systems, but only modest overgrowth.<sup>4,25</sup> On the other hand, patients with intragenic mutations may express less severe anomalies but demonstrate greater linear overgrowth.<sup>2,25</sup> Nevertheless, unrelated Sotos syndrome patients with identical mutations in *NSD1* may have different phenotypes.<sup>4</sup> In neonates with mutations in *NSD1*, birth length is substantially greater than in subjects with clinical Sotos syndrome but an intact gene.<sup>26</sup> Arm span relative to height and hand length relative to age are substantially greater in patients with Sotos syndrome (due to a mutation in *NSD1*) than in subjects with clinical

**Figure 3.**



NSD1 mutations in individuals with Sotos syndrome. **a**, BAC/PAC/cosmid map spanning the Sq535 breakpoint. Red and blue horizontal lines indicate clones spanning the breakpoint (detected by FISH analysis) and complete genomic sequences, respectively. Arrows indicate genes, and green boxes below *NSD1* represent exons 1, 2 and 3. **b**, Genomic structure of *NSD1* and four point mutations found in individuals with Sotos syndrome. Open and gray boxes and arrowheads indicate exons, the 5' and 3' untranslated regions, and start and stop codons, respectively. Specific domains are indicated by colored boxes, and sequence traces disclose mutations in lower row. **c**, FISH analysis of the affected individual harboring the deletion. Absence of a FISH signal for RP1-118m12 containing *NSD1* (green) along with the presence of 5pter signals (red) on the individual's chromosome 5 is apparent. **d**, Summary of FISH deletions in 20 affected individuals. Known genes, probes used and their genomic locations are indicated in the upper row. Numbers (1–20) and black and blue lines represent affected individuals, regions without deletion and those regions deleted, respectively.

manifestations of cerebral gigantism and normal *NSD1*.<sup>27</sup> Developmental delay may be more severe in patients with a mutation in *NSD1* than in those with clinically diagnosed cerebral gigantism.

Mutations or deletions of *NSD1* most often arise *de novo* and thus the risk of familial recurrence to phenotypically and genotypically normal parents is low. Nevertheless, a patient with Sotos syndrome due to an intragenic mutation has a 50% risk of transmitting this mutated gene to an offspring.<sup>7</sup> No instance of germline *NSD1* mosaicism has been observed to date. Intragenic mutations in *NSD1* have also been reported in patients with Weaver syndrome (OMIM 277590), an overgrowth disorder that is characterized by macrocrania, hypertelorism, large ears, retrognathia, hypotonia, developmental delay, loose skin folds, dysplastic deeply set nails, sparse hair, and hoarse cry as well as various skeletal anomalies.<sup>24</sup> Patients with Weaver syndrome very rarely develop tumors. In 2 out of 52 patients with the Beckwith-Wiedemann syndrome (BWS - OMIM 130650) who presented with *in utero* macrosomia, macroglossia, hemihyperplasia, and abdominal wall defects, mutations in *NSD1* were also reported.<sup>28</sup> A microdeletion of *NSD1* was detected in an infant girl with features of both Sotos and Nevo syndromes (OMIM 601451).<sup>29</sup> However, there is evidence that suggests that the Weaver, BWS, and Nevo syndrome patients studied in these reports were more likely to have had clinical variations of Sotos syndrome.<sup>6,30</sup> Anomalies of 11p15, the site of imprinting errors associated



**Table 1. Scoring System for Clinical Diagnosis of Cerebral Gigantism.**

| Criteria               |                                    | Score |
|------------------------|------------------------------------|-------|
| Facial characteristics | 5 or 6 present                     | 5     |
|                        | 2 to 4 present                     | 3     |
|                        | ≤1 present                         | 0     |
| Growth                 | Height SDS - TH SDS >2             | 2     |
|                        | Height SDS - TH SDS ≤2 (past data) | 1     |
|                        | Height SDS - TH SDS ≤2             | 0     |
| Head circumference     | ≥2 SDS                             | 1     |
|                        | <2 SDS                             | 0     |
| Development            | IQ <90 - delayed                   | 1     |
|                        | IQ ≥90                             | 0     |
| Bone age               | Consistently advanced              | 2     |
|                        | Adult                              | 1     |
|                        | Normal for age                     | 0     |

**Sum 9-11: Typical Sotos syndrome**

Adapted from reference 12.

Facial characteristics: frontal bossing, high hairline, dolicocephaly, prominent chin, highly arched palate, anti-mongoloid slant of palpebral fissures

Growth - all measurements before adult height

SDS - Standard deviation score

TH - Target height

**Table 2. Recommended Surveillance of Children With Cerebral Gigantism for Tumor Development: Yearly Frequency.**

| Age              | Physical examination | CBC | Abdominal ultrasound | Chest x-ray | α-Feto-protein | β-hCG | Urine catecholamines |
|------------------|----------------------|-----|----------------------|-------------|----------------|-------|----------------------|
| Birth to 4 years | 4                    | 3   | 2                    | 1           | 2              | 2     | 1                    |
| 4 to 10 years    | 3                    | 3   | 2                    | 1           | 2              | 2     | 1                    |

Adapted from reference 10.

with BWS, were identified in 2 out of 20 patients with cerebral gigantism, including one with paternal isodisomy of 11p15 of the *H19* locus and one with partial isolated demethylation of *KCNQ10T*, perhaps suggesting a functional relationship between *NSD1* and the imprinting centers on 11p15.<sup>2,28</sup>

**DIAGNOSIS AND MANAGEMENT**

The diagnosis of Sotos syndrome is established by clinical findings (characteristic facial features, prenatal and postnatal overgrowth, persistently enlarged head circumference, developmental delay, and advanced bone age [Table 1]) and confirmed by identification of a mutation in *NSD1*. Since this disorder is genetically heterogeneous, absence of a mutation does not negate the diagnosis; an abnormal methylation pattern on chromosome 11p15 might be investigated in patients with intact *NSD1*.<sup>28</sup> Cerebral gigantism is to be differentiated from Weaver syndrome and other overgrowth syndromes both on clinical grounds and by the presence of mutations in *NSD1*. Treatment is symptomatic and focuses on monitoring of growth and periodic surveillance

for associated systemic anomalies or development of neoplasms and on factors that may ameliorate developmental delay and behavioral problems. It has been recommended that children with Sotos syndrome be surveyed frequently for tumor development through 10 years of age. Complete physical examinations and blood counts should be done 3 to 4 times each year; abdominal ultrasound studies, α-fetoprotein, and β-hCG measurements twice yearly; and chest x-ray and urine catecholamine determinations once each year (Table 2).<sup>10</sup> Given the relatively low incidence of tumors in these patients, these recommendations may be excessive.<sup>11</sup>

**CONCLUSION**

Cerebral gigantism is an overgrowth syndrome characterized by increased *in utero* and postnatal growth, an adult height that is within or slightly above the upper normal range, macrocephaly, a characteristic face, and variable degrees of developmental delay. There is a high incidence of cardiovascular, central nervous and genitourinary malformations in these patients. Tumors occur in 2% to 4% of patients with cerebral gigantism, usually before 8 to 10 years of age. Mutations in *NSD1* seem to be quite specific for Sotos syndrome.<sup>6</sup>

Indeed, all subjects with documented mutations in *NSD1* have some manifestation(s) of cerebral gigantism. The majority of mutations in *NSD1* have occurred *de novo*, but the risk for development

of Sotos syndrome in the offspring of a patient with a mutation in *NSD1* is 50%. Periodic surveillance for tumor development is recommended in children with Sotos syndrome.

**Resources**

Sequence analysis of *NSD1* may be obtained through The Greenwood Genetics Center, Greenwood, SC.

The Sotos Syndrome Support association may be contacted at [www.well.com/user/ssa](http://www.well.com/user/ssa).

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Autoimmune Growth Hormone Deficiency: Whittling Away at Some of the Idiopathics

Lymphocytic hypophysitis is a recognized cause of growth hormone deficiency (GHD) in adults, either isolated or associated with deficiency of other pituitary hormones. Histopathological diagnosis on pituitary biopsy is considered the gold-standard test, though antipituitary antibodies (APA) have been recently identified in adults with idiopathic GHD (IGHD) or GHD and other autoimmune endocrinopathies. The APA-positive adults with IGHD all had documented childhood-onset GHD; thus the authors aimed to investigate the presence of APA in prepubertal children.

De Bellis and colleagues studied APA in 3 groups of prepubertal patients: 26 with IGHD, 60 with idiopathic short stature (ISS), and 33 with organic GHD (destructive lesions or developmental malformations of the hypothalamus-pituitary). The definition of IGHD was a height z score below -2 SD, growth velocity <25th centile, delayed skeletal development, and blunted GH response (<10 µg/L) on both arginine and insulin stimulation tests; all patients had GH peaks <5 µg/L and abnormally low IGF-I levels for age and gender. The definition of ISS was the same except that GH peaked at >10 µg/L on at least one stimulation test. Ten of the 60 ISS patients had abnormally low IGF-I levels. MRI was normal, as were all other pituitary hormone functions, in both groups. Sera from 40 age- and sex-matched normal children were collected as controls. The APA were detected by indirect immunofluorescence on cryostat sections of young baboon pituitary, with fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulins; positive samples were defined as titers >1:8. For sera that were APA positive, antibody specificity for the different anterior pituitary cell types was determined by a 4-layer double immunofluorescence technique, co-localizing the FITC-conjugated anti-human IgG antibody with rodamine-conjugated antibodies directed against each of the pituitary hormones.

The APA antibodies were positive in 27% of the children with IGHD and 23% of those with ISS, but were negative in those with organic GHD and in normal controls. The APA titers ranged from 1:32 to 1:128 in the former and 1:16 to 1:64 in the latter group. Immunostaining confirmed selectivity for pituitary somatotrophs with minor staining of lactotrophs but no other cell type. Three of the 7 APA-positive GHD patients and 8 of the 14 ISS patients had parents or first degree relatives with autoimmune endocrinopathy or non-endocrine disease. Within the ISS group, all 10 patients with abnormally low IGF-I levels were APA positive.

Nineteen of the 60 patients with ISS were re-evaluated 2 years later; 11 had originally been APA negative and 8 APA positive. All 11 negative patients remained APA negative, retained normal GH response to provocative testing, and normal age-dependent increase in IGF-I levels. In contrast, all 8 APA-positive patients demonstrated an increase in their autoimmunity, with titers ranging from 1:32 to 1:128. IGF-I levels remained abnormally low in all 8 patients. Furthermore, 7 developed failure on provoked GH testing. MRI was normal.

The authors concluded that APA against somatotrophs are present in 27% of children with IGHD and 22% of children with ISS. The APA may therefore indicate autoimmune hypophysitis despite the absence of MRI abnormalities. Furthermore, children with APA-positive ISS may represent an earlier stage of autoimmune hypophysitis in which GH reserve is still normal on provocative testing, but with time may develop into full GHD.

De Bellis A, Salerno M, Conte M, et al. Antipituitary Antibodies Recognizing Growth Hormone (GH)-Producing Cells in Children with Idiopathic GH Deficiency and in Children with Idiopathic Short Stature. *J Clin Endocrinol Metab.* 2006; *J Clin Endocrinol Metab.* 2006;91:2484-2489.

**Editor's Comment:** The authors pointed out the need for further testing in larger populations, as their study was not designed to establish predictive and/or pathogenic roles of APA. Nonetheless, this paper provides compelling data and a very plausible model that justifies pursuing this line of research. Endocrinologists certainly have precedent in using antibody titers to try to predict hormonal dysfunction and understand disease pathogenesis in disorders of the pancreas,<sup>1,2</sup> adrenals,<sup>3</sup> and thyroid.<sup>4</sup> From a practical perspective, measuring APA titers is a far more appealing diagnostic test than the inaccessible pituitary biopsy, for both clinicians and

their patients in search of a diagnosis, with the default option of an "idiopathic" non-diagnosis. Hopefully, APA testing will be available to clinicians soon.

Adda Grimberg, MD

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## Impaired Cognitive Function in Congenital Adrenal Hyperplasia

Cognitive function in individuals with congenital adrenal hyperplasia (CAH) is a topic of considerable interest. Effects of the condition or its treatment on cognitive function are plausible, ie, a permanent influence of sex steroid hormones *in utero* on brain development, the genetics of CAH or allied alleles, or the effects of under- or over-treatment with glucocorticoids during early postnatal period. Johannsen and colleagues conducted a case-control study of cognitive function in adult women with CAH. Participants included 35 women (84% of the eligible sample) diagnosed with CAH between 1953 and 2003 at a university hospital in Denmark. The patients with CYP21 mutations were grouped into salt wasters (SWs; n = 19, mean age, 31.2 yr; range, 19-46 yr), simple virilizers (SVs; n = 6, 34.6 yr, 23-51), late-onset (LO) CAH (n = 5, 25.5 yr, 19-36) and a mixed group of patients (mixed; n = 5, 28.8 yr, 17-49) with steroidogenic acute regulatory protein (StAR) deficiency (n = 3), CYP21 deficiency diagnosed in adolescence (n = 1), and 17-hydroxylase deficiency (n = 1). Patients with CYP21

deficiency were categorized by mutation severity, salt-wasting status, and clinical presentation. Control group participants were recruited through a general population registry of women born in the same month and year as the patient (response rate = 38%). The woman with the closest match on education was selected for pair-wise matching with the index patient. All participants received a medical interview, physical examination, psychological interview, cognitive assessment, hormone analyses and personality, sexual, and social functioning questionnaires. Five subtests (3 of 6 Verbal and 2 of 5 Performance) from the Wechsler Adult Intelligence Scale (WAIS) provided 3 indices of intelligence (IQ): full-scale IQs (FSIQ), performance (PIQ), and verbal (VIQ). (WAIS IQ scores are defined to yield a population mean of 100 [SD = 15].) Examiners were not blinded with respect to patients' diagnoses.

The combined CAH patient group achieved significantly lower FSIQ (84.5 vs 99.1), VIQ (86.6 vs 97.3) and PIQ scores (85.7 vs 101.3) than the pair-matched control group (Table). The same pattern was true for the

SW subgroup. The LO patients also achieved significantly lower FSIQ and VIQ scores than matched controls. Further, the mixed group received significantly lower scores than controls on all IQ indices. In contrast, the SV subgroup was not statistically different from control participants. The SW group received significantly lower FSIQ and VIQ than the SV group, and a nonsignificant trend was observed for PIQ. Patients with verified hyponatremic crises (n = 14) vs all other CAH patients (n = 21) revealed significantly lower FSIQ (78.6 vs 88.4) and VIQ (79.9 vs 91.0), but not PIQ (82.9 vs 87.6).

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Full-scale, verbal, and performance IQs in patients with CAH and matched controls.

|                                 | n  | Full-scale IQ<br>[mean $\pm$ SEM (range)] | Verbal IQ<br>[mean $\pm$ SEM (range)] | Performance IQ<br>[mean $\pm$ SEM (range)] |
|---------------------------------|----|---|---------------------------------------|--|
| All CAH patients                | 35 | 84.5 $\pm$ 2.1 (62–114) <sup>a</sup>      | 86.6 $\pm$ 2.0 (64–107) <sup>a</sup>  | 85.7 $\pm$ 2.4 (62–127) <sup>a</sup>       |
| All controls                    | 35 | 99.1 $\pm$ 2.1 (67–133)                   | 97.3 $\pm$ 2.1 (70–132)               | 101.3 $\pm$ 2.0 (73–122)                   |
| Salt-wasting CAH                | 19 | 81.2 $\pm$ 3.2 (62–114) <sup>b</sup>      | 84.7 $\pm$ 2.8 (66–107) <sup>b</sup>  | 81.5 $\pm$ 3.6 (62–127) <sup>a</sup>       |
| Salt-wasting CAH controls       | 19 | 96.5 $\pm$ 2.6 (67–113)                   | 95.4 $\pm$ 2.0 (70–115)               | 99.1 $\pm$ 2.7 (73–120)                    |
| Simple-virilizing CAH           | 6  | 92.8 $\pm$ 2.9 (83–103)                   | 95.5 $\pm$ 3.6 (84–103)               | 91.3 $\pm$ 5.1 (73–105)                    |
| Simple-virilizing CAH controls  | 6  | 95.7 $\pm$ 3.6 (85–110)                   | 92.7 $\pm$ 2.0 (86–101)               | 100.0 $\pm$ 5.4 (84–120)                   |
| LO CAH                          | 5  | 91.6 $\pm$ 4.0 (79–104) <sup>b</sup>      | 90.0 $\pm$ 3.6 (78–99) <sup>b</sup>   | 96.2 $\pm$ 4.0 (87–110)                    |
| LO CAH controls                 | 5  | 105.6 $\pm$ 5.6 (92–124)                  | 104.6 $\pm$ 4.4 (94–119)              | 105.0 $\pm$ 5.6 (91–122)                   |
| Mixed CAH <sup>c</sup>          | 5  | 80.0 $\pm$ 3.7 (67–88) <sup>b</sup>       | 79.4 $\pm$ 4.7 (64–90) <sup>b</sup>   | 84.8 $\pm$ 2.9 (78–92) <sup>b</sup>        |
| Mixed CAH <sup>c</sup> controls | 5  | 106.2 $\pm$ 7.7 (92–133)                  | 102.6 $\pm$ 8.5 (85–132)              | 107.8 $\pm$ 4.1 (99–122)                   |

Significance levels for differences between patients and matched controls are indicated as <sup>a</sup> P < 0.001 or <sup>b</sup> P < 0.05.

<sup>c</sup> Mixed CAH: three patients with STAR deficiency, one patient with 21OH deficiency diagnosed in adolescence, and one patient with 17OH deficiency.

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Johannsen TH, Ripa CPL, Reinisch JM, Schwartz M, Mortensen EL, Main KM. Impaired cognitive function in women with congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2006;91:1376–1381.

**Editor's Comment:** Pediatric endocrinologists have traditionally been taught that cognitive function and IQ in patients with CAH are not usually sources of concern. This study, and others,<sup>1,2</sup> suggest the contrary: individuals with CAH, particularly the SW-variant, are at risk for lower IQ. Elevated prenatal androgen exposure may affect later patterns of cognitive development and cerebral lateralization, thus individuals with CAH may exhibit a male-typical pattern of cognitive strengths and hemispheric lateralization,<sup>3–5</sup> although other research challenges this conclusion.<sup>6</sup>

This paper underscores the importance of partitioning the sample in data analyses according to genetic mutation and clinical sequelae. In particular, those individuals who had suffered multiple hyponatremic episodes should be considered a particularly high-risk group for neuropsychological sequelae. Although these investigators grouped CAH participants into categories according to corticosteroid replacement dose, it is puzzling that accompanying analyses were not reported. Nevertheless, the authors noted that glucocorticoids are important for normal maturation of the developing central nervous system and that excessive doses in infancy (reduced in current treatment recommendations)<sup>7</sup> might be partially responsible for the pattern of intellectual

deficits observed in this cohort.

This study and corroborating findings underscore the importance of surveilling cognitive function among children born with CAH. To this condition, one could add Turner, Noonan, and Klinefelter syndromes, congenital hypothyroidism, children born small for gestational age, early-onset diabetes, and many others who frequent pediatric endocrinology clinics. Forging collaborations between pediatric endocrinology and hospital-based pediatric psychology or child psychiatry programs that can offer neuropsychological evaluations would likely spare many youths (and their families) needless academic failure and frustration.

David E. Sandberg, PhD

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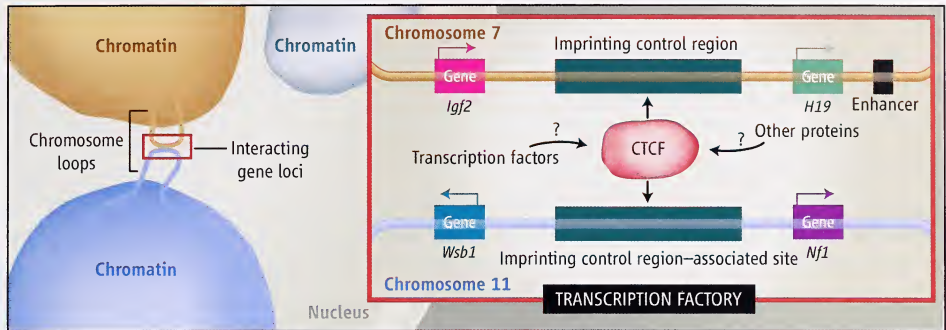
## Imprinting, Transcription Factories, and Igf2 Regulation

An important regulator of fetal growth, insulin-like growth factor 2 (Igf2), has received much attention in recent years because it is imprinted, ie, expressed only from the paternal allele, in contrast to the Igf2 receptor, which is expressed only from the maternal allele. New clues regarding regulation of Igf2 expression have emerged as further insight is gained into how gene expression is

regulated in general and how DNA and chromosomes are organized in the nucleus.

As commented upon by Spilnikakis and Flavell, DNA in higher organisms is organized with nucleoproteins into different kinds of chromatin from which chromosomes are constructed. Each chromosome resides in a specific region of the nucleus except during cell division.





Interchromosomal rendezvous. The interaction between two different gene loci on two different chromosomes is mediated by the transcriptional regulatory factor CTCF and perhaps other factors. This may occur on regions of the nucleus that are enriched with transcription machinery whereby the genetic elements on one chromosome regulate expression of genes on the partnering chromosome.

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Genes being actively expressed typically loop out from condensed chromatin into regions called “transcription factories” where the transcriptional machinery including factors that initiate and regulate transcription resides. Conventionally, this process was thought to be controlled by regulatory elements on the same chromosome as the gene being regulated—so-called *cis* regulation. However, there is growing evidence for genes on one chromosome being regulated by regulatory elements located on a different chromosome, ie, *trans* regulation, and this may help to explain the control of *Igf2* expression. A publication by Ling et al shows that a maternal gene locus on (mouse) chromosome 7 harboring 2 adjacent imprinted genes localizes with a paternal locus on chromosome 11 containing different genes in a manner that depends on a protein termed CTCF (CCCTC-binding factor).

More specifically, *Igf2* and *H19* are coordinately regulated, imprinted genes located ~ 80 kb apart on mouse chromosome 7 (Figure). An imprinting control region (ICR) located between them contains 4 binding regions for CTCF, a zinc finger-binding protein. Using a technique called chromosome conformation capture combined with fluorescence in situ hybridization, Reik and colleagues recently demonstrated that on the paternal chromosome, a differentially methylated region (DMR) loops out to interact with the methylated ICR pushing the *Igf2* promoter into contact with the *H19* enhancer resulting in *Igf2* expression.<sup>1</sup> On the maternal chromosome, a DMR interacts with the unmethylated ICR, partitioning the *Igf2* promoter into a silent loop.

Ling et al applied additional assays to show an interaction of DNA sequences mapped to ICR between the *Igf2* and *H19* loci on chromosome 7 with sequences that mapped to a region located between 2 genes—*Wsb1* and *Nf1*—on chromosome 11, which they called the ICR-associated site. They showed that CTCF binds only to the maternal allele of the *Igf2* ICR and only to the paternal

allele of ICR-associated site on chromosome 11 and that these specific interactions are required for co-localization and presumed interaction of relevant intrachromosomal loops from chromosomes 7 and 11.

Ling et al caution that while they cannot be certain, their evidence strongly argues that the genes on chromosomes 7 and 11 physically interact and regulate each other's expression. They note that transcription factories rich in preassembled transcription complexes are presumed to exist within the nucleus and suggest that since there are most likely fewer factories than transcribed genes, some genes would need to share a common factory. Given the strict parental allele specificity of CTCF binding, they further suggest that interchromosomal association plays an important role in the imprinting process. Spiliakakis and Flavell propose in their commentary that interchromosomal interactions may be a general phenomenon in gene regulation.

Spiliakakis CG, Flavell RA. Managing associations between different chromosomes. Science. 2006;312:207–208.

Ling JQ, Li T, Hu JF, et al. CTCF mediates interchromosomal co-localization between *Igf2/H19* and *Wsb1/Nf1*. Science. 2006; 312:269–272.

**Editor's Comment:** These papers add another chapter to the saga of imprinting and further insights into the complexity of gene regulation in higher organisms. It almost makes one long for the days when regulation could be explained one gene at a time with a relatively small number of transcription factors that turned them on and off. The new insights, however, provide a means to begin to explain observations, such as variable expression of genetic disease that we have never understood very well.

William A. Horton, MD

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## IGF-I During High-dose Growth Hormone Treatment of Children Born SGA

Growth hormone (GH) treatment in short children born small for gestational age (SGA) results in a significant improvement of final height, as most children reach normal stature defined as height above  $-2SD$ . This treatment is accepted by health authorities in both the US and Europe. However, a previous study by these authors had shown that only a slight and non-significant difference in adult height was observed when 2 GH doses ( $1 \text{ mg/m}^2/\text{d}$  [ $0.033 \text{ mg/kg/d}$ ] and  $2 \text{ mg/m}^2/\text{d}$  [ $0.067 \text{ mg/kg/d}$ ]) were compared.<sup>1</sup> Other reports have shown that the serum insulin-like growth factor (IGF)-I and IGF binding-protein (IGFBP)3 levels are particularly related to the GH dose. Therefore, this study aimed to document GH levels during an overnight profile and IGF-I / IGFBP3 levels before and after 6 months of treatment. This was performed in view of multiple epidemiological studies pointing at cancer risk in relation to high circulating GH and IGF-I levels.

Thirty-six prepubertal short children born SGA were stratified according to gender, and randomized into 2 groups according to dose of GH. The overnight GH profile after subcutaneous injection and IGF data were recorded before and after 6 months of treatment. Results were converted to SDS values when appropriate. Both groups had comparable baseline data. The growth response was significantly higher in the high-dose group after 6 months of treatment. This group had significantly higher GH levels overnight, whether considering area under the curve, mean GH, or maximal GH levels. IGF levels increased in the low-dose group from  $-1.6$  to  $0.2$  SD, while the change in the high-dose group was from  $-1.6$  to  $1.5$  SD. In the latter, 74% of the children had IGF-I levels in the highest quartile ( $>0.84$  SD) and 37% had levels above  $+2SD$ , compared with only 19% and 6% respectively, in children treated with the lower dose of GH treatment.

The short SGA children given the high dose of GH had evidence of higher circulating GH and IGF-I levels. The IGF-I/IGFBP3 ratio was also more elevated, suggesting higher values of circulating free IGF-I. A higher stimulation of the growth control axis for 6 months produced a significant increase in height,  $+0.7$  SD as compared to  $+0.5$  in the low-dose group. Of interest is that no correlation could be found between the growth response and the increase of GH or IGF-I/IGFBP3 levels. The authors suggested that this may reflect a reduced GH/IGF-I receptor sensitivity, eventually related to the SGA condition, and they recommended monitoring IGF-I levels during GH treatment to ensure that these remain within the normal range for age.

van Dijk M, Mulder P, Houdijk M, et al. High serum levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) during high-dose GH treatment in short children born small for gestational age. *J Clin Endocrinol Metab.* 2006;91:1390-1396.

**First Editor's Comment:** This is the first study comparing 2 doses of GH in short children with SGA and the effect on growth and IGF-I levels. The issue is important since there is a theoretical risk of cancer after prolonged exposure to higher circulating levels of IGF-I and IGF-I/IGFBP3 ratio. This has led to repeated recommendations for the evaluation of circulating IGF-I levels, at least yearly, during GH treatment. The present data document precisely the effect of the 2 most frequently prescribed doses of GH and provide unique data for an appropriate comparison at 6 months of treatment. However, the study does not elucidate the question whether the dose-related increase of IGF values remains the same at a later time, when the high-dose GH does not produce a higher growth rate anymore.

In any case, the authors challenge the recommended doses and focus on the efficacy of the medication. This paper should be read in relation to the previous study<sup>1</sup> by the same group showing a moderate, but not significant, increase in final height, when doubling the dose up to  $0.067 \text{ mg/kg/d}$ . It was shown that height gain was dependent on fewer amount of doses over the long-term than over the short-term. Therefore, the economical aspects of long-term administration of higher doses of GH should be considered. Interestingly, the present data again confirmed that during the first 6 months of treatment, there is a significant GH-dose effect allowing better and faster catch-up growth.

Even if a different approach is chosen by individualizing GH treatment to optimize height gain, one may still expect difficulties in adjusting GH dose when taking into account multiple factors such as differences between initial and later treatment periods, dose-related effects on IGF-I, individual susceptibility, and poor correlation between height gain and changes in IGF-I score. It will be of interest to determine whether long-term GH dose adjustments will cope with the observed changes of IGF-I levels and the need for maintaining them within safe limits.

Raphaël Rappaport, MD

**Second Editor's Comment:** Although most SGA children show catch-up growth and achieve normal height during the first 2 years of life, approximately 10% to 15% of them remain short with a height below  $-2$  SDS. Recent studies have demonstrated that GH treatment of short children born SGA results in the normalization of height during childhood. Van Pareren demonstrated that long-term treatment of short SGA children with a low dose of GH ( $1 \text{ mg/m}^2/\text{d}$ ,  $0.033 \text{ mg/kg/d}$ ) was as effective in attaining a normal final height as the treatment with a high dose of GH ( $2 \text{ mg/m}^2/\text{d}$ ,  $0.067 \text{ mg/kg/d}$ ).<sup>1</sup> In this study van Dijk et al showed that most SGA patients receiving a high dose of GH treatment had high GH levels for most of the day and IGF-I levels and IGF-I/IGFBP3 ratios in the upper quintile. In recent years,

concern regarding the detrimental effects of persistent high serum GH and IGF-I levels has been expressed in various studies. Of particular importance are the reports of an increased cancer risk (ie, breast, prostate, and colon cancer) in patients with IGF-I levels in the upper tertile to quintile, more so if accompanied by low IGFBP3 levels.<sup>2-4</sup> Recent studies have recommended beginning GH treatment of short SGA children at an early age.<sup>2-4</sup> Thus, GH and IGF-I levels may be elevated in many of these patients for a good part of childhood and adolescence, possibly placing them at an increased risk for complications later in their lives. The long-term deleterious effects of GH treatment in SGA children remain unknown. However, the use of an initially lower GH dose, which can then be individually adjusted and the monitoring of IGF-I and IGFBP3 during GH therapy,

in an attempt to maintain IGF-I concentrations in the upper half of the age-adjusted reference range, is strongly recommended.

Roberto Lanes, MD

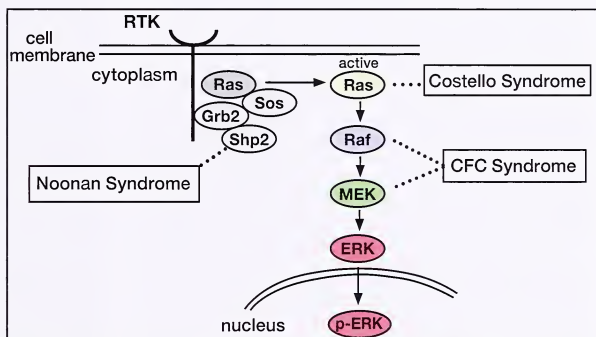
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## Germline KRAS, BRAF, and MAPK Mutations in Noonan and Cardio-Facio-Cutaneous-Syndrome

The mitogen-activated protein kinase (MAPK) intracellular signal transduction system is one of several signaling systems employed by growth hormone, prolactin, epidermal growth factor, and other mitogens (Figure). The MAPK pathway is important for cell proliferation, growth, aging, and apoptosis. After a growth factor binds to its specific receptor, GRB2 (growth factor receptor-bound protein 2; chromosome 17q23-q25, OMIM 108355), a cytosolic adaptor protein with SH2 and SH3 domains, complexes with the cytoplasmic domain of the activated growth factor receptor. Subsequently, GRB2 interacts with PTPN11 (protein-tyrosine phosphatase, non-receptor type 11; chromosome 12q24.1, OMIM 176876) through SH2-SH3 bonding and then binds to the guanine nucleotide

exchange factor-SOS1 (son of sevenless drosophila, homolog 1; chromosome 2p22-p21, OMIM 182530) to mediate growth factor-induced activation of RAS (rat sarcoma viral oncogene homolog; chromosome 11p15.5, OMIM 190020). The RAS family of GTP-binding proteins includes KRAS, NRAS, and HRAS, all composed of 189 non-identical amino acids. After activation by addition of GTP, RAS initiates signal transduction through a series of 3 tyrosine-serine/threonine kinases (phosphorylases) that culminates in phosphorylation and activation of several transcription factors such as activating protein-1 (AP-1), and signal transducer and activator of transcription (STAT) 5. Intrinsic RAS GTPase assisted by GTPase activating proteins degrades RAS-linked GTP to GDP, thus decreasing RAS signaling and depressing the activity of the MAPK pathway. The intermediary kinases



Ras/Raf/MEK/ERK signal transduction pathway and associated genetic syndromes. Noonan syndrome has also been associated with (K)RAS.

Shp2=PTPN11, MEK=MAP1K1 or MAP1K2, ERK=MAPK3 or MAPK1

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in the MAPK pathway include in sequential order:

- BRAF (V-RAF murine sarcoma viral oncogene homolog B1; chromosome 7q34, OMIM 164757) (there are additional RAF isoforms: ARAF and CRAF);
- MAP2K1 (mitogen-activated protein kinase kinase 1; chromosome 15q21, OMIM 176872) and related MAP2K2 (mitogen-activated protein kinase kinase 2; chromosome 7q32, OMIM 601263);
- MAPK3 (mitogen-activated protein kinase 3; chromosome 16p11.2, OMIM 601795) and related MAPK1 (mitogen-activated protein kinase 1; chromosome 22q11.2, OMIM 176948).

MAPK3 in turn phosphorylates AP-1, STAT-5, and other transcription factors. With somatic single point mutations at codons 12,13 or 61, RAS intrinsic GTPase activity is

diminished and the RAS proteins retain GTP, permitting them to become oncogenic by generating unbridled intracellular signaling that leads to unregulated cell proliferation and hematologic, lung, intestinal, pancreatic, thyroid, gonadal, and other neoplasms. Mutations in several of the genes involved in MAPK signaling have been identified and associated with clinical disorders.

Noonan syndrome (OMIM 163950) is an autosomal dominant disorder characterized by a "Turner-like" face, short stature, webbing of the neck, and right-sided anomalies of the heart as well as deafness, motor delay, and a clotting disorder. In approximately 45% of patients with Noonan syndrome, germline heterozygous gain-of-function missense mutations in *PTPN11* have been identified.<sup>1</sup> *PTPN11* (also designated SHP2) is an intracellular protein tyrosine phosphatase; adjacent to its catalytic domain are 2 tandem SRC homology 2 (SH2) domains that permit *PTPN11* to bind to other proteins with SH2 and SH3 domains and to remove phosphate groups from specific phosphotyrosine residues. Among the substrates of *PTPN11* is GRB2. Activating mutations in the SH2 or protein tyrosine phosphatase domains of *PTPN11* increase signal transduction through the MAPK pathway leading to the clinical manifestations of Noonan syndrome, although the cellular mechanism(s) by which they occur is (are) unknown at present.<sup>1</sup> (Heterozygous gain-of-function mutations within the protein tyrosine phosphatase domain of *PTPN11* have also been identified in the LEOPARD syndrome [OMIM 15100], an autosomal dominant disorder with café-au-lait spots and lentigines as well as features similar to those of Noonan syndrome.)

The Costello or facio-cutaneo-skeletal syndrome (OMIM 218040) is characterized by short stature, excessive skin of the neck (webbing), fingers, palms, and soles, curly hair, perioral and perinasal papillomata, developmental delay, and increased susceptibility to neoplasia. In the majority of patients with Costello syndrome, heterozygous gain-of-function mutations in *HRAS* (V-HA-RAS-Harvey Rat Sarcoma Viral Oncogene Homolog; chromosome 11p15.5, OMIM 190020) (*v.i.*) have been found.<sup>2</sup>

The 3 articles presently reviewed document overlapping clinical manifestations and mutations in several genes within the MAPK signal transduction pathway. Schubbert et al report that the clinical manifestations of Noonan syndrome can also arise as a consequence of gain-of-function mutations in *KRAS* (V-KI-RAS2 Kirsten Rat Sarcoma 2 Viral Oncogene Homolog, chromosome 12p12.1, OMIM 190070), a gene "downstream" of *PTPN11*. They identified *de novo* germline *KRAS* mutations in 5/174 subjects with Noonan syndrome without *PTPN11* mutations. The most common mutation (present in 3 patients) was substitution of isoleucine for valine at amino acid 14 (Val14Ile); this mutation depressed intrinsic GTPase activity of *KRAS*.

The cardio-facio-cutaneous syndrome (OMIM 115150) is associated with congenital heart disease (pulmonic stenosis, atrial septal defect, hypertrophic cardiomyopathy), distinctive face (high forehead, bitemporal narrowing,

hypoplastic supraorbital ridge, depressed nasal bridge, angulated ears), cutaneous abnormalities (sparse hair, ichthyosis-like thickening), and developmental delay. Schubbert et al found a heterozygous mutation in *KRAS* in 1/12 patients with this syndrome. Niihori and colleagues also identified 2 *de novo* germline heterozygous mutations in *KRAS* in 3/43 patients with the cardio-facio-cutaneous syndrome. These investigators further demonstrated 8 heterozygous mutations in *BRAF*-encoding the serine/threonine kinase most immediately responsive to *KRAS* (Figure) in 16/40 patients with the cardio-facio-cutaneous syndrome; 6/8 mutations were localized to the catalytic domain of *BRAF*. The majority of the mutations in *KRAS* and *BRAF* increased signal transduction through the MAPK pathway. These investigators identified no mutations in *PTPN11* in any patient with the cardio-facio-cutaneous syndrome nor did they find aberrations in *KRAS* or *BRAF* in any Noonan subjects. Rodriguez-Viciana and associates found 11 heterozygous gain-of-function *BRAF* mutations in 18/23 patients with the cardio-facio-cutaneous syndrome. They also identified 2 heterozygous, activating mutations in *MAP2K1* and one such mutation in *MAP2K2* in 3/5 patients with this disorder.

Schubbert S, Zenker M, Rowe SL, et al. Germline *KRAS* mutations cause Noonan syndrome. *Nature Genet.* 2006;38:331-336.

Niihori T, Aoki Y, Narumi Y, et al. Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. *Nature Genet.* 2006;38:294-296.

Rodriguez-Viciana P, Tetsu O, Tidyman WE, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. *Science.* 2006;311:1287-1290.

**First Editor's Comment:** The signal transduction pathway and associated genetic syndromes are shown in the figure. Mutations have now been found in several of the protein components of the MAPK signal transduction pathway. That Schubbert et al found 169 patients with clinical manifestations of Noonan syndrome without *PTPN11* or *KRAS* mutations demonstrates the substantial genetic heterogeneity of this disorder and leads one to anticipate the identification of gene mutations in other components of the MAPK signal transduction pathway, perhaps involving SOS, MAPK3, guanosine nucleotide exchange factors, and/or GTPase activating proteins. Indeed, neurofibromin, the neurofibromatosis type 1-associated tumor suppressor product of NF1, is a GTPase activating protein for RAS.

With the delineation of more and more specific gene mutations leading to clinically described disorders, it may well be time to redesignate such entities according to the gene mutation itself; eg, "Hyperactive RAS disease: type 1, 2 ..., "Hyperactive *PTPN11* disease: type 1, 2 ..., " or according to the genetic pathway involved, eg, "The MAPK syndromes." Indeed, all of the clinical disorders of this pathway share common features to a greater or lesser degree such as short stature, distinctive faces, developmental delay, congenital anomalies of the heart, and skin changes. With intimate knowledge of the basic



*abnormalities within the described syndromes, drugs might be devised that ameliorate the hyperactivity of the MAPK pathway and moderate its clinical manifestations. Prenatal diagnosis and perhaps even fetal gene therapy also loom as possible future therapeutic avenues.*

Allen W. Root, MD

*syndrome.<sup>3</sup> A similar resistance may also be present in other patients with syndromes with or without PTPN11 or KRAS mutations, as they all share common features and short stature. The availability of recombinant IGF-I and IGF-1/IGF BP3 may now allow treatment strategies not previously available.*

Fima Lifshitz, MD

**Second Editor's Comment:** *The reader is referred to Vol. 22, No. 2 of GGH for a review of 3 papers dealing with the increased growth hormone resistance of PTPN11 accounting for the short stature of patients with Noonan*

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## Idiopathic Short Stature: Psychosocial Development and GH Treatment

Visser-van Balen and colleagues presented a metanalysis of available research on the psychosocial functioning of medically referred children with idiopathic short stature (ISS) and the effects of growth hormone (GH) treatment. Specifically, the authors asked whether or not subgroups of medically referred children with ISS have specific risks and different outcomes when treated. Their search used the Medline and PsycInfo databases and included 11 studies that assessed psychosocial functioning. The results showed that according to parents, short children have lower social competence and more social problems than children with normal stature. The intelligence of the ISS children was within the normal range; however, they functioned on average between normal and below normal. Admittedly, the effect sizes were very small in these studies. Studies on the consequences of being short on psychosocial functioning in adulthood were inconclusive, as none of the adults in the studies had received GH. Two studies reported a relatively low percentage of marriages and relatively high percentage of unemployment and self-reported problems in social functioning among short adults. Other studies have not shown this effect. Of note, most of the studies among children only examined parental records. Studies using teachers and peers did not show lower social competence. Children's own reports regarding self esteem showed relatively few indications of psychosocial problems. The interpretation was that either these children are too young to give an adequate assessment of their own functioning, or they lack time perspective. There were no studies in which similar concepts were studied by both parents and children. The authors speculated that it was possible that medically referred children with ISS had psychosocial problems because they were short. It is also possible that children with psychosocial problems, who were also short, may be referred relatively often. Their conclusion was that medically referred children with ISS had on average more psychosocial problems than children with normal stature.

The review suggested that some risk factors for maladaptation in children with ISS include being teased, being juvenilised, being a boy, having a low intelligence,

having a younger but taller sibling, and being part of a low socioeconomic status family. Further studies on the impact of the degree of shortness did not find an effect. This may be because it was not actual height, but perceived height which was crucial in terms of psychosocial risk factors.

Finally, the effects of GH treatment on psychosocial factors were assessed in 9 studies in which the children had a mean height gain of at best 7 cm. On average, GH treatment did not improve psychosocial functioning and only a few studies showed improvement in problem behaviors. Although these pre- to post-treatment assessments with standardized questionnaires did not reveal changes in psychosocial functioning, a retrospective perception of GH treatment by parents and children was generally positive with parents reporting a positive change regarding social functioning and self-esteem of their children.

The 3 main conclusions of this review included: (1) parents of medically referred children with ISS ranked the behavior of their children on average between normal and below normal with more psychosocial problems, (2) some risk factors influencing adaptation in children with ISS have been found, and (3) GH treatment is a means to gain height, but not a means to solve psychosocial problems.

Visser-van Balen, H; Sinnema, G; Geenen, R. Growing up with idiopathic short stature: psychosocial development and hormone treatment; a critical review. *Arch Dis Child.* 2006;91:433–439.

**First Editor's Comment:** *This is a very interesting metanalysis, which is probably the first of many subsequent reports to be written concerning ISS and psychosocial functioning. There are many justifiable critiques of the data presented including the lack of control groups, lack of randomization, variable ages at initiation of therapy, and variable duration of treatment. These variables suggest the need for long-term prospective studies in children with ISS for whom treatment is initiated and for whom treatment is not given. It is hoped that one of the GH registries will initiate such a study and that sufficient numbers of children can be*



obtained to be able to adequately assess the influence of these variables on adult psychosocial functioning and adjustment.

William A. Clarke, MD

**Second Editor's Comment:** This review of the psychosocial development of medically-referred youths with ISS and the response to GH therapy is notable in that studies are summarized in the context of psychological theory—the disability-stress-coping model.<sup>1</sup> A theory-driven analysis offers the promise of accounting for variability in the experiences of youths with ISS. Most importantly, this strategy generates testable hypotheses regarding the relationship between short stature and quality of life which could be employed in the development of psychosocial treatments serving as an alternative (or adjunct) to medical intervention. Underscoring this point, the authors stated that "hormone

treatment is a means to gain height, but not a means to solve psychosocial problems."

As noted by the authors, the rigor of the research designs employed in assessing the psychosocial adaptation of short youths prior or subsequent to GH treatment is highly variable. Because of this, the studies conducted to date do not support firm conclusions regarding "risk factors" moderating the influence of short stature on psychosocial adaptation. Elements of research design pertinent to psychological studies of short stature have been discussed in this journal.<sup>2</sup>

David E. Sandberg, PhD

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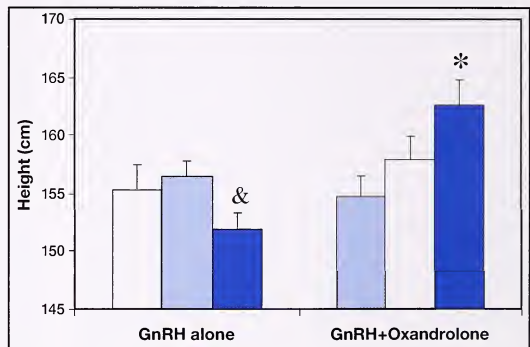
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## Final Height in Girls with Precocious Puberty Treated with GnRHa and Oxandrolone

Vottero et al assessed the benefits of adding oxandrolone (OX; 0.06 mg/kg/d orally) on the height outcome of girls with central precocious puberty (CPP) who received gonadotropin-releasing hormone analog (GnRHa) treatment (leuprolide acetate, 3.75 mg IM every 28 d) and whose height velocity decreased below the 25<sup>th</sup> percentile for chronological age. The adult height reached by 10 patients with CPP treated with GnRHa plus OX (group 1) was significantly higher than their pretreatment predicted adult height (PAH) ( $162.6 \pm 2.3$  vs  $154.8 \pm 1.7$  cm) and target height ( $162.6 \pm 2.3$  vs  $158.0 \pm 1.9$  cm), while 10 subjects with CPP treated with GnRHa alone (group 2) reached an adult height similar to the pretreatment PAH ( $151.9 \pm 1.2$  vs  $155.4 \pm 2.1$  cm), but significantly lower than target height ( $151.9 \pm 1.2$  vs  $156.6 \pm 1.4$  cm;  $P < 0.005$ ). The difference between final height and pretreatment PAH of patients in group 1 was significantly different from that in group 2 ( $7.8 \pm 2.3$  vs  $-3.8 \pm 2.3$  cm;  $P < 0.02$ ), as was the difference between final height and target height ( $4.6 \pm 1.8$  in group 1 vs  $-4.2 \pm 1.1$  cm in group 2;  $P < 0.005$ ) (Figure). No side effects were noted in either group of patients. The authors concluded that combined GnRHa and OX therapy is a viable treatment option for girls with CPP and marked growth deceleration during treatment with GnRHa alone.

Vottero A, Pedori S, Verna M, et al. Final height in girls with central idiopathic precocious puberty treated with gonadotropin-releasing hormone analog and oxandrolone. J Clin Endocrinol Metab. 2006;91:1284-1287.

**Editor's Comments:** It is well known that in some patients with CPP the growth deceleration during



□, PAH at start of GnRH; ■, target height; ■, final height. Results are shown as mean  $\pm$  SEM. \*,  $P < 0.05$  final height of patients treated with GnRH plus Ox vs. their PAH and target height; &,  $P < 0.05$  final height of patients treated with GnRH alone vs. their target height.

Adapted with permission Vottero A, et al. J Clin Endocrinol Metab. 2006;91:1284-1287. Copyright © 2006. The Endocrine Society. All rights reserved.

GnRHa therapy may be marked and may preclude an expected improvement in predicted adult height. The addition of growth hormone (GH) to the GnRHa therapy may result in increased final height.<sup>1,2</sup> In this study Vottero et al compared the final height of girls with CPP and growth deceleration while on GnRHa alone, who were subsequently treated with a combination of GnRHa and OX or GnRHa alone. The final height significantly exceeded the target height at the end of the combination treatment and was significantly higher than that of the GnRHa treated girls. Results of this study compare favorably with those obtained in other

studies<sup>1,2</sup> by the addition of GH to GnRHa. Oxandrolone, a non-aromatizable androgen with a high anabolic to androgenic ratio when compared to testosterone, has been used to stimulate growth in boys with constitutional growth delay and delayed puberty. The OX administration is oral, relatively inexpensive, and devoid of significant side effects. In contrast, GH treatment requires daily subcutaneous injections, is extremely expensive, and its use may be associated with rare, although substantial side effects. This study seems to demonstrate the effectiveness of oral OX for the treatment of patients with

CPP whose growth velocities during GnRHa treatment decline significantly; however, studies in a larger number of patients, including boys, will be necessary before this modality of therapy becomes established.

Roberto Lanes, MD

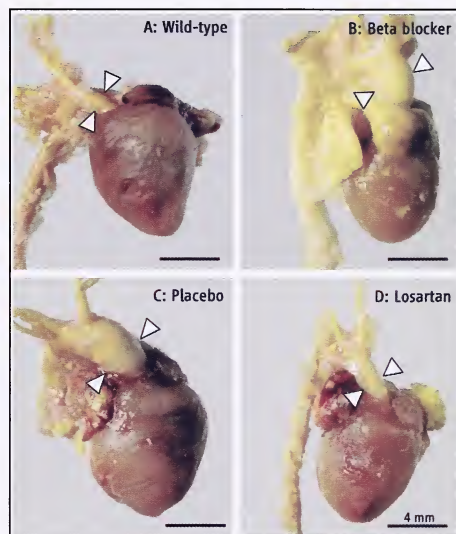
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## Treatment for Marfan Syndrome

The Marfan syndrome (MFS) was one of the first genetic conditions designated as an inherited disorder of connective tissue. Characterized by abnormalities mainly of the skeleton, eyes and heart, the most serious manifestations involve the aorta, namely, aortic dilatation and aneurysm. Heterozygous mutations of the gene encoding fibrillin-1 (*FBN1*) were identified more than 15 years ago. *FBN1* is a principal component of extracellular matrix microfibrils; thus, it was assumed that its function was primarily structural. However, it has recently become apparent that *FBN1* binds to and influences the local availability of the growth factor TGF- $\beta$ . In fact, evidence has emerged that at least some of the manifestations of MFS reflect excessive TGF- $\beta$  signaling. This is because most MFS mutations are believed to reduce *FBN1* in tissues; consequently, there would be less *FBN1* to sequester TGF- $\beta$  and keep TGF- $\beta$  signaling in check. Indeed, mice genetically engineered to have reduced tissue levels of *Fbn1* exhibit impaired pulmonary alveolar septation associated with increased TGF- $\beta$  signaling. This developmental defect can be corrected by administration of antibodies that neutralize TGF- $\beta$  signaling. Much of this work has been carried out by a group headed by Dietz at Johns Hopkins. The group has now directed their attention to the role of TGF- $\beta$  signaling in causing aortic aneurysm in MFS.

The authors studied mice heterozygous for an *Fbn1* mutation involving a cysteine substitution in one of the *Fbn1* epidermal growth factor-like domains; the mutation belongs to the most common class of mutations responsible for MFS. The mutant mice develop progressive aortic root dilatation evident as early as 2 weeks of age; the aortic roots of mutant and normal (wild-type [WT]) mice can be clearly distinguished by ultrasound at 7 weeks. Histologically, the aortic root of the mutant mice exhibits aberrant thickening of the media with disarray of elastic fibers and increased collagen deposition. Cells within the aortic media of the mutant mice also exhibit nuclear staining for phosphorylated Smad2 (pSmad2), which is only minimally detected in the WT mouse aortic root. Since phosphorylation of Smad2 and nuclear translocation pSmad2 are critical steps in TGF- $\beta$  signal transduction, detection of nuclear pSmad2 indicates TGF- $\beta$  signaling activity in these cells.



**Heart of the matter.** The aorta (arrows) of a normal mouse (A) and a losartan-treated mouse with a fibrillin-1 mutation (D) are indistinguishable, but those of mutant mice treated with a beta blocker (B) or placebo (C) have aneurysms.

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The 7-week-old mutant mice were treated with placebo or low- or high-dose TGF- $\beta$  neutralizing antibody. After 8 weeks of treatment, aortic root growth was no different between both antibody-treatment groups and WT controls in contrast to continued dilatation in placebo-treated mice. Histology revealed substantial normalization of vessel architecture with loss of pSmad nuclear staining in both antibody-treatment groups. These data were considered consistent with the notion that TGF- $\beta$  signaling contributes to aortic root dilatation in this mouse model and that TGF- $\beta$  antagonism represents a potential treatment strategy for aortic disease in MFS.

The group became interested in the drug losartan, an angiotensin II type I receptor (AT1) antagonist, not only because it lowers blood pressure—a desirable effect

in patients with aortic aneurysm—but also because it antagonizes TGF- $\beta$  in some circumstances. Accordingly, they initiated a therapeutic trial to determine if losartan could prevent the formation of aortic dilatation in the mutant mice. Either losartan or placebo was administered at 2-weeks gestation and continued until 10 months of age. To distinguish the effects of lowering blood pressure from those due to TGF- $\beta$  antagonism, the  $\beta$ -adrenergic blocker propranolol was given in doses that caused hemodynamic effects comparable to those of losartan. An important advantage of using propranolol as a control is that it is commonly employed to slow aortic growth in MFS. Upon analysis, aortic root dilatation with wall thickening and elastic fiber fragmentation was detected in the placebo- and propranolol-treated mutant mice, but not in the losartan-treated mice whose aortic root measurements were virtually indistinguishable from those of WT littermates (Figure).

A postnatal trial was also done since MFS is typically diagnosed after birth and also because losartan is contraindicated during pregnancy. The researchers compared placebo, propranolol, and losartan in postnatal mutant mice beginning at 7 weeks of age at which time the aortic root diameter was greater than for WT untreated mice. After 6 months of treatment, they observed that losartan treatment prevented elastic fiber fragmentation, which was found for placebo- or propranolol-treated mice. Aortic root growth was partially normalized by propranolol, but it was indistinguishable from WT controls for mice treated with losartan. Losartan-treated mice, but not propranolol-treated mice, showed a blunting of TGF- $\beta$  signaling in the aortic media cells. In short, the aortic root of losartan-treated postnatal mutant mice was comparable to that of WT control mice.

The group then showed that the distal alveolar airspaces in the lungs of postnatal losartan-treated mutant mice had sizes close to WT controls in contrast

to placebo-treated mutant mice whose airspace measurements were increased, as was expected for the mutant mice. This finding provided further evidence that the losartan effect on the aortic root is mediated by its antagonism of TGF- $\beta$  rather than some unappreciated hemodynamic effect; although the authors conceded that the mechanism by which AT1 blockade antagonizes signaling is not known.

Finally, the authors discussed the potential use of losartan for treatment of MFS. They point out that losartan is currently in widespread use for treatment of hypertension and prevention of strokes in both adults and children. In an accompanying editorial, Travis states that the NIH is finalizing plans for a multicenter clinical trial of losartan for children and young adults with MFS.<sup>1</sup>

Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science*. 2006;312:117–121.

**Editor's Comment:** *The results reported in this paper are both exciting and promising. As noted, prospects for effectively treating MFS were not good when it was viewed as a disorder of extracellular matrix structure. However, in its new light as a disease mediated at least in part by excessive signaling by a growth factor, the possibilities are much better as illustrated here. The authors showed in the mouse that TGF- $\beta$  antagonism can ameliorate manifestations of MFS in 2 organ systems: cardiovascular and lung. One wonders about disturbances of other organ systems, such as skeletal overgrowth. This work is a great example of successful translational research.*

William A. Horton, MD

## Reference

1. Travis J. *Science*. 2006;312:36–37.

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## MOLECULAR PATHOGENESIS OF ACHONDROPLASIA

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### INTRODUCTION

Achondroplasia (OMIM 100800) is by far the most common chondrodysplasia in humans with an estimated prevalence to be one in 15 000 to 40 000 live births. It is the prototype of short-limbed dwarfism and the archetype of a group of disorders that range from the much more severe thanatophoric dysplasia (TD) to the less severe hypochondroplasia.<sup>1</sup> These disorders share a common qualitative clinical phenotype dominated by short limbs, long trunk, large head with frontal bossing, and midfacial hypoplasia.<sup>2</sup>

Infants with achondroplasia typically present with mild-to-moderate limb

shortening, moderate craniofacial manifestations, and a lumbar gibbus. These features typically become more noticeable over time. The gibbus usually gives way to a lumbar lordosis, and infants and children with achondroplasia are at risk for spinal cord compression at the foramen magnum, as well as obesity. Average adult height for men with achondroplasia is 131 ± 5.6 cm; for women it is 124 ± 5.9 cm.

Thanatophoric dysplasia is much more severe in general. It is usually lethal in the perinatal period, but on rare occasions infants survive with a poor prognosis. Craniofacial abnormalities are much more dramatic. The thorax appears long but narrow and is associated with severe respiratory distress. Two types of TD (TDI and TDII: OMIM 18700 and 18760) can be distinguished radiographically. SADDAN dysplasia refers to a clinical phenotype

### From The Editor's Desk

Dear Colleague:

The latest issue of GGH includes the highlights of 2 important annual meetings in our field. The printed journal contains the highlights of the Endocrine Society's meeting held in June in Boston. The online journal also contains highlights from the European Society of Pediatric Endocrinology meeting held in July in Rotterdam. The lead article by Dr. William A. Horton, "Molecular Pathogenesis of Achondroplasia," elucidates the advances that have occurred in the understanding of the mechanisms of growth alterations of these patients. A look at the future with potential therapeutic considerations adds value to the clarification of the pathophysiology of the disease. Additionally, there are 17 reviews of recent papers that were selected by the Editorial Board. Altogether, the journal will stimulate you and enhance your continuous medical education efforts. I am very pleased to note that we continue to expand the content and size of the e-reviews; for example, this issue contains 11 reviews of papers with editorial comments. As well, new clinical practice guidelines continue to be added to the website. In order to provide more reviews, the index of volume 22 (2006) is now only online. Moreover, all issues and subjects are searchable online.

Finally, it is the time of year that I take the opportunity to wish you all the best for the holiday season and best wishes for the New Year.

Sincerely,  
Fima Lifshitz, MD

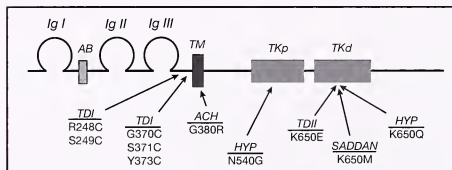
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intermediate in severity between TD and achondroplasia accompanied by developmental delay and acanthosis nigricans.<sup>3</sup> Patients with hypochondroplasia (OMIM 146000) typically present in mid childhood with mild short stature and a stocky build; the craniofacial manifestations may be minimal. Patients with hypochondroplasia blend in to the lower range of normal stature; many go undiagnosed or may be considered idiopathic short stature or be confused with another bone dysplasia.

## GENETICS

Achondroplasia was mapped to chromosome 4p16.3 in 1994, and heterozygous mutations of Fibroblast Growth Factor Receptor 3 (*FGFR3*) were identified shortly afterwards.<sup>4-6</sup> *FGFR3* mutations were subsequently discovered for the TDs and hypochondroplasia (Figure 1).<sup>7-9</sup> Remarkable degrees of genetic homogeneity and genotype:phenotype correlation soon became apparent as virtually all patients with classic achondroplasia were found to have the same Gly380Arg mutation in the transmembrane domain of this tyrosine kinase receptor.<sup>1,8</sup> Similarly, all infants with TDII had the identical Lys650Glu mutation in the distal kinase domain, whereas an Asn540Lys mutation in the proximal kinase domain was detected in most patients with hypochondroplasia.<sup>7-9</sup> Almost all infants with TDI have mutations that introduce free cysteine residues in the proximal extracellular ligand-binding domain of the receptor. Of note is that mutation of lysine 650 can produce 3 different clinical phenotypes: conversion to glutamic acid results in TDII, conversion to methionine causes SADDAN, and conversion to serine leads to hypochondroplasia.<sup>10,11</sup>



**Figure 1.** Domain structure of *FGFR3* and major sites of mutations. *Ig*: immunoglobulin, *AB*: acid box, *TM*: transmembrane, *TKp/d*: proximal and distal tyrosine kinase domains, *ACH*: achondroplasia, *HYP*: hypochondroplasia, *TD*: thanatophoric dysplasia, *SADDAN*: severe achondroplasia with developmental delay and acanthosis nigricans.

The penetrance of the achondroplasia mutation is 100%, meaning that individuals with *FGFR3* Gly380Arg mutation have achondroplasia. The vast majority of infants with *FGFR3* mutations are born to parents without *FGFR3* mutations, although there is a strong correlation with advanced paternal age (over 35 years). These findings were initially attributed to increased mutability of *FGFR3* during spermatogenesis. However, recent observations, including the detection of *FGFR3* in all germ cells except for elongated spermatids in adult men and failure to detect sufficiently high mutation rates in sperm from older males, have led to the alternative explanation that

sperm bearing mutant *FGFR3* have a selective advantage over sperm bearing normal *FGFR3* receptors.<sup>12-14</sup>

## MOLECULAR PATHOGENESIS

### a) Receptors

The *FGFR3* encodes one of 4 closely related FGF receptors (*FGFR1-4*) in mammals.<sup>15</sup> All have an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain that contains a split tyrosine kinase subdomain. The receptors differ in their temporal and spatial distribution of expression. Additional diversity is generated by alternative splicing that influences ligand specificity. Mutations similar to those in *FGFR3* have been observed in *FGFR1* and *FGFR2* in human craniosynostosis syndromes.<sup>16</sup>

After initial speculation that achondroplasia mutations cause loss-of-receptor function, it soon became evident they actually result in gain of *FGFR3* function, and the extent of this gain was found to correlate with the severity of the clinical phenotype.<sup>17</sup> The most compelling evidence came from genetic engineering experiments in mice in whom *FGFR3* was either inactivated or the receptor activated in cartilage by introducing achondroplasia or TD mutations, or by overexpressing ligands that activate *FGFR3*.<sup>18-23</sup> Mice in whom *FGFR3* was inactivated had long bones, while mice with excess *FGFR3* activation had short bones. Accordingly, *FGFR3* mutations associated with achondroplasia are often referred to as activating mutations.

Of interest is the fact that functions which are gained by activating mutations differ, depending on the cell type in which the *FGFR3* receptor is expressed. For instance, *FGFR3* activation promotes mitosis and blocks differentiation in many non-chondrocytic cell types. In fact, activating TD mutations have been found in colon and bladder carcinoma and multiple myeloma, as well as in benign adenoid seborrhoeic keratoses.<sup>24-27</sup> In growth plate chondrocytes, however, activation of *FGFR3* has the opposite effect as discussed below.

### b) Dimerization

The binding of FGF ligands to *FGFR3* monomers leads to receptor dimerization. Which of the 22 known FGFs is (are) the physiologic ligand(s) for *FGFR3* is (are) not known, although FGFs 2, 4, 9 and 18 are probably the best candidates based on the distribution of expression and ability to bind and activate *FGFR3* in *in vitro* assays.<sup>28,29</sup> It is also conceivable that different FGF ligands activate *FGFR3* in different physiologic situations. Heparin sulfate-bearing proteoglycans on the cell surface, such as syndecans, as well as alternative splicing of ligand-binding subdomains, influence binding specificity.<sup>30-32</sup>

Dimerization activates the intrinsic tyrosine kinase activity of the receptor and promotes transphosphorylation of

key tyrosine residues in the cytoplasmic domain. These residues serve as docking sites for adapter proteins and signal effectors that are recruited to the activated receptors and which propagate FGFR3 signals.<sup>33-36</sup>

### c) Signaling pathways

FGFR3 signals influence a variety of cellular events and processes largely through inducing or repressing expression of target genes in a cell-specific context. Four main signaling pathways have been identified to date to propagate FGFR3 signals: STAT, MAPK, PLC- $\gamma$ , and PI3K-AKT (signal transducer and activator of transcription 1, mitogen-activated protein kinase, phospholipase C gamma, phosphatidylinositol phosphate-3-kinase-serine/threonine kinase [protein kinase B]) with the first 2 receiving the most attention.<sup>31,37-42</sup> The most relevant signaling pathways are illustrated in Figure 2. STAT1 signals are thought to induce expression of mitotic inhibitors, such as the cdk inhibitor p21.<sup>40</sup> Using microarrays to assess changes in gene expression in chondrocytic cells, Dailey et al<sup>43</sup> showed that FGFs initiate signals in multiple pathways that result in the induction of antiproliferative functions and down regulation of growth-promoting molecules.

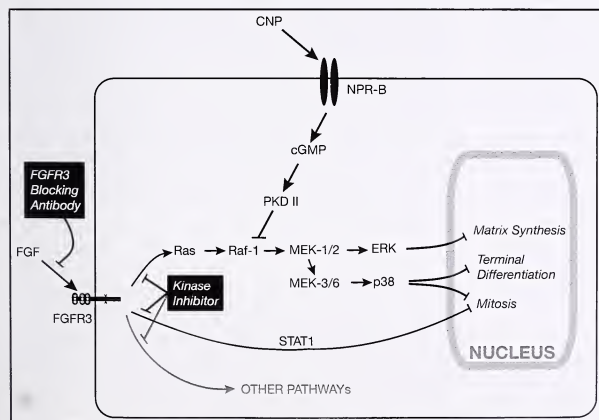
Two MAPK pathways have been implicated, the strongest evidence coming from transgenic mice in whom expression of constitutively active members of the 2 pathways was targeted to cartilage, including growth plate cartilage. Expression of activated MKK6, which specifically activates the MAPK-p38 pathway, inhibits chondrocyte proliferation in part through induction of the

transcription factor Sox 9.<sup>44</sup> Chondrocyte hypertrophy was also inhibited in these dwarf mice. Expression of constitutively active MEK1, which specifically activates the MAPK-ERK pathway, produced a similar dwarf phenotype, but through inhibition of terminal chondrocyte differentiation with no inhibitory effect on cell proliferation.<sup>45</sup> These observations underscore the importance of both chondrocyte proliferation and terminal (hypertrophic) differentiation in linear bone growth and the central role of FGFR3 in negatively regulating these events.

It is important to emphasize that FGFR3 is one of many physiologic regulators that modulate linear bone growth. Its normal function is as a negative regulator. The mutations associated with achondroplasia and related conditions are thought to act through exaggeration or enhancement of this normal physiologic function rather than through acquisition of new functions.

### d) Consequences of mutations

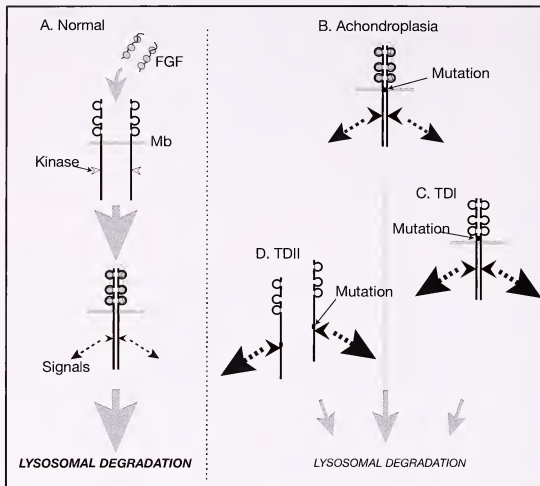
Several mutation-specific mechanisms have been proposed to explain how activating mutations of FGFR3 enhance FGFR3 signals (Figure 3).<sup>1,46</sup> The transmembrane achondroplasia mutation is thought to stabilize FGFR3 dimers following ligand-induced dimerization, although this mechanism has recently been challenged.<sup>47,48</sup> Monsonego-Ornan et al<sup>49</sup> have suggested that this mutation slows receptor internalization, leaving it on the surface to signal. The free cysteine residues introduced by the TDI mutations are believed to form disulfide bonds resulting in dimerization, which in turn activates the receptor.<sup>33</sup>



**Figure 2.** Signaling pathways and potential therapeutic strategies. FGFR3 signals are propagated through STAT1, MAPK-ERK, MAPK-p38 and probably other pathways which inhibit growth plate chondrocyte proliferation, post-mitotic matrix synthesis and terminal (hypertrophic) differentiation. The CNP-NPR-B pathway inhibits the MAPK pathways. Proposed therapeutic strategies include chemical inhibition of FGFR3 tyrosine kinase, antibody blockade of ligand-induced receptor activation, and enhancement of CNP-NPR-B signals.

The mutations of lysine 650 alter the conformation of the kinase domain, constitutively activating the intrinsic enzyme activity to different extents, corresponding with the severity of the clinical phenotype.<sup>34,46,47</sup> It is not clear if receptors carrying the lysine 650 mutations reach the cell surface to become activated by ligand. The receptor tyrosine kinase is also activated by the common (Asn540Lys) hypochondroplasia mutation, but presumably to a relatively low degree, ie, comparable to the Lys650Ser mutation that is associated with a hypochondroplasia phenotype.

A mechanism that seems to be relevant to all of the mutation types is delayed turnover of activated receptor, which increases the overall FGFR3 signal output.<sup>50</sup> Like most other transmembrane receptors, FGFR3 is internalized within endosomes relatively soon after



**Figure 3.** Proposed mechanisms by which mutations lead to gain of FGFR3 function. (A) Normally, ligand induces dimerization of receptor monomers, which activates kinase and initiates propagation of FGFR3 signals. Activated FGFR3 is targeted to and degraded by lysosomes relatively soon after activation. (B) FGFR3 dimers are stabilized by mutation (arrow) in transmembrane domain of the receptor in achondroplasia. (C) FGFR3 dimers are induced by formation of disulfide bonds in the proximal extracellular domain (arrow) in TDI. (D) Kinase is constitutively activated by mutation in TDII (and to lesser extent, in SADDAN and hypochondroplasia). (E) Lysosomal degradation is slowed in all 3 conditions. Mb: membrane.

activation. Since the intracellular “signaling” domain of the receptor has access to cytoplasmic signaling molecules, the endosomal-bound receptor continues to propagate signals until it is eventually degraded in lysosomes. Lysosomal targeting of receptors is mediated by the addition of multiple ubiquitin molecules to the activated receptor; the ubiquitin serves as a “lysosomal targeting signal.” FGFR3 ubiquitination is directed by the adapter protein c-Cbl, which functions as a ubiquitin ligase. c-Cbl activation occurs following FGFR3 activation; accordingly, the activated receptor directs its own degradation presumably as a negative feedback mechanism to keep its signaling output in check. In achondroplasia and related disorders, however, there is a defect in c-Cbl-mediated FGFR3 ubiquitination that leads to slowed receptor degradation and consequently, increased signal output.<sup>50</sup>

Another pathway that down modulates FGFR3 signaling involves C-type natriuretic peptide (CNP).<sup>51</sup> Through interaction with its receptor, natriuretic peptide receptor B (NPR-B), CNP induces accumulation of intracellular cGMP (Figure 2). Of interest is that mutations of NPR-B are responsible for acromesomelic dysplasia, type Maroteaux (OMIM 602875).<sup>52</sup> Both CNP and NPR-B are expressed in the proliferative and prehypertrophic zones of the growth plate, setting up a potential autocrine or

paracrine regulatory circuit.<sup>52</sup> Considerable evidence suggests that downstream signals from NPR-B antagonize FGFR3 downstream signals. More specifically, an increase in cGMP is known to activate a number of signaling mediators, including cGMP-dependent protein kinases (cGKs, or alternatively PKGs), one of which—cGKII (PKGII)—is thought to inhibit MAPK-ERK signaling at the level of Raf-1.<sup>53,54</sup> Probably most telling is a genetic study in which mice exhibiting dwarfism due to expression of the achondroplasia mutant *FGFR3* transgene in cartilage were mated to mice in whom CNP expression was also targeted to cartilage.<sup>55</sup> The dwarfism of the “achondroplasia” mice was rescued by expression of CNP in cartilage.

### THERAPEUTIC CONSIDERATIONS

As the molecular pathways involved in the pathogenesis of achondroplasia and related disorders have become clearer, a number of potential therapeutic strategies have emerged. Most of these approaches are similar to those used to treat cancer. This may seem odd, since the physiologic disturbances are in opposite directions, ie, too much growth in cancer, too little in achondroplasia. However, at the molecular level, the mechanisms are quite similar, ie, too much tyrosine kinase activity.

The most attention in achondroplasia has gone to inhibiting the FGFR3 tyrosine kinase through small chemical inhibitors. This approach has a strong rationale because essentially all of the cellular and higher level physiologic disturbances that interfere with bone growth seem to be driven by the excess in tyrosine kinase activity. For example, even the defect in lysosomal targeting and degradation of the activated receptor appears to be a downstream consequence of increased kinase activity. Selective FGFR3 kinase inhibitors have been developed and show promise in cell and organ culture experiments, but to date none has shown success in whole animals.<sup>56</sup>

An alternative approach has involved generating antibodies to block FGFR3 activation. Although highly specific humanized antibodies have been developed, there have been no reports to date of success beyond cell culture experiments in which they block receptor activation well.<sup>56</sup>

The therapeutic use of CNP or a CNP analog that could activate NPR-B signaling pathway to counter excessive FGFR3 signals transmitted through the MAPK-ERK and possibly MAPK-p38 pathways has been proposed.<sup>55,57</sup> This approach is appealing because other natriuretic peptides have been used clinically for their hemodynamic



effects in adults and even in children.<sup>57,58</sup> While they appear to be safe at least in the short term, a major drawback is their very short half-life requiring them to be administered by infusion, which would not be satisfactory for long-term treatment of achondroplasia.

A variation of this approach involves therapeutically targeting the NPR-C, another natriuretic peptide receptor that binds to CNP. The NPR-C, which is present on hypertrophic chondrocytes in the growth plate,<sup>52</sup> lacks the ability to increase intracellular cGMP and has been proposed to function as a clearance receptor to down regulate the effects of natriuretic peptides.<sup>57</sup> Theoretically, blocking NPR-C would lead to an increase in available CNP to bind to NPR-B in the growth plate, which in turn would be expected to antagonize FGFR3-MAPK-ERK/p38 signals as discussed above.

There are 2 considerations regarding molecular treatment of achondroplasia that deserve special attention. The first is that treatment would need to be long term, probably starting soon after birth when the diagnosis is made and lasting through puberty. This adds challenges to any form of treatment.

The second consideration relates to the difficulty in targeting therapeutic agents to the cartilaginous growth plate. Compared to most tissues, cartilage is avascular and the dense and highly charged extracellular matrix that surrounds chondrocytes represents a formidable barrier for drug delivery. Indeed, these factors may explain at least in part why treatments that have worked in cell and organ culture experiments, have failed in whole animals. Agents given systemically may need to be administered in higher doses than those used for most other tissues in order to achieve therapeutic levels in the growth plate, and this could create a predisposition to side effects in the other tissues. Accordingly, it may be necessary to develop means to target agents to growth plate chondrocytes to reach effective doses of drugs and to avoid adverse effects in other tissues. Concern over such adverse effects may be especially relevant to the central nervous system where FGFR3 is known to be expressed postnatally.<sup>59</sup>

## GENETIC IMPLICATIONS

The diagnosis of achondroplasia can usually be made clinically. In rare instances in which the patient is too young or exhibits atypical findings, it can be established by molecular genetic testing for the achondroplasia mutation.<sup>60</sup> There are a number of laboratories that carry out such testing, and these can be accessed through the GeneTest Laboratory Directory at [www.genetests.org](http://www.genetests.org). Given the virtual 100% penetrance of achondroplasia, the risk to family members who do not display clinical features of achondroplasia, ie, siblings and offspring of affected individuals, as well as siblings of parents,

is extremely low and testing is not ordinarily indicated. However, prenatal genetic testing may be useful in situations in which both parents have achondroplasia to identify fetuses with homozygous, or double-dose, achondroplasia. Such matings are at 25% risk for this much more severe form of achondroplasia.

Molecular genetic testing for hypochondroplasia may confirm a suspected diagnosis. However, only about 70% of individuals with typical findings of this condition are heterozygous for a mutation of FGFR3, presumably because mutations in genes other than *FGFR3* can result in the hypochondroplasia clinical phenotype.<sup>61</sup>

The position statement of the Little People of America regarding genetic discoveries in dwarfism may be reviewed online.<sup>62</sup>

## CONCLUSION

The tyrosine kinase-mediated transmembrane receptor FGFR3 is an important negative regulator of linear bone growth acting mainly through the STAT1, MAPK-p38, and MAPK-ERK signaling pathways to inhibit chondrocyte proliferation and terminal differentiation in the growth plate. Mutations that enhance these actions produce the qualitative achondroplasia clinical phenotype; the extent of this enhancement correlates with the severity of this phenotype. The mutations act through promoting or stabilizing the dimerization required for receptor activation, by directly activating kinase activity through conformational change of the receptor and by slowing of receptor degradation. Several strategies have been proposed to therapeutically counter the increased FGFR3 signal output, including chemical tyrosine kinase inhibitors and blocking antibodies, both selective for FGFR3 and activation of the CNP-NPR-B-cGMP pathway, which antagonizes MAPK-ERK/p38 signals downstream of FGFR3. All 3 strategies have shown success in cell and organ culture systems, but not yet in whole animal trials, perhaps because they may need to be targeted directly to growth plate chondrocytes to achieve therapeutic effect restricted to growing bones.

Research on achondroplasia and mutations of *FGFR3* has stimulated much interest in the molecular and cellular biology of both normal and abnormal linear bone growth. Indeed, many new genes whose products influence bone growth have been discovered or better delineated in the past several years, as have pathways that contribute to the regulation of bone growth. The hope is that these discoveries will lead to novel, safe, and effective therapies for disorders of linear bone growth within the next several years.

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Highlights of the Endocrine Society's 88th Annual Meeting, Boston MA, June 24-28, 2006

The Endocrine Society's Annual Meeting provided exciting presentations in plenary sessions and symposia, endocrine updates, endocrine debates, and special sessions, including multiple reviews of pediatric interest. However, I could not do justice to encapsulate those presentations in a succinct manner. Thus, only the highlights of the poster and oral presentations of the scientific papers that attracted my attention are summarized, particularly the research focusing on growth.

#### Adrenals

A novel mutation of melanocortin 2 receptor accessory protein (MRAP) gene was identified in 2 brothers with familial glucocorticoid deficiency by Li Chan et al. The first codon exon TAC-TAA lead to a severely truncated protein at position 11 of the MRAP gene, which rendered the adrenal gland unresponsive to ACTH.

#### Bone

How much of a work-up is needed when healthy children present with frequent fractures? That was the question addressed by Robert Olney et al. Sixty-nine patients with low-energy fractures were evaluated and compared to 56 controls. Those with fractures did not present low whole body bone mineral density (BMD) and did not ingest lower calcium or vitamin D. The researchers concluded that occult metabolic bone disease was not a common cause of repeated fractures in children.

Low-dose pamidronate treatment of osteoporosis in non-ambulatory children was shown to be effective by Horacio Plotkin et al. They administered pamidronate 4.12 mg/kg/yr intravenously (administered over 2 days every 4 months). There was a significant increase in BMD with treatment without significant side effects.

Recombinant human growth hormone (rhGH) induced a marked and sustained elevation in circulating levels of osteocalcin and c-terminal telopeptide of type I collagen. The increased levels exceeded the impact of bone fracture alone. This was reported by Jens Christiansen et al. The increased levels persisted for up to 12 weeks after cessation of rhGH treatment, suggesting that osteocalcin and type I collagen could be markers of illicit administration of rhGH.

#### Diabetes

Data on 5928 children with diabetes from 7 pediatric endocrinology centers in the United States were reviewed by Vanessa Davis et al. Approximately 10%

had type 2 diabetes mellitus (T2DM). Most of those with T2DM were initially treated with oral medications, but after 5 years with the disease, insulin became the most commonly used therapy. During the 5 year study, only 25% had an improved HbA1C, 46% worsened, 34% developed co-morbidities and complications, and 29% were unchanged. These data pointed out a worrisome trend of poor treatment adherence and an aggressive natural course of T2DM in children.

#### Growth

Humans with mutations in PROP1 present with hypopituitarism. Luciano Carvalho et al determined the molecular basis of the disease; they compared mice with a mutation of the PROP1 gene with Pit1 mutant mice. The mice with Prop1 mutation showed delayed vascular development, reduced cell proliferation, and elevated rate of apoptosis. These alterations may explain why the pituitary is very small in x-rays and MRI scans in patients with PROP1 hypopituitarism.

Did the small-bodied Hominis from Flores in Indonesia suffer from a molecular defect in the GH receptor (GHR) gene (Laron syndrome)? That provocative question was posed by Zvi Laron. Proposing a diagnosis due to a molecular defect in the GHR for the pathological findings of skeletons unearthed in Indonesia and whose age is 18 000 years is a challenge that may be resolved by DNA analysis; the possibility is interesting.

The response to rhGH therapy in 20 GH-deficient (GHD) patients receiving stimulant medication was assessed by Parm Gill et al. After one year of therapy, the growth rate of children treated with stimulant medication and rhGH was significantly lower than that of control patients with GHD, though the BMI of the 2 groups was similar.

The protein polymorphism of the GHR characterized by deletion of exon 3 has been linked to the magnitude of the response to rhGH treatment. Laura Audi et al demonstrated that the frequency of GHR polymorphism was higher among small for gestational age (SGA) patients than that of a control population with normal height. However, in 2 separate studies, Antonio Carrascosa et al and Veronica Mericq et al showed that in short SGA patients the response to rhGH treatment was not different among those with or without this genomic deletion. On the other hand, Gerhard Binder et al showed that Turner syndrome patients with a deletion of exon 3 presented a significantly reduced increment in height velocity during the first year of therapy with rhGH, as compared with Turner syndrome patients who

did not have this genomic mutation. The differences in height gain persisted until adult height was attained.

As MRI techniques have become more sophisticated, there are incidental findings detected in children undergoing assessment for short stature. Elena Sutu described 44 incidental findings in 38 children. Patients received further evaluations and none required treatment for the incidentalomas.

The prevalence of recurrences of craniopharyngioma in rhGH treated children was reported by Edward Laws et al. There were 51 recurrences among 773 patients with a prior history of craniopharyngioma. The risk ratio over a 7-year period was approximately 7%.

The diagnosis of GH deficiency (GHD) is often based on the response to pharmacological agents that stimulate GH release. Susan Rose and Melissa May demonstrated that children who ingested a dietary electrolyte drink (Diet Sprite™ >15 mL/kg) had improved tolerance to clonidine stimulation testing. These patients had less hypotension, higher blood pressure, and did not require any intravenous fluids during or after the test; patients were discharged earlier than those who did not ingest the hydration solution prior to the test.

The cause-specific mortality of all GHD children and adults in Denmark was reported by Kirstine Stochholm et al. During the years 1980 to 1999, there were 1823 patients with childhood and adult onset GHD; 581 of them died. The mortality rates were higher among all groups of GHD patients, as compared with appropriately matched controls. Furthermore, mortality due to cancer was increased; this was evident even in patients who had no evidence of cancer prior to rhGH treatment. Cardiovascular mortality was also increased.

The reproducibility of the insulin-like growth factor (IGF)-I generation test was assessed in 15 adults by Helena Gleeson and Stephen Shalet. Subjects were given rhGH 7 mg on 2 occasions separated by 4 weeks. The incremental response of IGF-I levels had a reasonable reproducibility and the test was considered to be a valid tool to measure GH responsiveness.

The targeting of IGF-I levels as a means to adjusting rhGH dosages in pediatric patients with short stature was studied by John Germak et al. The investigators assessed the effectiveness of an IGF-I-based dosing algorithm in rhGH therapy in a large group of short children. They concluded that the dosing algorithm was effective and allowed the titration of rhGH to the IGF-I levels. This permitted the treatment with rhGH based on the sensitivity of the individual patient. Patients with GHD demonstrated a greater increase in height and higher IGF-I levels as compared with idiopathic short stature patients.

There were several interesting papers regarding the newly available IGF preparations approved for the treatment of primary IGF-I deficiency. Susan Park et al characterized the structure and heterogeneity of mecasermin (rDNA) as a monomeric polypeptide containing 70 amino acid

residues and intramolecular disulfide bridges. Enona Gopinath showed that rhIGF-I injections supplied as a refrigerator-stable aqueous formulation had a long shelf-life (12 months) stored at 2°C–8°C.

In other studies, William Barr et al showed that the administration of a single dose of 0.5 mg/kg of rhIGF-I/rhIGFBP-3 to healthy adults was well-tolerated with no significant adverse events and produced a sustained elevation of serum IGF-I levels. These data supported the effectiveness of once-a-day dosing of this preparation. Additionally, Kenneth Attie et al demonstrated that the free levels of circulating IGF-I were sustained at a physiologic range following the administration of 0.5 mg/kg of rhIGF-I/rhIGFBP-3 given to adult volunteers. These data were in contrast to the substantial increase in free IGF-I levels induced by the administration of isolated rhIGF-I.

In a multicenter clinical trial, Cecilia Camacho-Hubner et al assessed the treatment with once daily-rhIGF-I/rhIGFBP-3 dosages on patients with severe primary IGF-I deficiency. There were 47 children from 13 countries given up to 1 mg/kg/day (low-dose group) or up to 2 mg/kg/day (high-dose group) titrated in accordance with the IGF-I levels. The height velocity in the low-dose group increased from 3.4 cm/yr to 6.4 cm/yr. The high-dose group increased the height velocity from a mean of 2.0 cm/yr to 8.3 cm/yr. The bone age advanced proportionately in both groups. Most patients developed antibodies to rhIGF-I/rhIGFBP-3, but this was not associated with adverse effects or growth attenuation. There were other adverse events that were considered mild, including hypoglycemia, headaches, erythema, and lipohypertrophy. Once-daily treatment with up to 2 mg/kg/day was effective and had an acceptable safety profile.

Patients with severe insulin resistance syndrome were treated with rhIGF-I/rhIGFBP-3 by Fiona Regan et al. The author reported improved glycemic control as well as growth in children with Leprechaunism and concluded that rhIGF-I/rhIGFBP-3 was an effective therapeutic agent.

### **Polycystic Ovary Syndrome (PCOS) and Metabolic Syndrome**

In a large scale population study, Mark Goodarzi et al genotyped 3 variants in the FEM1A gene located in chromosome 19 in a cohort consisting of 287 women with PCOS and 187 healthy controls; all subjects were white. The researchers showed that carriers of the allele of rs8111833 had an increased risk of PCOS (odds ratio 2:1), and suggested that the FEM1A gene modulates the development of PCOS.

Susanne Tan et al showed that PCOS patients had an increased risk of metabolic syndrome and Jennifer Wolfgang et al showed that lean, non-obese African-American women had double the prevalence rate of insulin resistance and cardiovascular risk factors



compared with Hispanic and non-Hispanic white female counterparts. Race played an important role independent of BMI.

Vitamin D deficiency was linked to metabolic syndrome by Jose Botella-Carretero et al. They demonstrated that 60% of such patients had low serum 25-hydroxyvitamin D concentrations which may contribute to insulin resistance.

### Pubertal Gynecomastia

Two ingredients common in many hair and skin products were linked to abnormal development of breasts in males with pubertal gynecomastia. Derek Henley et al showed that lavender and tea tree oil contained in

personal care products turned on estrogen-regulated genes and inhibited an androgen-regulated gene in human breast cancer line MCF-7. Patients' breast size decreased after they stopped using these products.

### Thyroid

Mario Salvi et al showed the immunosuppressive drug rituximab was shown to exert a significant positive effect in the treatment of Graves' ophthalmopathy. The monoclonal antibody rituximab blocked the production of B lymphocytes, particularly from the orbital area, thereby modifying the immune response and improving the ophthalmopathy.

Fima Lifshitz, MD

## Aromatase Inhibitor Effect on Near-final Height of Boys with Constitutional Delay of Puberty

Hero et al reported near-final height of boys with constitutional delay of puberty (CDP) treated during adolescence with the aromatase inhibitor, letrozole (Lz). Seventeen boys with CDP were randomized to receive testosterone (T) enanthate 1 mg/kg intramuscularly every 4 weeks for 6 months in combination with placebo (Pl; n = 8), or letrozole 2.5 mg/day orally (n = 9) for 12 months. Patients were followed to final height. Boys treated with T + Lz reached a higher mean near-final height than boys treated with T + Pl (175.8 vs 169.1 cm, respectively,  $P = 0.04$ ). Near-final heights of subjects treated with T + Lz did not differ from their mid-parental target height (175.8 vs 177.1 cm, respectively,  $P = 0.38$ ), while near-final heights of T + Pl-treated boys were lower than their mid-parental target height (169.1 vs 173.9 cm, respectively,  $P = 0.007$ ). Patients treated with T + Lz had a greater increment in height SDS than did T + Pl-treated boys (+1.4 vs +0.8 SDS, respectively,  $P < 0.03$ ). The authors concluded that an increase in adult height can be achieved by the use of aromatase inhibitors in adolescent boys with CDP.

Hero M, Wickman S, Dunkel L. Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. *Clin Endocrinol*. 2006;64:510-513.

**Editor's Comment:** Estrogens have been found to be important for bone maturation, growth plate fusion, and cessation of longitudinal growth in both boys and girls. By blocking estrogen biosynthesis in boys with the use of aromatase inhibitors, one could possibly delay bone maturation and improve their final height. Two studies<sup>1,2</sup> have demonstrated an improvement in predicted adult height of 5.1 cm and 5.9 cm following the administration of Lz for one or 2 years to boys with either CDP or idiopathic short stature. This study by Hero et al is the first to report an improvement in the near-final height of boys with CDP treated with T + Lz.

*The near-final height of subjects treated with Lz did not differ from their mid-parental target height, while the near-final height was found to be lower than the mid-parental target height in boys treated with placebo. It is of interest to note that the delay in bone maturation achieved during treatment with Lz was maintained after cessation of treatment, as indicated by the more delayed bone age at near-final height in the Lz-treated boys. In all 3 of these studies, Lz effectively inhibited estrogen biosynthesis, as indicated by low estradiol and elevated FSH, LH, and testosterone concentrations in the Lz-treated group. Six months after the cessation of treatment, the concentrations of gonadotropins, T, and estradiol did not differ among patients treated with Lz and Pl.*

*Larger numbers of patients, particularly short boys with idiopathic short stature and relatively early puberty, need to be studied to confirm these findings. Due to the gonadal androgen secretion noted during aromatase inhibition, careful follow-up of the progression of puberty, maturing spermatogenesis, and high-density lipoproteins of treated patients is necessary. In addition, the effect of low levels of estrogens on bone mass accrual during puberty and on body composition needs to be carefully followed. However, one could envision that this form of therapy could prove to be at least as effective as growth hormone and/or gonadotropin-releasing hormone analogs in increasing the final height of boys with idiopathic short stature entering into puberty at a relatively early age.*

Roberto Lanes, MD

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## Genetic Medicine: Dream, Reality or Something in Between?

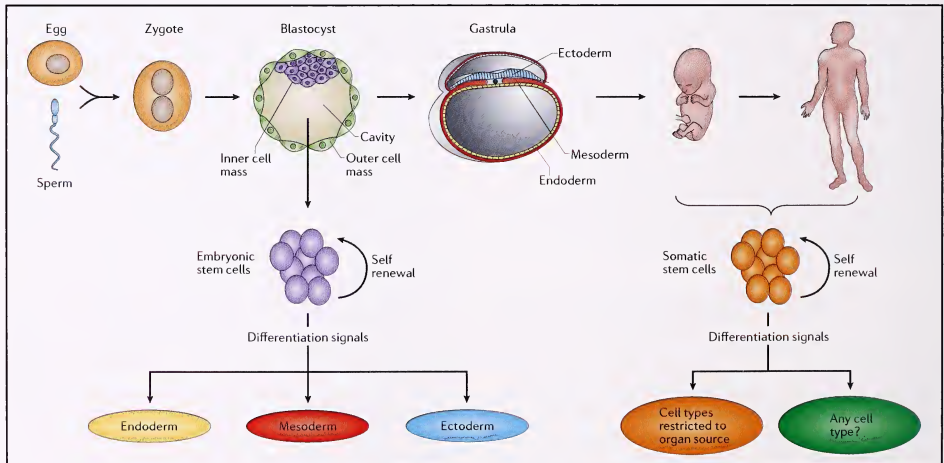
Genetic medicine has had its ups and downs and is often influenced more by rapid shifts in public sentiment than by scientific progress. Accordingly, a thoughtful and objective review of genetic medicine from O'Connor and Crystal is welcome and appreciated by those of us who are not familiar with the intricacies and recent progress in the field. Focusing on treatment of monogenic disorders, it examines the current status of 3 broad categories of genetic medicines: somatic stem cells, gene transfer, and RNA modification.

The review begins with the challenges facing genetic medicine. The main barriers are the delivery and maintenance of new genetic information. For stem-cell therapies, the major issues involve immune surveillance against foreign cells, providing a 'niche' and selective advantage for the transplanted cells and controlling and coordinating the proliferation, differentiation, location and survival of the stem cells and their progeny (Figure). For gene-transfer approaches, success requires circumventing immune defenses that arise against vectors that carry the therapeutic genes, transferring the gene to a sufficient number of cells to modify the mutant phenotype, and controlling expression of the new gene. For RNA-modification therapies, the principal challenge is delivery and, to some extent, specificity. The

authors stress that to overcome these challenges, it is essential to understand the target, including the molecular basis of the disorder, its mode of inheritance, the range of mutations and genotype-phenotype relationships that produce disease phenotypes, and how the manifestation of these phenotypes are influenced by age, location in the body, and modulation by other genes.

The review explains and illustrates the different therapeutic approaches and defines many of the terms commonly used in the world of genetic medicine. For example, differences between commonly used viral and non-viral gene-transfer vectors and their advantages and disadvantages are defined, as are the differences between antisense oligonucleotide, RNAi, ribozyme, and *trans*-splicing strategies to therapeutically alter mRNA transcripts harboring disease-causing mutations. Particularly interesting is a compilation and discussion of gene-transfer trials with a cautious assessment of their success in correcting the disease phenotype. The authors seem to be critical of the lack of success in many instances, but also optimistic that much has been learned to provide a basis for future progress.

With regard to future prospects, the authors ask the question: with all the human and financial resources



**Embryonic and somatic stem cells as a source of genetic medicines.** The fusion of sperm and egg gametes during human fertilization establishes a diploid zygote and initiates a series of cell divisions that result in a multicellular embryo. The blastocyst stage is characterized by the presence of a blastocyst cavity, outer cell mass and inner cell mass. Embryonic stem cells are derived from the inner cell mass of the blastocyst. Embryonic stem cells in culture are capable of self-renewal without differentiation and are able to differentiate into all cell types of the endoderm, mesoderm and ectoderm lineages using appropriate signals. In utero, the blastocyst implants and all three embryonic germ layers are formed during gastrulation. Somatic stem cells are present in many fetal and post-natal tissues. Somatic stem cells are also capable of self-renewal and, with appropriate signals, differentiate into various cell types from the organ from which they are derived. The extent to which they are capable of differentiating into cell types from alternative lineages is controversial.

Reprinted with permission from: O'Connor TP, Crystal RG. Nat Rev Genet. 2006;7:261-276. Copyright © NPG. 2006. All rights reserved.

that have focused on using genetic medicines to treat monogenetic disorders, why don't any of the therapies that have been tried alter disease phenotypes in a reproducible, efficacious manner, without significant toxicity? Their answer is that drug development takes years, averaging 12 to 15 years from concept to government approval. They also point to large societal hurdles that result in regulatory delays as well as economic barriers that must be overcome. They emphasize that despite its attention, the genetic medicine field is still young and that while genetic medicine is simple in concept, it is challenging to make it a reality. Indeed, they underscore the fact that paths for development of ground-breaking therapies taken as standard today, such as bone marrow and internal organ transplantation, and *in vitro* fertilization, were littered by disappointments and nay-sayers who predicted inevitable failure.

O'Connor TP, Crystal RG. Genetic medicines: treatment strategies for hereditary disorders. *Nat Rev Genet.* 2006;7:261–276.

**Editor's Comment:** *This is an excellent review for clinicians and other readers of GGH who want to catch up on the current status of genetic medicine. In most ways, the potential use of genetic medicine to treat problems of skeletal growth faces the same problems as mentioned for other areas. For instance, chondrocytes within the avascular growth plate represent a very difficult cell to target by any of the strategies mentioned in this review, as well as by more conventional therapies. There are social hurdles to developing growth-stimulating therapies in some segments of our culture, and there are enough differences between patients and animal models to make testing of new therapeutic approaches in animals challenging. Nevertheless, it seems highly likely that the general advances in genetic medicine predicted by the authors of this review will find their way to more effective ways to treat growth problems, especially in monogenetic disorders.*

William A. Horton, MD

## Catch-down of SGA and AGA Infants Born to Short-statured Parents

Völkl and colleagues reported their findings of a cross-sectional analysis of linear growth during the first 4 years of life. The 96 subjects (38 females) were children, between 5 and 10 years of age, who were born to short parents and presented to the pediatric endocrine clinic for evaluation of short stature. Endocrine disorders were excluded in each case. All the children had familial short stature (FSS) defined as a height SDS  $\leq -2.0$  but within the normal range of parental height according to the calculated target height. At least one of the parents had short stature (height  $\leq -2$  SDS). Children were divided into 2 groups according to birth size; 41 (19 female) were in the small for gestational age (SGA) group for whom birth length or birth weight was  $\leq -2.0$  SDS, and 55 (19 female) were in the group of appropriate for gestational age (AGA) infants. All children were born at  $>36$  weeks gestation and none were receiving any chronic medications. Cross-sectional data for length/height/weight/head circumference for the first 4 years of life were collected retrospectively from standardized German growth charts. The data analyses were performed at birth, 1, 2, and 4 years of age. The SDS of height and height velocity were calculated according to German and Swedish reference data.

There was a significant ( $p < 0.0001$ ) decline of height SDS within the first 2 years of life, which was more prevalent in the AGA children. These children started with a mean height of  $0.09 \text{ SDS} \pm 1.02 \text{ SDS}$  at birth and ended up with a mean height of  $-2.36 \pm 0.72 \text{ SDS}$  at 4 years of age. The growth pattern of the SGA group was similar, but the height loss was less than for the AGA group (mean  $-2.04 \text{ SDS}$  at birth,  $-3.05 \text{ SDS}$  at 4 years). Even though the height of the SDS did not decrease as much as that of the AGA children, there was a significant difference between the mean height SDS data

at all of the times studied. The absolute difference between height SDS values narrowed during the observation period. There was no significant difference in height and BMI SDS between those children having a father with short stature, compared with those with a mother with short stature. In addition, there was no relationship between the child's gender and the gender of the short parent.

The authors pointed out that there was selection bias inherent in their study since all children were initially identified in the pediatric endocrine clinic where they had been referred for evaluation of short stature. In addition, the short-stature children born SGA belonged to a subgroup of SGA children who did not experience postnatal catch-up growth. The authors stated that there is minimal information in the literature of spontaneous growth during the first years of life in children with idiopathic short stature born AGA. Of note, the SGA children increased their BMI to the same level as the AGA group after one year of age, but then these children tended to have a lower BMI SDS during the following years. This result was consistent with population-based data showing that SGA children weigh significantly less than AGA children at 3 to 6 years of age. The etiology of the growth failure in these children remains undefined.

Völkl TM, Haas B, Beier C, Simm D, Dorr HG. Catch-down growth during infancy of children born small (SGA) or appropriate (AGA) for gestational age with short-statured parents. *J Pediatr.* 2006;148:747–752.

**First Editor's Comment:** *Völkl and his colleagues have provided some very interesting data with regard to growth patterns in children identified as having familial short stature at 4 years of age. Both AGA and SGA children have losses in SDS over the first 4 years of life, but the loss appears to be greater in those children born SGA. The change in*

SDS is greater for those who were born AGA. As noted by the authors, the study was retrospective and it would be important to perform prospective studies on the children born SGA. Performance of these studies on the AGA children with short parents might prove more difficult, but the information to be gathered from such a study might be extremely important in understanding the auxiological changes that occur in these children, and might lend support to therapies for improving final adult height.

William L. Clarke, MD

**Second Editor's Comment:** The body weight and growth progression of patients with FSS with or without constitutional growth delay (CGD) was studied by Dr. Vaquero-Solans and me.<sup>1</sup> The linear growth in infancy was similar in both groups of patients. Infants with FSS and CGD showed a sharp parallel fall from the 50<sup>th</sup> percentile to -1 SD by 3 months of age and a more gradual, but steady, deterioration in length to -2 SD between 3 to 27 months of age. The z scores of height for age remained 2.0 - 2.5 SD below the mean until 12 years of age. In contrast, the body weight progression differed among the 2 types of patients. The CGD patients exhibited a marked impairment

in body weight gain as compared with the FSS. Patients with CGD had body weight deficits for stature, whereas the FSS patients did not. The differences were more marked during infancy. The CGD patients attained an appropriate body weight for height by 9 to 10 years of age, whereas the FSS patients presented body weight excess after 4 years of age and remained progressively overweight until 12 years of age. The catch-down pattern of growth in CGD patients during infancy has been observed by others.<sup>2</sup> The growth data of SGA and AGA infants in the paper by Völk et al was similar to the growth exhibited by FSS patients, though they did not assess bone development or weight and height progression after 4 years of age. The pattern of growth and weight gain during infancy and childhood has become more important as it may set the stage for obesity and adult-onset disease.<sup>3,4</sup>

Fima Lifshitz, MD

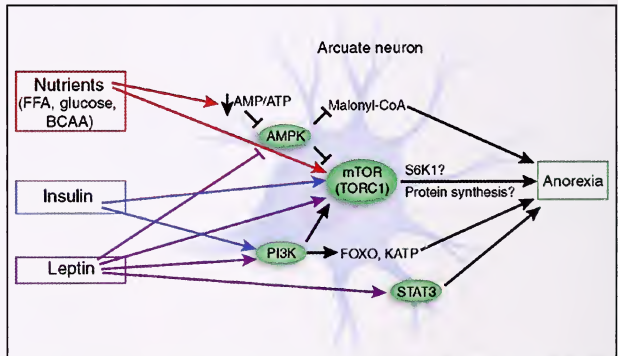
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## Interleukin Deficiency Leads To Hyperphagia, Obesity, and Insulin Resistance

Serum concentrations of interleukin 18 (IL-18) OMIM 600953, chromosome 11q22.2-q22.3), an interferon- $\gamma$ -inducing factor that augments natural killer cell activity and perhaps contributes to chronic inflammatory disorders such as Crohn's disease, are increased in patients with obesity, type 2 diabetes mellitus, and polycystic ovarian syndrome. Interleukin-18 is synthesized and secreted by hepatic Kupffer cells and macrophages. The biologic effects of IL-18 are mediated by its binding to a specific cytokine receptor (IL-18R1; OMIM 604494, chromosome 2q12) and receptor accessory protein (IL-18RAP; OMIM 604509, chromosome 2q12). The biologic activity of IL-18 is inhibited by binding to an IL-18-binding protein (IL-18BP) OMIM 604113, chromosome 11q13) which prevents the interaction of IL-18 with IL-18R1.

Netea et al demonstrated in the mouse that loss ("knock out") of IL-18 (IL-18<sup>-/-</sup>), or its receptor (IL-18R<sup>-/-</sup>), or excessive ("knock in") production of IL-18bp (thus neutralizing endogenous IL-18) results in hyperphagia and obesity associated with hyperinsulinemia and insulin resistance primarily confined to muscle



**Converting metabolic signals into anorectic (appetite-suppressing) responses in the hypothalamus.** Major classes of anorectic signals in the hypothalamus include nutrients such as free fatty acids (FFA), glucose, leucine and other branched-chain amino acids (BCAA), and hormones such as insulin and leptin. Cota et al<sup>1</sup> show that BCAA potentially activates signaling through the mTOR complex (TORC)-1. FFA and glucose may also regulate TORC1 in the arcuate nucleus, either directly or indirectly (via cellular AMP/ATP levels and AMPK activity). The regulation of cellular malonyl-coenzyme A levels may mediate a component of feeding control by AMPK in parallel with AMPK effects on mTOR. In addition to potentially regulating TORC1 indirectly through the inhibition of AMPK, insulin and leptin may also control mTOR via the PI3K or other pathways. Regulation of FOXO-dependent transcription and ATP-dependent potassium (KATP) channels probably also contributes to PI3K-dependent anorexia. Activation of STAT3-dependent transcription by leptin is a crucial short- and long-term regulator of feeding. Although the mediators of TORC1-dependent anorexia are not clear, S6K1 and downstream events such as protein synthesis are likely to be involved.

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and adipose tissues, hyperglucagonemia, hyperglycemia and impaired glucose tolerance, increased hepatic glucose output, hyperlipidemia, and vascular atherosclerosis. Thus, *IL-18<sup>-/-</sup>* mice had characteristics of metabolic syndrome. In *IL-18<sup>-/-</sup>* mice, relative to wild-type (wt) mice, body weight was normal at 3 months of age but substantially elevated by 6 months, and became progressively greater thereafter. The increased weight of the *IL-18<sup>-/-</sup>* mouse was due to excessive caloric intake and augmented fat accumulation, while basal metabolic rate remained normal. Peripheral administration of leptin and central injection of recombinant IL-18 decreased appetite; peripheral administration of IL-18 restored glucose homeostasis in the *IL-18<sup>-/-</sup>* mouse. The increase in hepatic glucose production in the *IL-18<sup>-/-</sup>* mouse was due to decreased phosphorylation of the transcription factor—signal transducing and activation of transcription (STAT)3—that resulted in accentuated gluconeogenesis due in part to increased expression of phosphoenolpyruvate carboxykinase (PEPCK-1). The investigators concluded that IL-18 is another component of the complex of factors that regulate appetite and energy metabolism.

Netea MG, Joosten LA, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nature Med.* 2006;12:650–656.

**Editor's Comment:** To the enlarging list of anorexigenic factors (insulin, leptin,  $\alpha$ -MSH, cocaine and amphetamine regulated transcript [CART], branched chain amino

acids, and other nutrients) that regulate appetite and energy expenditure, IL-18 may now be added. One could speculate that an analogue of this cytokine might be an effective therapeutic agent for the management of patients with obesity and/or metabolic syndrome. Recent studies have further defined cellular mechanisms involved in appetite regulation. The serine-threonine kinase mTOR (mammalian target of rapamycin) has been identified as a critical regulatory factor in the integration of peripheral hormonal and nutritional (glucose, fatty acids, amino acids) signals (Figure) that decrease appetite.<sup>1,2</sup> Leptin, insulin, and various nutrients suppress appetite in part by activating mTOR. This protein is a component of the multi-protein complex TORC1 that senses energy availability; when energy is sufficient, TORC1 permits cell growth and enables leptin production by the white fat cell. The TORC1 is particularly active in the arcuate nucleus, the site in which the central regulation of energy balance is present. Leptin also decreases appetite and energy utilization by inhibiting synthesis of orexigenic agouti-related peptide (Agrp) in the arcuate nucleus, an activity mediated through phosphatidylinositol 3 kinase (PI3K) but antagonized by the forkhead box-containing protein of the O subfamily (FOXO1), a DNA binding protein.<sup>3</sup>

Allen W. Root, MD

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## Insulin and Sulfonylureas in Diabetic Patients with Kir6.2 Mutations

Neonatal diabetes mellitus is a rare disorder and about half of those diagnosed before 6 months of age develop permanent diabetes. The most frequently identified genetic cause is related to heterozygous activating mutations in the *KCNJ11* gene encoding the Kir6.2, a subunit of the ATP-sensitive potassium ( $K_{ATP}$ ) channel. The activity of this channel in the pancreatic beta cell regulates insulin secretion. These activating mutations cause 30% to 58% of the cases of diabetes mellitus diagnosed in infants. Diabetes results from a failure of this channel to close in response to increased intracellular ATP, leading to impaired insulin secretion. Sulfonylureas, a class of drugs used to treat type 2 diabetes mellitus, close this potassium channel by an ATP-independent route, causing insulin secretion. Thus, this drug represents an alternative therapy to insulin in these patients. The first cases treated with sulfonylureas were reported 2 years ago; this study by Pearson et al is the first to assess the sustained response to sulfonylureas in a large cohort of patients who were initially treated with insulin.

A total of 49 consecutive patients who had been diagnosed at less than 6 months of age with Kir6.2 mutations were switched from insulin to sulfonylurea therapy. An adequate dose of sulfonylureas was defined

as a dose of glyburide (also known as glibenclamide) of at least 0.8 mg/kg/day. The change was considered to be successful if the patient was able to stop insulin treatment completely. Additionally, insulin secretory responses were assessed in subgroups receiving intravenous or oral glucose, a mixed meal, or intravenous glucagon before and after treatment with glyburide.

Switching was successfully accomplished in 44 patients regardless of the type of sulfonylurea used, suggesting a class effect. The oldest patient was 36 years of age and the youngest was 3 months of age. The mean glycated hemoglobin level improved in all subjects and fell from 8.1% during insulin therapy to 6.4% after a mean of 12 weeks of sulfonylurea treatment and cessation of insulin. The initial improvement was sustained in the 12 patients who were insulin-independent for more than one year. The longest duration of insulin independence was 2.0 years, with a glycated hemoglobin level of 5.7%.

Switching to sulfonylureas was unsuccessful in only 5 patients (10%). Of these patients, 4 (80%) had severe neurological features, including severe developmental delay, epilepsy, and neonatal diabetes, known as DEND syndrome. These neurological features occurred in only 6 patients (14%) who were successfully treated with



sulfonylureas. In 2 families, the mothers were unable to switch from insulin therapy, even though their affected children were able to do so. Only 5 patients had transitory diarrhea while on sulfonylureas. The treatment had no detrimental effect on growth. There were no patient reports of severe hypoglycemia.

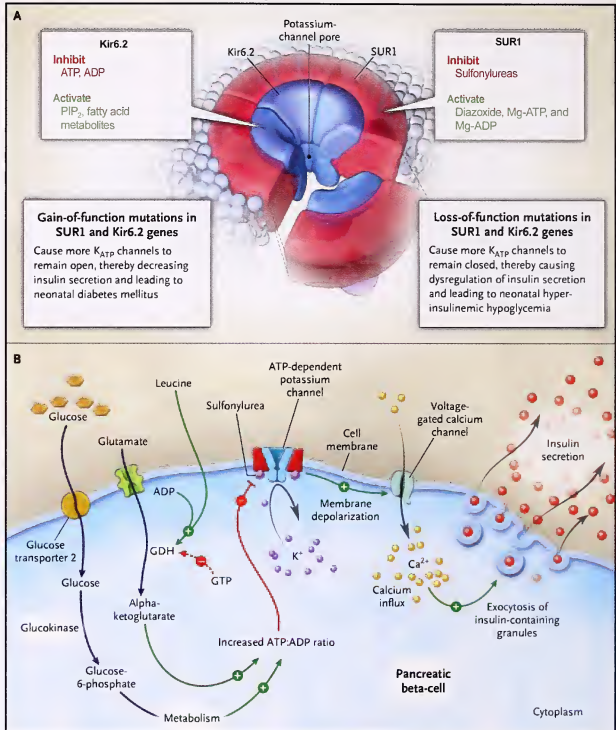
In physiological studies sulfonylurea treatment increased insulin secretion. This was more highly stimulated by oral glucose or a mixed meal than by intravenous glucose. Exogenous glucagon increased insulin secretion only in the presence of sulfonylureas.

The successful switch from insulin to sulfonylureas was reflected *in vitro* in xenopus oocytes with the same  $K_{ATP}$  channel mutation. Tolbutamide blocked more than 75% of the  $K_{ATP}$  current. The relatively high doses of sulfonylureas used in the treatment of these patients appeared to be safe on the short term. There was no increase in mild-to-moderate hypoglycemia, and a near-to-normal glycated hemoglobin level was achieved. The improved insulin secretory response to oral glucose and to mixed meals was interpreted as an effect of the sulfonylureas on the K channel, allowing the membrane to become depolarized, thereby the beta cell was able to respond to endogenous incretins (glucagon-like peptides). Because of the reported important therapeutic implications, the authors recommended a molecular diagnosis in all patients with neonatal diabetes mellitus diagnosed before the age of 6 months. It is also stressed that a longer follow-up is required to fully appreciate this progress in therapy of a genetic form of diabetes.

Pearson ER, Flechtner I, Njolstad PR, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med*. 2006;355:467-477.

**Editor's Comment:** This large collaborative study by the neonatal diabetes international collaborative group has confirmed and extended our knowledge of the treatment of the most frequently occurring genetic form of permanent neonatal diabetes resulting from activating Kir6.2 mutations of an ATP-sensitive potassium channel of the beta cell (Figure). This novel pharmacogenetic approach was based on a model of regulation of insulin

secretion involving a  $K_{ATP}$  channel. Interestingly, inactivating mutations of the components of this channel have been identified as the cause of hyperinsulinemic hypoglycemia of infancy; an opposite condition, activating mutations causing diabetes mellitus by limiting insulin secretion. The authors not only carefully investigated the new treatment with sulfonylureas, but also established physiologic evidence that this treatment restored insulin secretion in



**Regulation of insulin secretion.** The Kir6.2-SUR1 complex and its regulation and genetic variability. Panel A shows the detailed subunit structure of the  $K_{ATP}$  channel. Panel B shows the regulation of insulin secretion by glucose or amino acids (glutamate is used in this example). The beta cell senses the concentration of glucose or amino acid, or both, and converts their metabolism to energy in the form of ATP. In turn, ATP is converted to changes in the electrical membrane that regulate voltage-gated calcium channels to permit the influx of calcium and thereby insulin secretion. Central to these processes is the  $K_{ATP}$  channel, which is composed of four small subunits, Kir6.2, that surround a central pore and four larger regulatory subunits constituting SUR1. In the normal resting state, the potassium channel is open, modulated by the ratio of ATP to ADP. Hence, the beta-cell membrane is hyperpolarized, and the voltage-gated calcium channel (L type) remains closed. With the ingestion of food, the glucose concentration rises and enters the beta cell by way of the non-insulin-dependent glucose transporter 2. Glucose is rapidly phosphorylated by glucokinase, yielding glucose-6-phosphate, and further metabolism yields energy-rich ATP. The now altered ratio of ATP to ADP closes the  $K_{ATP}$  channel, causing the accumulation of some intracellular potassium, membrane depolarization, opening of the voltage-regulated calcium channel, and triggering of insulin exocytosis.  $PIP_2$  denotes phosphatidylinositol-4,5-bisphosphate.

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relation to glucose metabolism by closure of mutant  $K_{ATP}$  channels; it also amplified the effect of incretins levels that are stimulated by nutrient ingestion.

In an accompanying editorial, Sperling<sup>1</sup> recommended that a test for this genetic mutation be included as part of routine newborn screening programs. In all cases, newborns with this disease should be tested for activating mutations affecting Kir6.2, an approach facilitated by the one exon structure of the gene. Furthermore extensive familial studies are needed and other phenotypes may be expected as a consequence of mutations with milder activity. Another cause of permanent neonatal diabetes

was also reported by Babenko et al.<sup>2</sup> A careful history is needed in all patients with the onset of diabetes in infancy. It is remarkable that some, but not all, adult patients were responsive to the treatment switch from insulin to sulfonylureas. More information is needed regarding the failures observed in about 10% of the patients with the same genetic mutations.

Raphaël Rappaport, MD

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## Acidosis and Protein Kinase: A Novel Mechanism of Growth Failure

Chronic acidosis is known to cause growth failure by an effect on the bone end-organ, but the exact mechanism has remained elusive. Goldberg and colleagues have recreated growth retardation of endochondrial ossification centers *ex vivo* by culturing murine mandibular condyles in medium with 2.4 mM HCl, to lower the pH to 7.1 – 7.15. In previous studies, this group found that acidosis led to decreased expression of both insulin-like growth factor (IGF)-I and its receptor (IGF-IR) as well as markers of differentiation like type II collagen and cartilage proteoglycans. They also found that the acidotic growth inhibition could be prevented by local application of low concentrations ( $10^{-10}$  M) of parathyroid hormone (PTH).

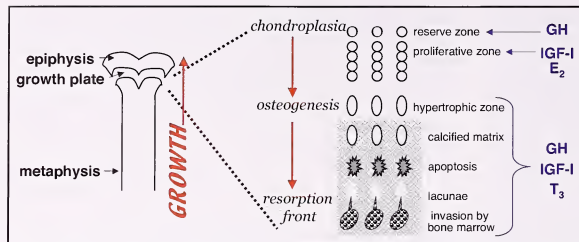
PTH works through 2 main signaling pathways: Gq protein/protein kinase C (PKC) and Gs protein/adenylate cyclase/protein kinase A (PKA) pathway. Goldberg and colleagues previously showed that acidosis represses PKC expression, an effect partially inhibited by PTH. However, the PKC agonist PMA succeeded in protecting condyles against acidotic differentiation arrest (increased expression of type II collagen and proteoglycans) but not the acidotic suppression of IGF-I and IGF-IR expression.<sup>1</sup> Therefore, the researchers sought to examine the possible role of the PKA pathway in acidosis-induced growth retardation.

In contrast to the reduction in PKC levels, PKA $\alpha$  protein levels were increased by acidosis; both levels were normalized by adding PTH to the acidotic cultures. A specific PKA inhibitor, H89, prevented the acidosis-induced reductions in expression of IGF-I, IGF-IR, and aggrecan (the core protein of cartilage-specific proteoglycans in chondrocytes). Using the converse approach, the cAMP regulating factors 8Br-cAMP (a cAMP analog), iso-butyl methyl xanthine ([IBMX] a phosphodiesterase inhibitor), and forskolin (an adenylylase cyclase analog), all reproduced the morphologic changes seen in acidotic growth plates: decreased condylar length with loss of the chondroblast population, leaving the mature hypertrophic cell layer adjacent to the chondroprogenitor zone which is

itself wider due to differentiation arrest. Chondrocyte proliferation was also reduced by acidosis, IBMX, and forskolin, as evidenced by decreased expression of proliferating cell nuclear antigen (PCNA), a cell cycle marker. Acidosis and IBMX also decreased expression of IGF-IR in the chondroblasts and chondrocytes. Using mandibular condyle-derived primary cultures of chondrocyte (MCDC) cells, the temporal cascade of endochondrial ossification was reproduced. When grown in acidotic conditions for one week, MCDC cells showed less proliferation and developed fewer cartilaginous nodules. The possibility of toxic effects of acidosis acidifying the intracellular cytoplasm was neatly ruled out by comparing the fluorescence pattern of a pH-dependent fluorescent dye, acridine orange; intracellular pH looked normal despite acidotic culture conditions, but reflected intracellular acidosis with the addition of nigericin (an ionophore that equalizes intracellular and extracellular proton concentrations). Involvement of the entire PKA pathway by acidosis was demonstrated by the increased ratio of phosphorylated- (activated) to total cAMP-responsive element binding protein ([CREB] the transcription factor which is a major PKA substrate) at one end, and the increased expression of Gs $\alpha$  protein at the other. Despite acidosis, the Gs inhibitor GDP $\beta$ S allowed normal condyle development.

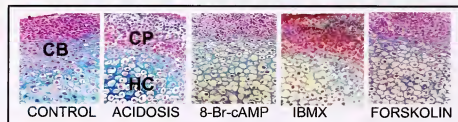
Thus, the authors developed a model of growth plate chondrocytes whereby acidosis induces Gs $\alpha$  protein, which in turn activates adenylylase cyclase and hence PKA, leading to phosphorylated CREB and altered gene expression. Both genes of differentiation and IGF-IR are ultimately down-regulated. PTH inhibits the acidotic growth retardation through both its PKA and PKC signaling pathways. The authors speculated that the activation of Gs protein by acidosis was mediated through proton-sensing receptors rather than ligand binding.

Goldberg R, Reshef-Bankal E, Coleman R, Green J, Mao J. Chronic acidosis-induced growth retardation is mediated by proton-induced expression of Gs protein. J Bone Miner Res. 2006; 21:703-715.



**Figure 1. Growth plate and process of growth.** Reprinted with permission from: Grimberg A, De Leon D. Disorders of Growth. In Moshang T, Ed., *Requisites in Pediatrics - Pediatric Endocrinology*, Elsevier, Inc., Philadelphia, 2005; 127-167. Copyright © Elsevier, 2005. All rights reserved.

**Editor's Comment:** I am always delighted when the underlying mechanisms of long-standing clinical observations are finally elucidated. To remind our readers, the anatomy of the growth plate and the growth process are depicted in the schematic<sup>2</sup> of Figure 1. The



**Figure 2. Effects of 8Br-cAMP, IBMX, and forskolin (cAMPPrf) on the development of the mandibular condyle.** Condyles derived from 6-day-old ICR mice were cultured for 72 h under normal, acidic condition (2.4 mM HCl) or treated with the following cAMP-inducing factors (cAMPPrf): 0.05 mM 8Br-cAMP (a cAMP analog), 0.05 mM IBMX (a phosphodiesterase inhibitor), or 1  $\mu$ M forskolin (adenylyl cyclase analog). Chondroblastic cell layer (CB) is absent in the acidosis and cAMPPrf cultures, leaving the hypertrophic cells (HC) adjacent to the chondroperichondrium zone (CP), which is larger than that of the control.

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chondroblasts absent in the condyle cultures with acidosis or cAMP regulating factors in the current paper (Figure 2) correspond to the proliferative zone in Figure 1. This zone is regulated primarily by IGF-I and estradiol. Instead, the hypertrophic zone was seen adjacent to chondroprogenitors (Figure 2) in an expanded reserve zone (Figure 1) that failed to fully differentiate.

Although showing a role of PKA in acidosis-induced growth retardation in the growth plate is certainly novel, this is not the first paper to demonstrate regulation of the IGF axis by PKA. cAMP/PKA induced IGF-I expression in primary rat osteoblasts<sup>3</sup> and cultured embryonic mouse mandibular mesenchymal cells,<sup>4</sup> IGF binding protein (IGFBP)-1 in hepatocytes,<sup>5</sup> and IGFBP-3, -4 and -5 in periosteal and osteoblast bone cell cultures.<sup>6</sup> PKA inhibitors interfered with the induction of IGF-I and IGFBP-3 by growth hormone in porcine ovarian granulosa cells,<sup>7</sup> IGFBP-3 by FSH also in porcine ovarian granulosa cells,<sup>8</sup> and IGFBP-4 by platelet-derived growth factor-BB in fetal rat lung fibroblasts.<sup>9</sup>

Adda Grimberg, MD

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### POTENTIAL NON-GROWTH USES OF rhIGF-I

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#### INTRODUCTION

Recombinant human insulin-like growth factor (rhIGF)-I, singly or in combination with its binding protein (recombinant human IGF binding protein [rhIGFBP]-3), was recently approved by the U.S. Food and Drug Administration for the treatment of severe short stature (height  $<-3$  standard deviations) caused by primary IGF-I deficiency. In addition to its role as the principal mediator of somatic growth in humans (together with growth hormone [GH]), IGF-I exerts multiple metabolic and organ-specific effects. IGF-I acts by binding the type 1 IGF receptor (IGF1R), an  $\alpha_2\beta_2$  transmembrane tyrosine kinase

receptor, leading to phosphorylation cascades involving the mitogen-activated protein (MAP) kinase pathway and the phosphoinositide 3 (PI3) kinase/Akt pathway and, through the latter, the mammalian target of rapamycin (mTOR) pathway. Growing appreciation for the pleiotropic actions of IGF-I has expanded its potential therapeutic usefulness beyond height enhancement. The ongoing trials to assess the role and efficacy of this agent and the current state of investigation regarding IGF-I in experimental models are shown in the Table. We herein review the highlights of some pertinent trials of the potential non-growth uses of rhIGF-I.

#### ENHANCING INSULIN SIGNALING

The effects of rhIGF-I and rhIGF-I/rhIGFBP-3 have been studied in patients with type 1 diabetes (T1DM)<sup>1-5</sup> and type 2 diabetes (T2DM).<sup>6-9</sup> The rationale for the use of these agents in diabetes is based on the

#### From The Editor's Desk

Dear Colleague:

In the first issue of 2007, volume 23 number 1, the lead article by Drs. Kim and Grimberg deals with a clinical experimental area of interest—namely, the non-growth uses of IGF-I currently under investigation. Most of the ongoing research trials in this field are at the pre-clinical stage. These protocols should be of academic interest to the readers of GGH; perhaps in the future, some of them may be of potential clinical application if proven safe and efficacious. I also want to bring to your attention a most interesting ethical dilemma posed by the paper "Growth Attenuation in Developmental Disabilities." This paper has been widely discussed in the media and lay press (ie, "A Convenient Truth" by Peter Singer, *The New York Times*, January 26, 2007) and in several blogs on the Internet. The lucid editorial comments by Dr. Sandberg bring forth the ethical considerations in a succinct manner. Please let me know if you have encountered similar situations and/or treated such patients.

The printed version of the journal includes 7 additional reviews and the online version includes 10 additional reviews of importance in the field. In addition, please note the book review written by Dr. Robert Blizzard in the e-section of the journal. The book is titled *Size Matters: How Height Affects the Health, Happiness, and Success of Boys—and the Men They Become* by Steven Hall (Houghton Mifflin, 2006). This book should be a great resource for you and your patients with short stature. There are few books like this one, although another that I have enjoyed and have recommended to my patients was written several years ago, *The Height of Your Life* by Ralph Keys (Warner Books, 1982).

Sincerely,  
Fima Lifshitz, MD  
Editor-in-Chief  
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disruptions of the GH/IGF axis associated with diabetes mellitus (Figure). In T1DM, GH levels are elevated,<sup>10,11</sup> yet IGF-I is low,<sup>12,13</sup> indicating a potentially impaired hepatic response to GH. Portal insulinopenia is thought to contribute to this impairment of GH signaling.<sup>14</sup> Decreased delivery of insulin to the liver also produces an increase in IGFBP-1 synthesis, a phenomenon observed in both T1DM and T2DM.<sup>12,15-17</sup> In turn, elevated IGFBP-1 concentrations decrease the bioavailability of IGF-I, further diminishing IGF-I signaling. The loss of negative feedback by IGF-I on GH secretion results in even greater GH secretion, which itself is known to cause insulin resistance.<sup>18-21</sup> Low IGF-I exacerbates hyperglycemia by increasing hepatic glucose output.<sup>22</sup> Given the evidence that GH therapy can cause or worsen diabetes,<sup>23</sup> trials of rhIGF-I and rhIGF-I/rhIGFBP-3 have been carried out in patients with T1DM and T2DM. The results of the larger randomized clinical trials of rhIGF-I and rhIGF-I/rhIGFBP-3 are discussed here.

### Type 1 Diabetes

Acerini et al<sup>2</sup> reported on 53 young adults with T1DM randomized to 24 weeks of placebo, or 20 or 40 µg/kg daily of rhIGF-I administered as a single evening injection in addition to their usual multiple-injection insulin regimen. Patients receiving 40 µg/kg/day had an approximately 0.5% lower HbA1c at the end of the 24-week treatment period compared to placebo. There was no difference in retinopathy, hypoglycemia, or any other adverse event.

Thraikill et al<sup>3</sup> found a similar degree of benefit in a randomized, placebo-controlled trial of 223 subjects with T1DM aged 11 to 66 years. For 12 weeks, patients received 2 injections a day of placebo, or rhIGF-I at doses of 40/40, 80/40, or 80/60 µg/kg (AM dose/PM dose). All patients continued their usual split/mix insulin therapy. At the end of 12 weeks, the HbA1c was 0.5% lower in groups treated with rhIGF-I compared to placebo treatment. Treated groups also experienced a reduction of their daily insulin requirements. The number

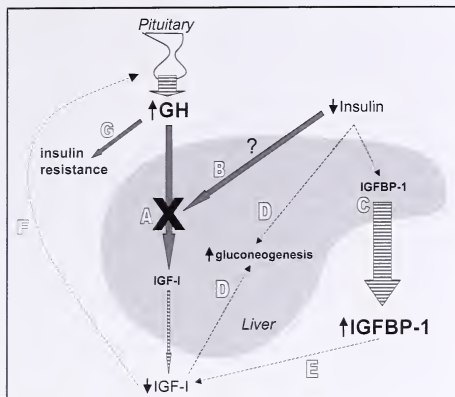
of hypoglycemic events per person per day increased with increasing dose although the differences were not statistically significant (0.14 and 0.23 episodes per subject per day in the placebo and highest dose groups, respectively). These episodes were defined as a blood glucose ≤60mg/dL, or symptoms of hypoglycemia without a blood glucose measurement. Treated groups had more frequent edema, peripheral edema, jaw pain, headache, and arthralgia, which occurred in a dose-related fashion. Although these were considered minor side effects, they were cited as the cause for the higher drop-out rates in the highest dose groups (21% and 29% in the 80/40 and 80/60 groups, respectively, compared to 15% in the placebo group). Also of concern was that 16 of 199 subjects studied had worsening of diabetic retinopathy, and 13 of these 16 were in the 2 highest dose rhIGF-I groups. Furthermore, 17 patients developed new optic disk swelling, an appearance which can be caused by diabetes or by pseudotumor cerebri.

A 2001 subgroup analysis of the 1999 Thraikill et al study<sup>24</sup> focused on the younger patients (age 11-21 years) and found a similar degree of HbA1c lowering (about -0.7%,  $P < 0.05$ ). Again, insulin requirements were reduced in the rhIGF-I treated groups. Worsening of diabetic retinopathy was also observed in this subgroup. Overall, the reports on the 2 largest controlled trials<sup>2,3</sup> of rhIGF-I suggest improved glycemic control albeit—particularly with higher doses—a high frequency of worsening diabetic retinopathy and other adverse effects often leading to discontinuation of treatment.

The effects of rhIGF-I/rhIGFBP-3 have also been studied in T1DM.<sup>4,5</sup> In April 2000, Clemmons et al<sup>4</sup> published results of their randomized cross-over study of 12 adults with T1DM randomized to 2 weeks of placebo or rhIGF-I/rhIGFBP-3 (2mg/kg/day, composed of an equimolar concentration of IGF-I and IGFBP-3, equaling a ratio of 1:4 by weight) delivered by continuous subcutaneous infusion. All subjects continued their home insulin treatment and measured 4 daily blood glucoses. After a 2-week wash-out period, the patients received the opposite therapy. Investigators and subjects were masked to assignment. At the end of the trial, insulin dose decreased 49% in the treatment group compared to placebo, and mean glucose decreased 23%. The HbA1c did not change during the short trial. There was no difference in hypoglycemic events. Edema, arthralgias, and jaw pain did not occur. Retinal exams were not conducted. The authors concluded that rhIGF-I/rhIGFBP-3 may provide improvements in glycemic control without the adverse

Table. Potential Non-growth Uses of rhIGF-I and Their Level of Development

| Potential indication                   | Preclinical data | Non-randomized trials | Randomized controlled trials |
|--|------------------|-----------------------|------------------------------|
| Enhancing insulin signaling            |                  |                       |                              |
| Type 1 Diabetes mellitus               | ✓                |                       | ✓                            |
| Type 2 Diabetes mellitus               | ✓                |                       | ✓                            |
| Type A Insulin Resistance              | ✓                | ✓                     |                              |
| Rabson Mendenhall Syndrome             | ✓                | ✓                     |                              |
| Lipodystrophy                          | ✓                | ✓                     |                              |
| Diseases of the Central Nervous System |                  |                       |                              |
| Dementia                               | ✓                |                       |                              |
| Hearing loss                           | ✓                |                       |                              |
| Spinal cord injury                     | ✓                |                       |                              |
| Cardiovascular disease                 | ✓                |                       |                              |
| Osteoporosis                           | ✓                |                       | ✓                            |



**Figure.** Disrupted GH/IGF-I signaling in diabetes exacerbates hyperglycemia. Stimulatory pathways are indicated by thick arrows, and inhibitory paths by dashed lines. Striped arrows indicate secretion. **A.** In diabetes, IGF-I is low relative to the GH hypersecretion, ie, a state of GH resistance (indicated by **X**). **B.** GH resistance may be related to deficient insulin delivery to the liver. **C.** Insulinopenia directly causes increased IGF-BP-1 production. **D.** Low IGF-I levels have a permissive effect on hepatic gluconeogenesis, as does insulinopenia itself. **E.** Elevated IGF-BP-1 levels decrease the bioavailability and hence activity of IGF-I. **F.** Reduced IGF-I synthesis and bioavailability produce less negative feedback inhibition on GH secretion from the pituitary. **G.** Resultant elevation of GH itself promotes insulin resistance.

effects associated with rhIGF-I alone. However, long-term studies will need to be performed in a larger number of patients to carefully document retinal changes and other side effects, in addition to measures of efficacy.

### Type 2 Diabetes and Insulin Resistance

In 2005, Clemmons et al<sup>3</sup> reported on the short-term use of rhIGF-I/rhIGFBP-3 in patients with T2DM. They enrolled 58 adult patients with long-standing (mean 17.1 years) T2DM treated with insulin alone (44 patients) or insulin plus oral hypoglycemic agents (14 patients). The mean baseline HbA1c was 8.2%, fasting glucose was 211 mg/dL, and body mass index was 32 kg/m<sup>2</sup>. Subjects were randomized to one of 4 treatment groups: (1) continuous infusion of rhIGF-I/rhIGFBP-3 (2 mg/kg/d); (2) 6h infusion between 2000 and 0200h of 2 mg/kg/d; (3) twice daily subcutaneous injection of 1 mg/kg; or (4) a single evening injection of 1 mg/kg. Patients were hospitalized for the entire 2-week intervention, and all continued their usual home regimen of injected insulin and any oral agents. If needed, insulin was adjusted to "maintain glycemic control." There was no placebo group, and assignment was not masked. Diets were standardized for protein and caloric content, and physical activity was limited to less than 1 mile per day of walking. The mean insulin dose for days 10-14 was reduced in all groups by 54% to 82%. The decrease was significant compared to baseline for all arms, but did not differ from each other, so no difference

by delivery method or dose was observed. The mean fasting glucose for days 2-14 decreased by 32% to 37% in all groups. Side effects were frequent, including headache in 19%, hypoglycemia in 10%, back pain in 15%, nausea in 12%, and jaw pain in 4%. Bell's palsy occurred in 1 patient. Six patients dropped out. No retinal exams were conducted. The authors concluded that combination therapy with rhIGF-I/rhIGFBP-3 improves glycemic control in T2DM. The brevity of the study may have reduced the apparent frequency of adverse side effects. However, the major concerns regarding this study are the absence of a placebo group and lack of masking. It is possible, and perhaps likely, that the stringent in-patient protocol consisting of frequent blood glucose monitoring, ensuring compliance with medications, and strict control of diet, by itself would lead to reductions of fasting glucose and insulin dosage in any patient with suboptimal diabetes control.

### Other Insulin-Resistant States

Recombinant IGF-I has been studied in metabolic diseases characterized by markedly impaired insulin signaling, including type A insulin resistance,<sup>25-27</sup> Rabson Mendenhall syndrome,<sup>28</sup> and congenital lipodystrophy.<sup>27</sup> These reports uniformly show improvements in parameters of glucose homeostasis, although the numbers of patients studied have been small. Recombinant IGF-I is a logical candidate therapy to enhance glucose homeostasis in these conditions because it acts primarily through the type 1 IGF receptor. Thus, it may provide an "alternate pathway," circumventing a defective or impaired insulin receptor and improving glucose homeostasis through the mechanisms described previously.

Lipodystrophy associated with human immunodeficiency virus (HIV) anti-retrovirals is characterized by insulin resistance, abnormal fat distribution (central fat accumulation and peripheral lipodystrophy), and dyslipidemia. This syndrome is associated with impaired GH and IGF-I secretion.<sup>29,30</sup> Clinical trials have shown that rhGH can induce hyperglycemia while reducing visceral and subcutaneous fat,<sup>31-33</sup> although low doses of rhGH may be a less diabetogenic option.<sup>34</sup> In contrast, rhIGF-I could enhance glucose homeostasis while also improving fat distribution, given evidence that IGF-I may be adipogenic.<sup>35,36</sup> The latter effect could benefit patients with the most severe peripheral lipodystrophy. Currently, the use of rhIGF-I or rhIGF-I/rhIGFBP-3 in the setting of HIV-associated lipodystrophy awaits further study.

### THE CENTRAL NERVOUS SYSTEM

IGF-I is essential for normal central nervous system development<sup>37</sup> and function throughout the lifespan, including neuronal plasticity and neuroprotection against potentially pathological disturbances.<sup>38</sup> IGF-I may serve as a regenerative agent in the central nervous system due to its mitogenic and anti-apoptotic actions, which

stimulate progenitor cell proliferation and the formation and survival of new neurons, oligodendrocytes, and blood vessels.<sup>39</sup> Because IGF-I and insulin are both actively transported from the circulation across the blood-brain barrier,<sup>40</sup> intracranial administration of rhIGF-I, as performed in some experiments, may not be required. Clinical efficacy of rhIGF-I treatment, systemic or otherwise, remains to be established.

## Dementia

Alzheimer disease (AD), the most common form of age-related dementia, is characterized by: (1) extensive brain atrophy from neuronal loss; (2) accumulation of neuritic plaques (deposits of amyloid beta protein); (3) neurofibrillary tangles (aggregates of hyperphosphorylated tau, which misfolds and dissociates from the microtubules); and (4) neuroinflammation surrounding the plaques and tangles. IGF-I has been implicated in affecting all 4 components.<sup>41</sup> Analysis of frontal lobe tissue from brains of AD patients and age-matched controls found that as clinical severity of AD increased, there was decreased expression of insulin, IGF-I, IGF-II, their receptors, tau and Hu D (a neuronal RNA-binding protein that inhibits the decay of labile mRNAs that contain AU-rich elements and affects their nuclear export and translation), but increased expression of disease-related amyloid beta protein precursor and glial fibrillary acidic protein. Choline acetyltransferase expression in insulin receptor and IGF1R positive neurons was also reduced in AD, and increased with insulin or IGF-I stimulation.<sup>42</sup>

The neurodegenerative changes of AD were replicated in vivo by intracerebral streptozotocin (ic-STZ) injection in rats. Without altering peripheral glucose, insulin, or pancreatic status, ic-STZ chemically depleted insulin and IGF signaling and induced oxidative injury within the brain. Brains of rats treated with ic-STZ had reduced size, immunohistochemical changes of neurodegeneration (cell loss, gliosis, and increases in p53, active glycogen synthase kinase [GSK]-3 $\beta$ , phosphorylated tau, and amyloid beta), and changes in gene expression profiles consistent with disease activity.<sup>43</sup> The neurodegenerative changes of AD were also replicated in vivo by blocking IGF1R in the choroid plexus. These rats developed cognitive disturbances, gliosis, synaptic protein loss, brain amyloidosis, and deposits of hyperphosphorylated tau. Restoring IGF1R function mostly corrected these disturbances, and blocking IGF1R exacerbated AD-related pathology in older, already affected mutant mice.<sup>44</sup>

Mechanistically, megalin/low-density lipoprotein receptor-related protein-2 (LRP2) is a multicargo transporter expressed by the choroid plexus that is involved in IGF-I transport into the brain and mediates IGF-induced clearance of brain amyloid beta. Levels of choroid plexus megalin/LRP2 in normal animals were reduced by aging and increased by physical exercise.<sup>45</sup>

Premature cerebral accumulation of amyloid beta was found in mice with hepatic-specific *IGF1* gene deletion, while subcutaneous chronic infusion of IGF-I to aged rats promoted amyloid beta levels to decrease in the brain parenchyma and increase in the cerebrospinal fluid.<sup>46</sup> IGF-I treatment was also able to reduce amyloid beta levels in the brains of mice over-expressing mutant amyloid.<sup>46</sup> In addition to megalin/LRP2, albumin and transthyretin have been implicated in IGF-mediated clearance of brain amyloid beta through the choroid plexus, and this function was inhibited by intracarotid injection of tumor necrosis factor alpha (TNF- $\alpha$ ), a pro-inflammatory cytokine involved in neurodegeneration.<sup>46</sup> Regarding neurofibrillary tangles, IGF-I can reduce tau phosphorylation directly or indirectly through its effects on amyloid beta.<sup>41</sup> IGF-I also promotes neurogenesis and neuronal survival in adult brains.<sup>47</sup>

The administration of IGF-I showed promising results in several animal models of dementia. Over-expression of mutant amyloid beta precursor protein (APP) and presenilin (PS)2 in transgenic mice results in AD-like cognitive deficits and severe brain amyloidosis. Systemic, slow-release IGF-I treatment of one-year-old, neurologically affected APP/PS2 mice improved cognitive performance, increased levels of synaptic proteins, and decreased brain amyloid beta load and its associated gliosis.<sup>44</sup> A 14-day infusion of amyloid beta 25-35 into the cerebroventricles of rats decreased somatostatinergic signaling in the temporal cortex (a system commonly affected in AD), decreased levels of phosphorylated Akt and increased cell death; these changes were prevented by simultaneous subcutaneous infusion of IGF-I.<sup>48</sup>

Brain atrophy and dementia have also been associated with the catabolic state of diabetes. Like AD, it has been associated with lower IGF-I levels. Subcutaneous infusion of IGF-I in rats with 12 weeks of uncontrolled STZ-induced diabetes partially prevented the loss of brain protein content (by 27.3%), despite ongoing hyperglycemia.<sup>49</sup> Subcutaneous IGF-I infusion in diabetic rats also improved learning/memory performance without ameliorating the hyperglycemia, catabolism or reductions in both total brain and hippocampal weights induced by subcutaneous STZ injection.<sup>50</sup>

## Hearing Loss

In addition to short stature and neurodevelopmental delays, sensorineural deafness has been reported in individuals with *IGF1* gene mutation<sup>51</sup> or homozygous partial deletion.<sup>52</sup> Homozygous *IGF1*<sup>-/-</sup> mice had all-frequency bilateral sensorineural hearing loss and delayed response to acoustic stimuli that increased along the auditory pathway, thereby indicating involvement of both cochlear and central nervous system function.<sup>53</sup> A biodegradable hydrogel, immersed with rhIGF-I or saline for control, was applied to the round window membranes



of Sprague-Dawley rats, 3 days before 2 hours of exposure to 120 dB of white noise. The local rhIGF-I administration significantly blunted the noise-induced elevation of threshold on auditory brain stem response testing (a marker of cochlear function) one week and one month later, and significantly prevented loss of outer hair cells in the temporal bones.<sup>54</sup> These preliminary results suggest IGF-I may be protective for the hearing apparatus against noise-induced damage. These studies also raise speculation about the possible contribution of the age-related decline in circulating IGF-I levels to presbycusis.

### Spinal Cord Injury

Moderate voluntary physical exercise can be induced in rats through enriched housing, wherein water and food are placed on opposite sides of the cage and additional attributes, such as running wheels, climbing frames and tubes, are provided.<sup>55</sup> Enriched housing also stimulates the recovery of locomotion after spinal cord injury in rats by inducing voluntary locomotor training. Locomotor training in turn provides locomotor-specific sensory feedback to the central pattern generators that stimulate remodeling of the central nervous system pathways. Subcutaneous rhIGF-I treatment improved locomotor recovery after spinal cord injury in rats, compared to control rats receiving saline infusion, while neutralization of circulating IGF-I with a chronic infusion of anti-IGF-I serum inhibited the benefits of enriched environment on functional recovery.<sup>56</sup>

### CARDIOVASCULAR DISEASE

The importance of the GH/IGF axis for cardiovascular health was first indicated by the increased cardiovascular mortality of individuals with GH deficiency.<sup>57</sup> In vitro mechanistic studies further suggested potential benefits from rhIGF-I administration in myocardial disease. IGF-I treatment of cardiac myocytes in culture was shown to attenuate apoptosis induced by hyperosmotic stress; the protective effects of IGF-I required the CREB transcription factor, which was itself activated through the MAP kinase, PI3 kinase, calcium/calmodulin kinase, and calcineurin systems.<sup>58</sup> IGF-I was also shown to protect adult rat ventricular myocytes from high glucose-induced contractile impairments; this required the PI3 kinase/Akt/mTOR pathways but not the calcineurin system.<sup>59</sup>

While GH/IGF-I deficiency portends a worsened cardiovascular prognosis, so too does GH/IGF-I excess; ventricular hypertrophy is a recognized complication of acromegaly<sup>60</sup> and pituitary gigantism.<sup>61</sup> Thus, the conceptual approach of rhIGF-I treatment for cardiovascular remodeling and regeneration, such as the use of rhIGF-I as a myocyte survival and mitogenic factor after myocardial infarction, may be limited by dose-response or situation-dependent toxicities. For example, hypertrophy can be physiologic and adaptive (response to aerobic exercise) or pathologic (response to pressure

or volume overload). An intriguing checkpoint has recently been identified. In cultured rat neonatal cardiomyocytes exposed to cyclic mechanical stretch, IGF-I was shown to mediate the induction of myostatin through the stress-activated p38 MAP kinase.<sup>62</sup> A member of the transforming growth factor (TGF)- $\beta$  superfamily, myostatin is a negative regulator of myocyte growth. Thus, IGF-I and myostatin form a negative feedback loop to regulate cardiac tissue size.<sup>63</sup> Understanding how this balance is achieved and how it can be manipulated will be important for developing effective and safe therapeutic strategies.

Studies of GH, IGF-I, or GH-releasing peptides to treat cardiomyopathies are summarized elsewhere.<sup>64</sup> While there have been clinical trials of rhGH, hexarelin, and ghrelin, studies of rhIGF-I, either singly or in combination with rhGH, are limited to rats following left coronary artery ligation, a common experimental model of post-myocardial infarction heart failure. These studies have shown that in rats with prior coronary artery ligation, rhIGF-I can increase ventricular mass and cardiac output, and lower systemic vascular resistance.<sup>65-68</sup> Results in humans, however, have not been published.

Another role of IGF-I may be to stimulate cardiac tissue regeneration in the setting of stem cell transplantation. Heterologous bone marrow cells were transplanted into the myocardial scars of Lewis rats 3 weeks following experimental ischemia; co-transfection of the donor cells with the genes for IGF-I and vascular endothelial growth factor (VEGF) resulted in better transplanted cell survival, lower apoptosis, and greater left ventricular ejection fraction than cells transfected with either gene singly or control transplantation with cell-free medium.<sup>69</sup> Cardiomyocytes derived from human embryonic stem cells proliferate in vitro, slowing gradually with increasing differentiation. In vitro proliferation of human stem cell-derived cardiomyocytes was inhibited by IGF1R-neutralizing antibodies and dose-dependently enhanced by IGF-I or IGF-II treatment.<sup>70</sup>

### OSTEOPOROSIS

IGF-I plays a role in promoting bone anabolism.<sup>71</sup> A pilot, randomized, double-blind, placebo-controlled trial of short-term, continuous subcutaneous infusion of rhIGF-I/rhIGFBP-3 was performed in older women (65-90 years of age) recovering from recent hip fracture. The infusion, administered via portable mini-pump, was initiated within 72 hours of the fracture event and continued for 8 weeks after hip fracture surgery.<sup>72</sup> Thirty patients were randomized 1:1:1 to higher dose (1 mg/kg/day), lower dose (0.5 mg/kg/day) or placebo infusions, and evaluated 6 months post-operatively (ie, 4 months after discontinuation of the infusion). Following the immediate post-operative loss of hip bone density (measured in the contralateral side), the high dose group regained femoral bone density while the placebo group remained



with a deficit at 6 months' follow-up; changes from baseline were  $-2.6\%$  ( $P = 0.53$ ) in the former and  $-6.1\%$  ( $P < 0.05$ ) in the latter. The high dose group also benefited from an  $11.4\%$  increase in grip strength ( $P < 0.05$ ), while the placebo group lost  $11.6\%$  ( $P = 0.16$ ), which further contributed to their functional recovery. The rhIGF-I/rhIGFBP-3 was tolerated well at both doses.<sup>72</sup>

Studies of rhGH and/or rhIGF-I, alone or in combination with anti-resorptive drugs, in the treatment of osteoporosis were recently summarized.<sup>73</sup> Controlled trials establishing an effect on fracture incidence are still needed. Also, with multiple options available for the treatment of osteoporosis, cost-effectiveness analyses of using rhIGF-I will need to be considered. For example, a similar short-term, randomized, double-blind, placebo-controlled study was done to address the protein malnutrition that is frequently found in osteoporotic, elderly individuals.<sup>74</sup> Subjects with recent osteoporotic hip fracture ( $n = 82$ , mean age  $80.7 \pm 7.4$  years) received 550 mg/day calcium supplementation and a single 200000 IU dose of vitamin D at baseline, and were then randomized to receive 20 gm/day protein supplementation or an isocaloric placebo for 6 months. The protein-supplemented group had significant increases in their serum IGF-I levels ( $85.6 \pm 14.8\%$ , vs  $34.1 \pm 7.2\%$  among controls;  $P < 0.005$ ) at 6 months, less proximal femoral bone mineral loss at 12 months ( $-2.29 \pm 0.75\%$  vs  $-4.71 \pm 0.77\%$  among controls;  $P < 0.05$ ), and shorter median stay in rehabilitation wards (33 vs 54 days;  $P < 0.05$ ). Thus, there may be less expensive interventions than rhIGF-I or rhIGF-I/rhIGFBP-3 to achieve similar therapeutic goals.

### NEGATIVE CLINICAL TRIALS OF rhIGF-I

Despite promising animal data or theoretical appeal of rhIGF-I for certain conditions, the results of some clinical trials have shown no benefit. For example, rhIGF-I was shown to enhance recovery in a rat model of acute renal failure.<sup>75</sup> However, a clinical trial of rhIGF-I showed no benefit in human subjects with delayed graft function following cadaveric renal transplantation.<sup>76</sup> Another trial of rhIGF-I was conducted based on the association of aging with lower IGF-I levels. In this study, 16 healthy, non-obese, post-menopausal women (mean age 71 years) were randomized to 1 year of rhIGF-I at a dose of  $15 \mu\text{g/kg}$  given twice daily. The mean circulating IGF-I increased from 66 ng/ml at baseline to 298 ng/ml at 12 months. However, at the end of the study there were no significant differences in bone mineral density, muscle mass, or cognitive function.<sup>77</sup> These studies highlight the critical importance of rigorous testing of all potential uses of rhIGF-I and rhIGF-I/rhIGFBP-3 by randomized, controlled trials.

### POTENTIAL SAFETY ISSUES

The adverse effect most commonly encountered in trials of rhIGF-I is hypoglycemia. This can be mitigated by taking the medicine with food. The greatest theoretical

risk of rhIGF-I treatment is that of cancer. Not only have higher circulating IGF-I levels been associated with increased risk of multiple cancers, but mechanistically, IGF signaling can contribute to all stages of the neoplastic process.<sup>78</sup> Current evidence supports a permissive—not causal—role for either GH or IGF-I in cancer development.<sup>79</sup> This has been borne out in formal carcinogenicity studies; over-expression of IGF-I in animals or the administration of rhIGF-I increased food intake, body size, and the growth rate of existing tumors, but did not increase tumor incidence.<sup>80</sup> One must keep this effect in mind when considering IGF-I as potential treatment for mature individuals at ages in which cancer incidence is highest to begin with. Careful screening and patient selection, as well as a thorough risk-benefit analysis for each patient, are warranted.

### SUMMARY

Recombinant human IGF-I therapy, singly or in combination with rhIGFBP-3, remains experimental for non-growth indications (Table). In diabetes, the best studied of the non-growth clinical indications, rhIGF-I clearly showed enhanced glycemic control in T1DM, but the benefit is offset by a worsening of diabetic retinopathy and a high frequency of other adverse effects including jaw pain, arthralgias, and edema. This is especially concerning at higher doses. In limited studies, low dose rhIGF-I therapy ( $40 \mu\text{g/kg}$  daily or twice daily) appears to improve glycemic control to a similar extent as higher doses but with fewer side effects. Given the risks of high dose rhIGF-I therapy, robustly designed long-term clinical trials of low-dose rhIGF-I therapy would be required to ensure its efficacy and safety. Combination rhIGF-I/rhIGFBP-3 therapy appears to improve glycemic control in patients with T1DM, although its efficacy in T2DM remains somewhat undefined. The safety of rhIGF-I/rhIGFBP-3 when used for more than 2 weeks is not clear and requires further study. Longer-term, placebo-controlled, double-masked efficacy studies need to be conducted before rhIGF-I is considered for either T1DM or T2DM. Any such studies should include careful interim assessments of microvascular complications and other adverse effects. Additionally, these agents are particularly appealing for insulin-resistant conditions such as lipodystrophy and when there is defective insulin receptor signaling. IGF-I may play a role in other conditions where undesired lean mass catabolism occurs with insulin resistance, as in low-calorie diets for obesity, and where there is severe systemic stress, such as renal disease, burns, cachexia, and other critical illnesses. However, there are no published studies available to date.

Potential use of rhIGF-I for central nervous system disease is promising, especially in light of the paucity of current treatment options. The fact that IGF-I stimulates clearance of brain amyloid beta, thereby correcting what is believed to be the primary pathogenic event, makes

it an especially appealing candidate treatment for AD. However, current evidence is still limited to preliminary preclinical data. While there are a few favorable trials of rhIGF-I in animal models of ischemic myocardial disease, much work needs to be done to determine the patient characteristics and treatment parameters that optimize efficacy and safety. Administration of rhIGF-I improves bone mineral density in some studies, but impact on fracture incidence and cost-effectiveness still needs to be resolved. Other conditions for which rhIGF-I has been found to be unhelpful are in the recovery of renal function after ischemia and as an anti-aging medicine. Potential benefits of IGF-I therapy must always be weighed against the potential risks of raising IGF-I levels. Thus, clinical use of rhIGF-I, singly or in combination with rhGFBP-3, for non-growth indications should be limited to well-designed clinical studies until adequate evidence supporting efficacy and safety can be collected.

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Growth Hormone Deficiency and Antipituitary Antibodies in Celiac Disease

Iughetti et al studied 130 patients (59 males, age  $5.67 \pm 3.6$  years, height  $0.32 \pm 1.25$  SDS) who had been diagnosed with celiac disease (CD) based on the presence of anti gliadin, antiendomysial, and antitransglutaminase antibodies, as well as endoscopic biopsies of the distal duodenal mucosa. These children had a poor clinical response to a gluten-free diet (GFD) and a growth hormone deficiency (GHD); they presented to the pediatric clinic at the University of Modena and Reggio Emilia, Italy between 1999 and 2004. Their growth velocity was determined yearly and serum endomysial antibodies were measured after at least 12 months on a GFD. Those children showing no catch-up growth on a GFD were evaluated to exclude possible GHD. Studies included measurement of basal serum GH, insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3, free T<sub>3</sub>, free T<sub>4</sub>, TSH, prolactin, cortisol, ACTH, LH, FSH, estradiol or testosterone, and repeat studies for antibodies. In addition, antipituitary and antihypothalamus antibodies were measured. On different days, arginine and L-dopa GH stimulation tests were performed in all 7 of the children identified as having poor catch-up growth. Bone age was determined as well. A diagnosis of GHD was based on short stature, decreased growth velocity, delayed skeletal maturation, and blunted GH response ( $<10 \mu\text{g/L}$ ) to the 2 pharmacological tests. Antipituitary antibodies were detected by an immunofluorescent method that had been previously described. MRIs were performed in these 7 patients.

Five of the 7 patients showed a blunted GH response to the different stimuli and met the criteria for GHD. Four of the 5 had high titers of antipituitary antibodies, 2 were additionally positive for antihypothalamus antibodies. Antipituitary antibodies were also positive in low titers in 3 out of 25 (12%) children with CD only, and in 2 out of 58 (3.4%) control children. None of the 7 children had any pituitary abnormalities on MRI.

The authors stated that in the past an insufficient GH response to hypoglycemia had been reported in

children with CD, which subsequently improved with a GFD. The hypothesis that autoimmunity could involve the pituitary gland was reported about 40 years ago; however, the nature and significance of antipituitary antibodies in GHD patients is still being discussed. The authors stated, however, that high titers of antipituitary antibodies could explain some cases of apparent idiopathic GHD. In patients with multiple autoimmune abnormalities, such as the children with CD, these antibodies may explain their GHD.

Iughetti L, De Bellis A, Predieri B, et al. Growth hormone impaired secretion and antipituitary antibodies in patients with celiac disease and poor catch-up growth after a long gluten-free diet period: a causal association? *Eur J Pediatr*. 2006;165:897-903.

**Editor's Comment:** *Whether or not one subscribes to the significance of antipituitary antibodies (and/or antihypothalamic antibodies) and the development of isolated GHD, the finding that 5 of 7 children with CD who failed to have catch-up linear growth after 12 months of a GFD met all criteria for GHD is an important finding. Indeed, it is tempting to ascribe failure of catch-up growth following initiation of a GFD to lack of compliance with the meal plan; but, GHD may be present in those children. It is important for pediatric endocrinologists to perform stimulation tests to identify this deficiency. Indeed, low IGF-I levels would not be a surprising finding in children newly diagnosed with CD, as their nutritional status is often poor. However, the failure of IGF-I levels to rise when antiendomysial antibody levels have fallen (at approximately 12 months after the initiation of a GFD) should raise suspicions as to an additional cause for growth failure. As more and more children are being diagnosed with CD, it becomes even more important for the pediatric endocrinologist to be aware of other endocrinologic abnormalities that might be associated with this disease; any of these may be autoimmune in origin.*

William L. Clarke, MD

### Genetics of Height Variation

A large number of human genetic disorders including chromosomal and single gene disorders have short stature as a significant component. Aside from these conditions, genetic factors have long been known to influence height within the normal range (ie, short parents have short children). Linkage studies have pointed to a number of chromosomal regions that contain one or more gene(s) that affect height, but the identities of

specific genes and how they influence height have eluded investigators. Liu and colleagues offer evidence that height is affected by the interactions of genes located in 2 different chromosomal regions, a phenomenon referred to as epistasis.

Height information was collected on 3726 Caucasians from 434 pedigrees as part of ongoing studies in the Osteoporosis Research Center of Creighton University to



identify genes that contribute to common human traits. Although most of the kindreds contained less than 10 individuals, many were larger, and 14 families had over 40 members who were studied. The many large families provided a large number of relative pairs for height comparisons, which increased the statistical power of the linkage analysis.

Genotyping was performed with microsatellite markers spaced on average about 9 cM apart. The initial analysis was designed to identify individual chromosomal regions linked to height variability. More specifically multipoint and two-point LOD scores were calculated using Sequential Oligogenic Linkage Analysis Routines (SOLAR). The results reported in early 2006 (Liu et al, Hum Genet 2006) suggested linkage for chromosome regions 9q22 and Xq24 and possible linkage at 6p21 and 2q21.

In the second publication reviewed here (Liu et al, J Clin Invest 2006), the investigators further analyzed their data for 3 loci (9q22, 6p21, 2q21) using statistical tests for pairwise epistatic interactions between the 3 loci under different hypothetical models. This approach allowed them to compare the effect of the individual loci with the additive effects of paired loci and with the interactive or epistatic effects of the paired loci. The epistatic model implies a genetic influence that is greater than simply adding the influences of the paired loci together.

The results revealed the most likely model to explain the results is a 2 locus epistatic model involving chromosome regions 6p21 and 2q21. In other words, the analysis suggests that a functional interaction between genes residing in these 2 regions somehow influences height.

After making statistical corrections, the authors suggested that the fraction of height variation due to the interaction between these 2 regions is approximately 20%.

The authors discussed the specific gene loci that map to the 6p21 and 2q21 chromosome regions. For example, 6p21 contains genes for the  $\alpha$ -2 chain of type XI collagen, the transcription factor, RUNX2 and the retinoid X receptor- $\beta$ , RXRB, all of which have known functions in linear skeletal growth. No obvious growth-related genes have been mapped to region 2q21.

Liu YZ, Xiao P, Guo YF, et al. Genetic linkage of human height is confirmed to 9q22 and Xq24. Hum Genet. 2006;119:295-304.

Liu YZ, Guo YF, Xiao P, et al. Epistasis between loci on chromosomes 2 and 6 influences human height. J Clin Endocrin Metab. 2006;91:3821-5.

**Editor's Comment:** *This report is very interesting, but not very surprising. It is becoming clear from investigations of the skeletal growth plate that its biology and function are regulated by mechanisms that typically involve molecular interactions of multiple gene products (proteins) often in the form of linear pathways, such as signal transduction pathways or in multicomponent complexes, such as signaling platforms and transcriptional complexes that act like complicated machines. Such pathways and complexes provide means for products of different genes to interact functionally, which is presumably what happens in this case, with proteins whose genes map to chromosomes 2 and 6.*

William A. Horton, MD

## Adiponectin Suppresses GH and LH In Vitro

Leptin is the prototypic adipokine, a newly recognized class of hormones originating in adipose tissue. Leptin relays the degree of body fatness back to the hypothalamus as part of the homeostatic mechanisms for regulating body energy balance. In addition to its hypothalamic effects, leptin directly stimulates pituitary secretion of luteinizing hormone (LH) and growth hormone (GH), facilitating reproductive, growth, and anabolic functions during times of nutrient abundance.

The more recently discovered adiponectin is the most abundantly secreted of the known adipokines. Adiponectin expression has also been detected in human and murine skeletal muscle, cardiac myocytes, osteoblastic cells, placenta, and chicken pituitaries. Adipocyte expression of both adiponectin and its receptor (AdipoR) have been shown to be regulated, at least in part, by GH.<sup>1,2</sup> Thus, Rodriguez-Pacheco et al sought to investigate whether adiponectin plays a central role, akin to leptin, in regulating somatotroph and gonadotroph function. Anterior pituitary glands were isolated from male Sprague-Dawley rats, minced, enzymatically dissociated and mechanically

dispersed to create in vitro cultures of adenohypophyseal cells. After 3 days in culture, and 2 hours in serum-free medium, the pituitary cells were switched to fresh medium and experimental conditions.

Growth hormone secretion was decreased by 34% to 52% after 4 hours incubation in adiponectin at concentrations of  $10^{-9}$  to  $10^{-7}$  M. After 24 hours exposure, only the highest dose of adiponectin ( $10^{-7}$  M) changed GH secretion, and that change was a doubling. Focusing on the short-term (4 hours), adiponectin exposure ( $10^{-7}$  M) inhibited the stimulation of GH release by  $10^{-8}$  M ghrelin, but not that of  $10^{-8}$  M GH releasing hormone (GHRH). This adiponectin dose increased pituitary cell expression levels of ghrelin receptor (GHS-R) by 34% and GHRH receptors (GHRH-R) by 448%; in the short-term  $10^{-8}$  M adiponectin also significantly induced GHRH-R expression. In contrast, long-term (24 hour) exposure to adiponectin at concentrations of  $10^{-9}$  to  $10^{-7}$  M did not alter expression levels of either GHS-R or GHRH-R.

Like GH, LH secretion was suppressed by 4-hours incubation in adiponectin at concentrations of  $10^{-8}$  to



$10^{-7}$  M, and the suppression was gone by 24 hours. Four hours of  $10^{-7}$  M adiponectin caused a 74% reduction in the LH secretion stimulated by  $10^{-8}$  M gonadotropin-releasing hormone (GnRH). At 4 hours GnRH receptor expression was halved by adiponectin at concentrations of  $10^{-9}$  to  $10^{-7}$  M, but only the highest concentration of adiponectin significantly reduced GnRH receptor expression at 24 hours.

Rodriguez-Pacheco et al also examined the pituitary adiponectin system. Expression of adiponectin and its 2 receptors (AdipoR1 and AdipoR2) were demonstrated by RT-PCR in extracts of rat and human pituitaries. Returning to the rat pituitary cell culture model, the authors found that 4 hours of adiponectin exposure at concentrations of  $10^{-9}$  to  $10^{-7}$  M increased its own expression by almost 70% (at the highest dose only), but did not alter the expression levels of either of its 2 receptors. However, after 24 hours' exposure, adiponectin ( $10^{-8}$  M only) increased its own expression (by 300%), decreased expression of AdipoR1 (by  $10^{-8}$  M only), and increased expression of AdipoR2 (by  $10^{-7}$  M only).

Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, et al. Regulation of pituitary cell function by adiponectin. *Endocrinology*. 2007;148:401-10.

**Editor's Comment:** Rodriguez-Pacheco et al showed that short-term adiponectin exposure suppressed both basal and stimulated (by ghrelin [but not GHRH] and GnRH) secretion of GH and LH, respectively, by rat pituitary cells *in vitro*. They further laid the groundwork for a pituitary adiponectin autocrine/paracrine system in which both adiponectin and its receptors are expressed and further modulated by adiponectin exposure. Thus, adiponectin seems to serve like the classic adipokine leptin, in centrally linking growth, anabolic, and reproductive

function to fat cell activity. These relationships warrant *in vivo* confirmation. From the evidence so far, it seems that neither endocrine leptin nor endocrine adiponectin underlie the old clinical observation that obesity suppresses GH secretion; circulating leptin levels are increased in obesity but leptin stimulates GH release, and although adiponectin suppresses GH secretion, as shown in this paper, circulating adiponectin levels are reduced in obesity.

Nonetheless, adiponectin attracts tremendous clinical interest. Adiponectin seems to do what clinicians are desperately seeking to accomplish in the obesity epidemic: adiponectin acts as an insulin-sensitizing, anti-atherogenic, anti-inflammatory, anti-angiogenic, and anti-tumoral agent. The sooner we learn about adiponectin physiology, the sooner it can inspire novel therapeutic approaches.<sup>3,4</sup> For example, it turns out that thiazolidinediones up-regulate adiponectin. Adiponectin's reported insulin-sensitizing activities are multiple and peripheral: it enhances hepatic insulin action and decreases endogenous glucose production; it increases glucose uptake by adipocytes and myocytes, and it increases fatty acid oxidation in muscle. If the *in vitro* findings of this paper are confirmed *in vivo*, then we can add one more mechanism to the list: adiponectin centrally inhibits secretion of the counter-regulatory GH. Further, adiponectin was shown to decrease body weight in mice by stimulating energy expenditure.<sup>5</sup> Not everything from fat is bad.

Adda Grimberg, MD

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## Vitamin D Receptor in Idiopathic Short Stature

Stature is a highly heritable trait, but beyond those genes known to cause specific disorders in which short stature is a major component, the genetic factors responsible for variation in height are poorly understood. As reported by Dempfle et al, genome-wide linkage scans of adult height have been performed in at least 22 separate samples and the results summarized in 12 publications. Although these studies, most of which have been performed on relatively small samples, yielded divergent results and no chromosomal region was highlighted across all scans, evidence for linkage is convincing for some regions, in particular regions on chromosomes 6, 7, 9, and 12.

Building on these studies, Dempfle et al carried out a genome-wide scan on 92 families, each with 2 affected children with idiopathic short stature (ISS), which they defined as including constitutional delay of growth and puberty, familial short stature, and ISS in its more narrow meaning. For inclusion, each family had one child whose

height was below the 5<sup>th</sup> percentile and a second child with height less than the 15<sup>th</sup> percentile. Only Caucasian families were included, and all but 2 parents were of German origin.

Linkage analysis using 511 short tandem repeat markers revealed the highest LOD score (3.18 [and only LOD score >3]), which is usually accepted proof of linkage, at chromosome 12q11. This is the region to which adult height has been linked and which contains the vitamin D receptor (*VDR*) gene that has been previously implicated as a factor in adult height variability. In fact, as noted in a 2005 GGH abstract,<sup>1</sup> a single nucleotide polymorphism (SNP) at the *VDR* locus has been associated with variation in adult stature. The same association was found in ISS in this investigation.

The *VDR* polymorphism involves the substitution of a G base for an A base at a particular nucleotide; it is called the G allele. The G allele was detected more often

than the A allele in children and adolescents with ISS. The substitution maps to the VDR start codon where it abolishes the first translation initiation site, resulting in a peptide lacking 3 amino acids, which increases the transcriptional activity of the gene. The more active allele was over-transmitted to affected children in the sample giving estimates of relative risks for ISS of 1.33 and 1.9, respectively, for heterozygotes and homozygotes for the allele. The authors suggested that on the population level, the G allele might be responsible for 34% of ISS cases.

The genomic scan did not detect evidence of linkage to other sites that have been implicated by other investigators in ISS, including the *SHOX* and *NPR2* loci.

Dempfle A, Wudy SA, Saar K, et al. Evidence for involvement of the vitamin D receptor gene in idiopathic short stature via a genome-wide linkage study and subsequent association studies. *Hum Molec Genet.* 2006;15:2772-83.

**Editor's Comment:** Readers may ask: if linkage to the VDR locus and association with the G allele of VDR has been established for adult height, why repeat the genomic scan in children with ISS? The reason is that the findings in the adult study could be explained by the effects of several genes, each having a small impact on stature or a small number of genes having a larger impact. Finding a similar effect in a small subset of individuals, ie, those with ISS, argues for a larger effect of a smaller number of genes, of which VDR is one. The next step will be to delineate how the more transcriptionally active VDR allele actually affects linear bone growth.

William A. Horton, MD

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## Hyperinsulinemia, Impaired Glucose Tolerance, and T2DM in Cancer Survivors

The occurrence of hyperinsulinism and type 2 diabetes mellitus (T2DM) has been identified in survivors of childhood malignancy, particularly after bone marrow transplantation (BMT). Only small numbers of patients had been studied and evaluated long-term. The recent study of Hoffmeister et al<sup>1</sup> dealing with a population of children followed after hematopoietic cell transplantation, showed a 3-fold increase rate for T1DM and T2DM. The study of Neville et al focused on the predisposing factors and early markers of DM, a critical issue for the development of prevention strategies. This group studied 248 survivors of childhood cancers; half of them were adults at the time of evaluation. The median duration after diagnosis was 12.9 years. They grouped hyperinsulinism (HI), impaired glucose tolerance (IGT), and T2DM

together for analysis of potential risk factors. Body mass index (BMI) and abdominal adiposity were potential markers. In this population, which is often growth-retarded, the waist-to-height (W/H) ratio correlated well with the volume of visceral fat as measured by CT scan. A ratio of >0.5 was considered a good predictor of complications of obesity.

The mean BMIs of both prepubertal and pubertal subjects were similar compared with controls, but the mean W/H ratio was higher, with a doubling of the percentage in children with abdominal adiposity. In all groups, there was a tendency for accumulating abdominal fat. In pubertal and adult subjects, abdominal adiposity was predictive for the occurrence of biochemical markers for metabolic abnormalities (insulinemia and lipid profiles). Fasting insulin concentrations were higher in prepubertal and pubertal subjects, compared with their controls. Hyperinsulinism, IGT, or DM were detected in 18% of pubertal and adult subjects. Eleven percent of this group had IGT/DM ( $p < 0.001$ ). In the group with BMT, conditioning with total body irradiation (TBI) increased the risk (Table).

This study confirms the risk factors previously identified, with a strong focus on the BMT group. Total body irradiation turns out to be a major risk factor for metabolic abnormalities. Differences with previously reported studies could be accounted for by the prospective approach, the

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**Table. Significant risk factors in cancer survivors for the development of HI, IGT, or DM**

|   | Odds ratio<br>(95% CI) | P<br>value |
|---|------------------------|------------|
| BMT (54) vs. all others (158)             | 6.6 (3.1–13.9)         | <0.001     |
| ALL BMT (16) vs. no BMT (82) <sup>1</sup> | 25.6 (6.6–100)         | <0.001     |
| TBI                                       | 13.8 (5.7–34.3)        | <0.001     |
| Pituitary irradiation ( $\geq 30$ Gy)     | 4.5 (2.1–10.0)         | <0.001     |
| GH deficiency                             | 5.1 (2.3–11.3)         | <0.001     |
| Untreated hypogonadism                    | 21.1 (6.4–69.7)        | <0.001     |
| Untreated hypothyroidism                  | 19.7 (2.1–181.2)       | 0.009      |
| Overweight or obese (BMI)                 | 5.3 (2.5–11.4)         | <0.001     |
| Abdominal adiposity <sup>2</sup>          | 14.5 (4.9–42.8)        | <0.001     |
| Family history of dyslipidemia            | 2.1 (1.0–4.2)          | 0.04       |
| Hypertension                              | 2.6 (1.1–5.8)          | 0.03       |
| BMT survivors only (54)                   |                        |            |
| TBI                                       | 7.6 (2.2–26.2)         | <0.001     |
| Busulphan                                 | 0.2 (0.1–0.8)          | 0.02       |

<sup>1</sup> Patients with acute lymphoblastic leukemia (ALL) with and without BMT.

<sup>2</sup> Abdominal adiposity defined as a waist-to-height ratio more than 0.5.

(n)=number

Modified from Neville KA, et al. J Clin Endocrinol Metab. 2006;91:4401-7.

broad ranging diagnoses, and the grouping together of the 3 metabolic criteria. Interestingly, hypogonadism also emerged as an independent risk factor, and W/H ratio was a more important marker than BMI. In keeping with these data, it is suggested that the use of conditioning with TBI for BMT deserves reconsideration and underlines the need for regular and long-term clinical and metabolic follow-up.

Neville KA, Cohn RJ, Steinbeck KS, Johnston K, Walker JL. Hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus in survivors of childhood cancer: prevalence and risk factors. J Clin Endocrinol Metab. 2006;91:4401-7.

**Editor's Comment:** Diabetes mellitus has not been considered a significant risk in the follow-up of cancer survivors. Initially, treatment with asparaginase suggested a rare immediate risk. Thereafter, the higher frequency of moderate—but significant—overweight observed in patients with leukemia suggested such a risk. In the present prospective study of a large group of etiologies, a new vision is emerging. Of note, some factors did not turn out to be significant: asparaginase-related hyperglycemia, diagnosis, small birth size, abdominal or testicular irradiation. The group at risk had BMT with TBI as conditioning, as opposed to busulfan conditioning, which had no significant effect on the metabolic outcome. The authors suggested that the pancreatic beta cell is an unlikely target, and instead focused on the effect of irradiation on the muscle mass by unknown mechanisms, one possibly being mitochondrial dysfunction. Little is known about the outcome of the irradiated adipose tissue and possible inflammatory processes.

This study provides some clinical clues such as early correction of hypogonadism and careful follow-up of W/H ratio. In the population at risk because of TBI, appropriate nutritional and lifestyle control may not be sufficient. More long-term studies are needed to help understand the mechanism(s) of these adipose—and possibly muscular—changes to help prevent metabolic syndrome and DM.

Raphaël Rappaport, MD

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## Growth Attenuation in Developmental Disabilities

Caring for nonambulatory children with profound developmental and cognitive disabilities becomes more difficult as the child grows. Treatment with high-dose estrogen, can arrest further growth and facilitate the option of continued care in the home. This case report discusses medical and ethical considerations of such an intervention strategy and describes a comprehensive program including reviews by pediatric specialists in endocrinology, neurology, development, surgery, and ethics.

A 6-year, 7-month-old girl was referred to pediatric endocrinology for early pubertal development. She had a 1-year history of pubic hair and a 3-month history of breast budding. Static encephalopathy with marked global developmental deficits was previously diagnosed. Motor and cognitive development never progressed beyond that of an infant; at 6 years of age

she could not sit up, ambulate, or use language. She was gastrostomy-tube dependent for nutrition and responded to others by vocalizing and smiling. The consensus of the specialists was that there would be no significant future improvement in cognitive or neurological function.

The onset of puberty roused parental fears that they would not be able to continue to care for their daughter at home, despite their desire to do so, as she continued to grow. A plan to attenuate growth using high-dose estrogen was developed along with pretreatment hysterectomy. An institutional ethics committee met with the family, patient, and patient's physicians and reached consensus that requests for growth attenuation and hysterectomy were ethically appropriate in this case. Plans were instituted to convene an interdisciplinary review panel that included pediatric specialists in



endocrinology, neurology, development, surgery, and ethics. After a uneventful surgery and a little more than a year of daily transdermal estradiol (400 µg), the patient approached the end of her linear growth.

Gunther and Diekema reviewed the history of growth-attenuation therapy, in particular its application to tall adolescent girls. Most reported decreases in adult height between 2 cm and 10 cm, with greatest reductions observed the earlier the treatment was initiated. The authors speculated that "treatment beginning in a 5-year-old boy of average height and weight might result in a reduction in final length of as much as 24 inches (60 cm) and in weight of more than 100 pounds (45 kg)." What of the risks of treatment? Based on experiences in treating girls for constitutional tall stature, known short-term risks of high-dose estrogen treatment include mild nausea, headache, and weight gain. Long-term effects on fertility have recently been raised, although this risk did not apply to the case(s) in question. The effects of high-dose estrogen in young prepubertal children includes gynecomastia in boys, and rapid advancement of secondary sexual characteristics in girls, including uterine bleeding which can be controlled with injections of depot medroxyprogesterone acetate (DMPA) or hysterectomy. Concerns over the risk of thrombosis were not thought to be a reason to withhold this intervention. The authors acknowledged historical controversies associated with hysterectomy but, in profoundly impaired children, careful ethical and legal deliberations are needed.

Ethical factors in the decision to employ high-dose estrogen treatment to attenuate growth in a profoundly impaired individual included past abuses against this population justified by the benefits to society or the caretakers, rather than the individual. The authors discouraged overgeneralizing from past abuses directed toward mildly- to moderately-impaired individuals to the potential benefits of such interventions for those who are nonambulatory, profoundly cognitively and neurologically impaired, and wholly dependent on others for all their needs. Two major considerations in determining whether it is ethical to attenuate growth in this population exist: does growth attenuation offer the patient benefit, and does growth attenuation do any harm to the patient? A thoughtful discussion was presented with the thrust pointing in the direction of benefits to the child and family through the reduced physical burden on aging parents in attending to the changing physical needs of their child in the home. The authors addressed medical and psychosocial harm associated with this treatment, and concluded that patients such as the one being discussed would not be placed unduly at risk.

Gunther DF, Diekema DS. Attenuating growth in children with profound developmental disability: a new approach to an old dilemma. Arch Pediatr Adolesc Med. 2006;160:1013-17.

**Editor's Comment:** In an editorial accompanying this article, Brosco and Feudtner<sup>1</sup> acknowledged the

predicament facing parents of children with profound cognitive and physical disabilities who are considering continuing care in the home. In evaluating the reasoning of Gunther and Diekema's strategy, they posed 4 questions: (1) Will early high-dose estrogen treatment enable such children to remain home under the care of their parents for longer periods of time? Will this improve the quality of their lives? What if the height-attenuated child continues to gain weight; wouldn't this effect partially offset the benefits of shorter stature? These questions, in addition to concern over the association between low-dose estrogen therapy and seizures, lead the commentators to call for a rigorous investigation of assumptions underlying this intervention and examination of unforeseen risks; (2) Is it acceptable to manipulate a person's height? Here, the authors have little trouble dispensing with such concerns; they opined that to not do so implies that a person's value as a human being is dependent upon their physical size; (3) Will this treatment be misused? The history of the eugenic movement in the first half of the 20<sup>th</sup> century and more recent practices (eg, sterilization of individuals with mental retardation) that have come to be rejected, lead the authors to call for stiff safeguards and protections; and (4) Is the proposed treatment an attempt at a simple technical fix to a far more complex problem, that being the plight of families caring for such children without adequate societal support?

The authors concluded that attempts to attenuate growth in such cases are ill-advised. Instead, they claim what is needed are more funds for home-based services. They warn clinicians that adopting medical interventions, even in the context of interdisciplinary review with ethical oversight, will ultimately be judged "in the social-political context of both the disability rights movement and the woefully impoverished options for high-quality, long-term residential care of children or adults with profound developmental disabilities."

Personally, I am particularly sympathetic to the fourth point of Brosco and Feudtner. It appears that in our effort to help struggling families, we may narrow our intervention options to those perceived to be in our control, in this case, high-dose estrogen therapy to attenuate growth. Adopting such an approach to achieve the desired outcome of allowing the parents to continue to care for their child at home seems far more attainable than trying to modify the state of home-based services. Besides, strictly speaking, we are not paid to do the latter. However, if there is validity to this line of reasoning, then how do we avoid choosing the expedient and readily available when it clashes with the just course of action?

David E. Sandberg, PhD

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## Sox2/SOX2 Mutation and Abnormalities in Hypothalamo-pituitary-gonadal Axis

Many transcription factors are required for normal development of the adenohypophysis, including *LHX3*, *LHX4*, *HESX1*, *PROP1*, *POU1F1*, *SF1*, *SOX3*, and others. To this growing list may now be added *SOX2* (OMIM 184429, chromosome 3q26.3-q27), another member of the family of transcription factors that contain an SRY-related, 79 amino acid, high-mobility group box (HMG) DNA-binding domain. The 20 members of the SOX gene family encode proteins that are necessary for neuroepithelial cell differentiation. Humans with heterozygous mutations of *SOX2* have bilateral anophthalmia/microphthalmia, sensorineural hearing loss, anomalies of the male genital tract, short stature, and developmental delay. To this clinical picture, the present authors have added hypogonadotropic hypogonadism. The investigators examined the *SOX2* genotype in 235 patients (143 male) with congenital hypothalamic-pituitary disorders (97 subjects with congenital hypopituitarism and no midline cranial or eye defects; 126 patients with septo-optic dysplasia [SOD], 12 patients with anophthalmia/microphthalmia). They identified heterozygous loss-of-function mutations in *SOX2* in 8 patients, 6 of whom had bilateral or unilateral anophthalmia/microphthalmia and 2 of whom had SOD (Table). The 6 patients with congenital defects of eye formation had isolated hypogonadotropic hypogonadism, one subject with SOD had deficiencies of growth hormone (GH), TSH, and ACTH, while the second patient with SOD was short but pituitary function had not been evaluated. Depending on the site of the *SOX2*

mutation, there was defective nuclear localization of the *SOX2* protein, impairment of its binding to DNA, or decrease in its ability to transactivate target genes.

The investigators then evaluated mice in which *Sox2* had been partially inactivated. Homozygous loss of *Sox2* was lethal, while partial heterozygous loss of *Sox2* led to impaired growth and subfertility in males, which was associated with decreased pituitary content of GH and LH (but no ocular abnormalities). The adenohypophyses of these animals were small, the morphology of the somatotrophs and gonadotrophs abnormal, and the number of these cells low compared to wild-type animals. The pituitary contents of TSH and prolactin were variably low in the heterozygous mice, but that of ACTH was normal. In addition, *Sox2* heterozygous male mice were subfertile; many had small testes with abnormal spermatogenesis. The *Sox2* heterozygous female mice had normal fertility. The data indicate that *Sox2* is an important transcription factor for development of the anterior pituitary (and testes/spermatogenesis). The pituitary expression of *Sox2* normally declines as the adenohypophysis develops, but its expression is maintained in the hypothalamus, suggesting that an abnormality of hypothalamic function may also be present in mice with heterozygous mutations in *Sox2*. (With more stringent *Sox2* deficiency, ocular anomalies can be produced experimentally.)

Kelberman D, Rizzotti K, Avilion A, et al. Mutations within *Sox2/SOX2* are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. *J Clin Invest*. 2006;116:2442-55.

**Table. Clinical phenotype in patients with *SOX2* mutations**

| Pt | Mutation  | MRI   | Ocular phenotype                                       | Other features  |
|----|-----------|---|--|---|
| 1  | c.60insG  | Hippocampal abnormalities, small corpus callosum, hypothalamic hamartoma, APH, generalized reduction of white matter bulk, absent optic nerves  | Bilateral anophthalmia                                 | HH, learning difficulties, spastic diplegia, esophageal atresia                                 |
| 2  | c.70del20 | Hippocampal abnormalities, abnormal anterior pituitary, absent left optic nerve   | Left anophthalmia, right microphthalmia                | HH, learning difficulties   |
| 3  | c.387delC | Hypoplastic corpus callosum, APH, hypothalamic hamartoma, small left optic nerve and chiasm, generalized lack of white matter bulk, hippocampal abnormalities with small and rotated mesial temporal structures | Left microphthalmia, right coloboma                    | HH, cryptorchidism, micropenis, learning difficulties, mild spastic diplegia                    |
| 4  | Y160X     | Partial agenesis of corpus callosum, small anterior pituitary, hippocampal abnormalities, generalized reduction in white matter   | Bilateral microphthalmia                               | HH, cryptorchidism, micropenis, severe learning difficulties, spastic and dystonic quadriplegia |
| 5  | Q177X     | Not done  | Bilateral anophthalmia                                 | HH, cryptorchidism, micropenis, severe learning difficulties, mild facial dysmorphism           |
| 6  | c.479delA | APH, small hippocampus, thin corpus callosum, cavum septum pellucidum, absence of optic nerves and chiasm   | Bilateral anophthalmia                                 | HH, small testes, micropenis, learning difficulties, sensorineural deafness                     |
| 7  | G130A     | Absent septum pellucidum, bilateral optic nerve hypoplasia, bilateral schizencephaly, right porencephalic cyst, normal anterior and posterior pituitary   | Roving nystagmus with bilateral optic nerve hypoplasia | Short stature with a normal growth velocity; endocrine status not investigated                  |
| 8  | A191T     | Absent septum pellucidum, small optic chiasm, absent infundibulum, severe APH, ectopic-undescended posterior pituitary  | Roving nystagmus with bilateral optic nerve hypoplasia | GH, TSH, and ACTH deficiency  |

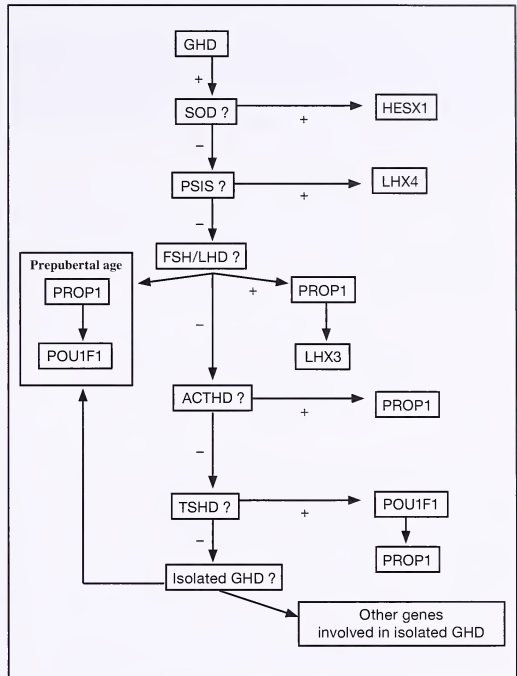
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**Editor's Comment:** Loss of SOX3 (OMIM 313430, chromosome Xq26.3) activity in man is associated with X-linked GH deficiency and mental retardation.<sup>2</sup> Reynaud and co-workers<sup>3</sup> reported the distribution of mutations in PROP1, POU1F1, LHX3, LHX4, and HESX1 in a population of 165 unrelated families (195 patients) with deficiencies of multiple anterior pituitary hormones (combined pituitary hormone deficiency [CPHD]) with or without SOD or pituitary stalk interruption syndrome (PSIS). Overall mutations in one of the 5 transcription factor genes examined were found in 22 of 165 index patients (13.3%). CPHD was familial in 21 families, with mutations identified in 10 of these 21 families (52.4%). Homozygous or double heterozygous mutations in PROP1 were identified in 20 patients, in 8 of whom CPHD was familial. A mutation in POU1F1 or LHX4 was identified in only one patient each, and no mutations in LHX4 or HESX1 were found in this CPHD population. Although mutations of HESX1 have been found in patients with SOD, none were identified in the report of Reynaud and co-workers. It would be of interest to analyze SOX2 in these subjects. Reynaud et al also correlated phenotype with genotype and outlined a schematic algorithm through which gene analysis of patients with CPHD and associated anomalies might be pursued (Figure).

Allen W. Root, MD

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3. Reynaud R, Gueydan M, Saveanu A, et al. *J Clin Endocrinol Metab.* 2006;91:3329-36.



**Figure.** Algorithm of CPHD genetic screening. FSH/LHD, FSH and LH deficiencies; ACTHD, ACTH deficiency.

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## Clinical Significance of a 6 Hr Exon 3-deletion Polymorphism

Audi and colleagues from the Spanish SGA Study Group reported the relative frequencies of the deleted and full-length exon 3 growth hormone receptor (GHR) polymorphisms in 247 short stature children and adolescents with birth weight small for gestational age (SGA) and 289 normal stature adult control subjects. The homozygous or heterozygous inheritance of the exon 3 deleted isoform has been reported to enhance GH action, although the significance of this genotype on GHR function is unknown. There was a 2-fold increase of the biologically less active homozygous full-length exon 3 isoform genotype in the SGA subjects. In the control population, there was no relationship between the height phenotypes and genotypes of the subjects. Therefore, it is suggested that in short stature SGA subjects, the presence of the full-length isoform may have impeded post-natal catch-up growth.

Carrascosa et al, also from the Spanish SGA Hormone Study Group, reported the results of GH

therapy in patients from the same cohort of SGA subjects as described by Audi and colleagues. Previous reports have demonstrated an increased growth response to GH therapy in SGA subjects who have the deleted exon 3 isoform, compared to those with the full-length receptor.<sup>1</sup> In contrast, this paper reported no differences in first- or second-year growth velocity and height gain between the different genotypes of 86 GH-treated SGA subjects. These patients were treated with a GH dose of 66 µg/kg/day, an amount that is at the upper end of the recommended dose and higher than in other reported series. It was suggested that these high GH doses might over-ride a more subtle effect reported with lower GH regimens.

Jorge and colleagues from São Paulo, Brazil performed a retrospective genetic analysis for the retained or deleted exon 3 GHR genotypes in 75 patients with severe isolated or combined GH deficiency. Clinical and laboratory data were similar at baseline in patients with different

genotypes. However, patients on GH therapy who were carrying at least one GHRd3 allele demonstrated a higher first-year height velocity ( $P<0.05$ ), compared to those with the full-length isoform. Final height was also greater in the GHRd3 subjects. No parental height data were given. Jorge et al hypothesized that manipulation of GH dose following genotype characterization might become a reality in the future.

Blum and colleagues from Eli Lilly in Germany studied 107 patients with severe idiopathic GH deficiency. In contrast to the Jorge group, they found no difference in growth responses to GH therapy between the subjects with the d3-GHR allele and those with the full-length receptor.

Audi L, Esteban C, Carrascosa A, et al. Exon 3-deleted/full-length growth hormone receptor polymorphism (d3/ff-GHR) genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents ( $n=247$ ) and in an adult control population ( $n=289$ ) show increased ff/ff in short GSA. *J Clin Endocrinol Metab.* 2006;91:5038-43.

Carrascosa A, Esteban C, Espadero R, et al. The d3/ff-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational-age children: results from a two-year controlled prospective study in 170 Spanish patients. *J Clin Endocrinol Metab.* 2006;91:2381-6.

Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold J. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab.* 2006;91:1076-80.

Blum WF, Machinis K, Shavrikova EP, et al. The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. *J Clin Endocrinol Metab.* 2006;91:4171-4.

**Editor's Comment:** *Several large studies that look at the possible influence on responses to GH therapy of homozygous or heterozygous inheritance of the deleted exon 3 GHR isoform have now been performed. The results are conflicting in SGA subjects as no difference in growth response was found in the Spanish study, contrasting with the original description of an apparent growth-enhancing effect shown in the French study. However, Binder et al<sup>2</sup> reported significantly increased responses in both SGA and Turner syndrome patients carrying the exon 3-deleted isoform. Now, Jorge and Blum have reported different outcomes and conclusions in patients with GH deficiency.*

*With such differing conclusions, it is hard to imagine that an effect of real clinical relevance exists from the inheritance of the deleted isoform. No doubt further studies will be published with probably differing conclusions. At this stage, the prospective genotyping of short patients in order to optimize their responses to GH therapy seems premature.*

Martin O. Savage, MD

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## GROWTH IN OSTEOGENESIS IMPERFECTA

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### INTRODUCTION

Osteogenesis imperfecta (OI), or brittle bone disease, is a rare disorder with congenital bone fragility caused by mutations in the genes that codify for type I pro-collagen production in osteoblasts (*COL1A1* and *COL1A2*), located in chromosomes 7 and 17.<sup>1</sup> Numerous mutations have been described as causing the condition.<sup>2</sup> In the vast majority of cases, OI is inherited in a dominant fashion, or caused by a new mutation. The prevalence of OI is estimated to be 1 in 20 000 to 50 000 infants.<sup>3</sup>

Besides brittle bones, clinical characteristics and severity of OI are widely variable. There

may even be a different degree of severity in different members of the same family.<sup>4,5</sup> Clinical features that may be present include bone fragility, joint hyperlaxity, muscle weakness, chronic unremitting bone pain, and skull deformities (eg, posterior flattening) due to bone fragility in infants with severe OI. Fractures may still occur after puberty,<sup>6</sup> with bone fragility persisting throughout life. Individuals with mild forms of the disease may have normal stature with no deformities or fractures at all, and the condition would be diagnosed only when an x-ray is obtained for other reasons. People with severe OI may have extreme short stature and severe deformity of the long bones. Exercise tolerance and muscle strength are significantly reduced in patients with OI, even in the mild forms.<sup>7</sup>

Osteogenesis imperfecta can affect several organs and systems. For example, hearing loss may be present in about 50% of the

### From The Editor's Desk

Dear Colleague:

You may be aware that our former sponsor, Inmed, settled a patent infringement dispute and no longer promotes IGF-1/IGFBP-3 to patients with severe primary IGF-1 deficiency or other short stature indications. Therefore, they no longer provide an educational grant to the GGH journal. Consequently, Pediatric Sunshine Academics, Inc., a 501(c)(3) non-profit organization, is funding the cost of this issue of GGH without prior anticipation or alternative funding sources available.

However, I am committed to seek new grants that will allow us to continue publishing this journal. I am grateful to the editorial board for their strong support; they have all pledged to contribute with their usual efforts and expertise while we seek more stable times. Since its inception 23 years ago, GGH has improved and expanded; it is held in high regard and enjoys over 11 000 subscribers. We all feel obliged not to let you down.

In order to forge ahead GGH will need the support of its readers while we elicit educational grants. You can help us during this transition by contributing to Pediatric Sunshine Academics, Inc. an organization whose mission is to support research and education in pediatric endocrinology and nutrition. Your fully tax deductible donation to **Pediatric Sunshine Academics, Inc., P. O. Box 3208, Tallahassee, FL 32315-3208**, either by check or online at [www.PedSacademics.org](http://www.PedSacademics.org) will be used entirely for the continued publication of GGH. Pediatric Sunshine Academics, Inc.'s federal EIN is 65-0854085.

On behalf of the editorial board, I thank you in advance for your donations and support. I will keep you apprised of our quest to elicit new grants and sponsorships for the continuation of the publication of GGH.

Fima Lifshitz, MD  
Editor-in-Chief



individuals with mild forms of OI after the third decade of life.<sup>8</sup> The incidence of congenital malformations of the heart in children with OI is probably similar to that of the normal population,<sup>9,10</sup> but respiratory complications secondary to kyphoscoliosis are common in individuals with severe OI.<sup>11</sup> Joint hyperlaxity is also a common occurrence in patients with OI,<sup>12</sup> and may lead to dislocation of hips and radial heads, sprains, and flat feet. Constipation and hernias are also a common complication of OI.<sup>13</sup> Dentinogenesis imperfecta (DI), caused by an abnormal dentin while enamel remains normal,<sup>14,15</sup> is prevalent in about 28% of OI patients.<sup>16</sup> Life expectancy in subjects with non-lethal OI appears to be the same as that in the normal population,<sup>17</sup> with the exception found in cases of very severe OI with respiratory or neurological complications.<sup>18</sup>

Histomorphometric analysis of the bone in patients with OI shows decreased trabecular bone volume, possibly secondary to the formation of fewer trabeculae, and to a lack of thickening of trabeculae with growth. There is evidence of defects in modeling of external bone size and shape, production of secondary trabeculae by endochondral ossification, and thickening of secondary trabeculae by remodeling.<sup>19</sup> Contrary to the common conception of attributing the defect in OI to the osteoclast, OI should be regarded as a disease of the osteoblast. Collagen plays an essential role in forming an interactive network between the cells by making extracellular matrix and noncollagenous proteins that lead to proper mineralization of the bone. When the fundamental structure of the collagen helix is disturbed by a mutation, a complex series of secondary changes to the bone develops, leading to increased bone fragility.

### GROWTH IN CHILDREN WITH OI

Severely affected patients may be short because of vertebral compression fractures, severe scoliosis, lower limb deformities, and disruption of growth plates.<sup>20</sup> However, growth can also be delayed in the absence of these abnormalities. The most commonly used classification divides OI into 4 types. Type I patients do not have bone deformities and may have normal height, but fractures may range from very few to dozens over a lifetime. Type II is the most severe, with patients usually not surviving the perinatal period. Patients with type III have a characteristic triangular face, very short stature, and severe bowing of long bones; they typically suffer many fractures throughout their life. Type IV is not clearly defined. Patients with this type of OI are generally short, although there is no consensus regarding the specific characteristics of this type. Other types have been described, but there is controversy because they actually represent syndromes resembling OI.<sup>1</sup> According to one study, during the first 10 years of life the number of fractures, extent of skeletal deformities, and growth retardation do not differ between OI types III and IV.<sup>10</sup> This is surprising, as individuals with type III OI usually have

very short stature, whereas individuals with type IV OI may have mild-to-moderate short stature. Furthermore, according to some authors, individuals with type IV OI may have normal stature.<sup>21</sup> This highlights the inaccuracy of classifying this disease into 4 types.<sup>22</sup> I will, therefore, refer to OI "severity" throughout this article, instead of OI "type."

The mean standing height of patients with OI is lower than that of their unaffected first degree family members, regardless of severity. Truncal height is reduced and head size increased in one third of the patients, more so in individuals with moderate or severe OI (Sillence's types IV and III). During childhood, there appears to be no difference between the standing heights of girls and boys, but women had lower height z-scores than men. The reduction in arm span z-score generally follows the same pattern as for height: individuals with moderate or severe OI tend to have lower z-scores than individuals with mild OI. The arm span/height ratio appears to be increased in children with moderate or severe OI, but not in those with mild OI. Mean concentrations of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 are generally normal, in the low range of age-specific reference values.<sup>10,21</sup> Growth hormone (GH) deficiency is very rare in patients with OI. In a group of 22 children tested by Marini et al.,<sup>23</sup> none fulfilled the standard criteria for GH deficiency. A few children in that study had a blunted response to GH-releasing hormone or failed to double their serum IGF-I in a 5-day somatomedin generation test. However, there was no consistent relationship between those responses or between the responses and type of OI.

The etiology of the growth restriction in children with moderate and severe OI is not entirely clear. It has been suggested that it could be viewed as a self-protective mechanism: a given mechanical load creates smaller stresses in a short bone than in a long bone, thus a short bone will break less easily.<sup>24</sup> People with severe OI have a typical deformity of the growth cartilage, defined as "popcorn" appearance of the metaphysis. Microfractures of the growth cartilage may play a role in the growth problems experienced by these patients. There are no reports on the effects of puberty and hormonal changes on growth in children with OI.

### USE OF BISPHOSPHONATES IN CHILDREN WITH OI

Bisphosphonates are synthetic drugs with a chemical structure based on pyrophosphate,<sup>25</sup> and have been used to treat osteopenia of primary and secondary origin in both children and adults.<sup>26</sup> Effects on both osteoblasts<sup>27,28</sup> and osteoclasts<sup>29,30</sup> have been shown, although the mechanism through which bisphosphonates increase bone mineral density (BMD) is not clear (Figure 1). Likewise, effects of bisphosphonates on growth have been documented, but the mechanism of those effects has not been elucidated. There are differences

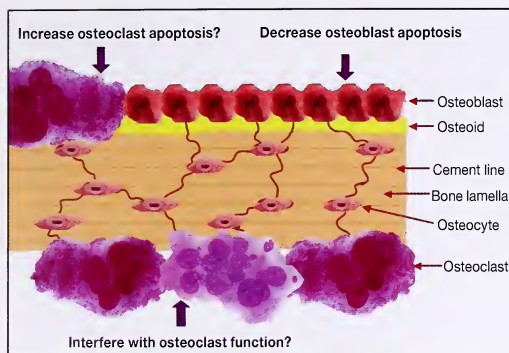


Figure 1. Possible mechanisms of action of bisphosphonates on bone.

among the bisphosphonates that may influence their mechanisms for binding and inhibiting bone crystal growth and dissolution. This may explain differences in potency among different bisphosphonates, such as the apparently more prolonged duration of action of alendronate and zoledronic acid, compared with the more readily reversible effects of risedronate.<sup>31</sup>

Different treatment protocols recommend the use of different bisphosphonates (ie, pamidronate, risedronate, alendronate, olpadronate, neridronate), and at different dose regimens for the pediatric population. For example, pamidronate doses range from 4.5 mg/kg/yr<sup>32,33</sup> (Tables 1 and 2) to 9 mg/kg/yr.<sup>34,35</sup> Children treated with high-dose pamidronate experience dramatic increase in BMD, with changes of as high as 200% per year.<sup>35,36</sup> Other positive effects observed include increase of the cortical width of the metacarpals, and increased vertebral height in previously fractured vertebrae. The incidence of fractures

decreases as well. Fracture healing does not appear to be impaired in patients with OI when compared to untreated OI patients.<sup>37,38</sup> There is a striking disappearance of bone pain and decreased fracture incidence noted with intravenous treatment. This may contribute to greater mobility,<sup>39,40</sup> an essential factor for the development of the skeletal system.<sup>41</sup> A lower fracture incidence, despite higher risk of injury due to increased mobility, suggests a direct effect of the therapy. These effects contribute to an improvement in the quality of life of patients with OI who are receiving treatment.

A side effect of high doses of pamidronate (9 mg/kg/yr) is retention of calcified cartilage within secondary spongiosa in children with OI.<sup>30</sup> Higher doses have caused osteopetrosis in a patient with no diagnosis.<sup>42</sup> Retention of calcified cartilage within secondary spongiosa is a hallmark of osteopetrosis, this suggests a dose-related effect of pamidronate. Studies using oral bisphosphonates for the treatment of OI (olpadronate,<sup>43</sup> alendronate) showed no differences between the drugs and placebo on functional outcome, anthropometrics, fracture incidence, or vertebral height, although it has been suggested that oral alendronate may improve quality of life in this group of patients.<sup>44</sup>

## EFFECTS OF BISPHOSPHONATE TREATMENT ON GROWTH IN CHILDREN WITH OI

The effect of treatment with bisphosphonates on longitudinal bone growth in children has been a concern among clinicians.<sup>45</sup> Bone resorption is an essential part of the normal endochondral ossification process,<sup>46</sup> and of the bone modeling and remodeling process. Despite the fact that the mechanism of long bone growth relies upon clonal expansion and subsequent hypertrophy of chondrocytes, endochondral bone growth requires resorption of the septa of calcified cartilage at the chondro-osseous junction of the growth plate by chondroclasts, permitting vascular invasion of the hypertrophic cell lacunae.<sup>47</sup> Drugs interfering with this mechanism could potentially cause impairment of the bone elongation process. Bisphosphonates interfere with osteoclast function<sup>48</sup> or survival,<sup>49</sup> and could, therefore, have a deleterious effect on bone growth. This undesired effect has actually been shown in animal studies.<sup>49</sup> High doses of alendronate (>2.5 mg/kg/wk) inhibited long bone length in the OIM mice (a model of OI) through alteration of the growth plate and possibly reduced resorption at the chondro-osseous junction.<sup>50</sup> Furthermore, lower doses of alendronate do not appear to have a detrimental effect on growth in oim/oim mice,<sup>51</sup> suggesting another dose-related effect of bisphosphonates. On the other hand, bisphosphonates do not appear to be detrimental for growth in human subjects at the doses currently used (Figure 2).<sup>24,52-55</sup> Each time a patient receives a pamidronate infusion, a new sclerotic line appears in the

Table 1. Protocol for administration of low-dose IV pamidronate treatment.

| Age group     | Dose                      | Interval |
|---------------|---------------------------|----------|
| <2.0 years    | 0.37 mg/kg/day for 2 days | 2 months |
| 2.0-3.0 years | 0.56 mg/kg/day for 2 days | 3 months |
| >3.0 years    | 0.75 mg/kg/day for 2 days | 4 months |

Table 2. Suggested dilution and infusion rates for IV pamidronate treatment.

| mg of pamidronate | mL of normal saline | mL/hr |
|-------------------|---------------------|-------|
| 0-5               | 50                  | 15    |
| 5.1-10            | 100                 | 30    |
| 10.1-15           | 150                 | 45    |
| 15.1-25           | 250                 | 75    |
| 25.1-45           | 500                 | 150   |



**Figure 2.** Lower limbs x-ray of a child with severe OI before (a) and after 12 months (b) of treatment with low-dose pamidronate (4.5 mg/kg/yr). Treatment was started at 18 months of age. Note longitudinal growth. Fractures do occur under treatment as evident in the panel on the right, but at much lower rate than before treatment.

metaphysis of long bones. The distance between these lines reflects longitudinal bone growth (Figure 3).

Pamidronate in high doses (9 mg/kg/yr) does not appear to negatively affect growth. Height z-score actually increased in a group of patients with OI who had started treatment before 3 years of age.<sup>35</sup> After one year of pamidronate therapy, height z-scores increased significantly in a group of children with severe OI and did not change in children with mild and moderate OI.<sup>24</sup> After 4 years of therapy with the same dose regimen of pamidronate, mean height z-scores increased significantly in patients with moderate OI, whereas non-significant trends to increase were seen in patients with mild and severe OI.<sup>24</sup> Low doses of pamidronate appear to have a similar effect (data not published) (Figure 3).

Low doses of pamidronate elicited no short-term evidence of growth impairment in children with a variety of pathologies leading to osteoporosis, including OI. A median annualized change in height SDS of 0 (range, -0.4 to 0.5) was noted in that group.<sup>56</sup> As expected, growth changes are greater in children with milder OI than

in those with more severe forms of the condition when receiving therapy with alendronate or pamidronate.<sup>57</sup>

One study showed that patients treated with high doses of pamidronate (9 mg/kg/yr) had similar growth plate width but wider metaphyses when compared with



**Figure 3.** Distal femur of a pediatric patient with OI receiving treatment with IV pamidronate. Note the sclerotic lines, each representing an infusion. The distance between lines reflects longitudinal bone growth in a 2-month period.

untreated OI patients who were matched for OI type and age, despite the lack of detrimental effects of bisphosphonates on longitudinal growth,<sup>58</sup> suggesting an effect of the high dose on bone remodeling. A different study showed that metaphyseal modeling in the distal femur is constant in children on bisphosphonates—with slight variation between sexes—resulting in a similar shape of the distal femur throughout childhood when looking at the modeling process.<sup>59</sup> Noteworthy, the observed positive effect of pamidronate on bone growth does not appear to be secondary to acceleration of bone age.<sup>24</sup>

Infants with OI appear to grow better when treatment with neridronate is started soon after birth, rather than at 6 months of age.<sup>60</sup> Older children with OI receiving neridronate grew faster than controls in one study.<sup>61</sup> At the microscopic level, the size of iliac crest bone biopsies is not significantly different before or after treatment in children



with OI. Changes are seen in cortical width, which increased by about 90%. Cancellous bone volume increases by about 45% with treatment. This change is due to higher trabecular number, with no change in trabecular thickness.<sup>30</sup> Importantly, there is no evidence for a mineralization defect in children with OI treated with high doses of pamidronate.<sup>30</sup> Growth in children continues after treatment with pamidronate is stopped, and the newly-formed bone will be unprotected and prone to fractures (Figure 4).

### LONG-TERM EFFECTS OF BISPHOSPHONATES ON HEIGHT

In one study, mean height z-scores of subjects with all OI degrees of severity tended to increase after 4 years of pamidronate therapy when compared with baseline. However, the change in height z-scores was significant only for the group with moderate OI, but not for mild or severe OI.<sup>24</sup> It is of note that these comparisons were done against normal growth charts designed for healthy children. To more accurately assess the growth rate of children with OI undergoing treatment, the same group compared their growth with that of a group of children with OI who were not receiving treatment with bisphosphonates. In that study, each height measurement of patients was expressed as a percentage of the mean value expected for untreated OI patients. During 4 years of pamidronate therapy, height significantly increased above the values expected for untreated patients.<sup>24</sup>

### EFFECT OF PAMIDRONATE ON FINAL HEIGHT

There is very little information about final height in children with OI treated with bisphosphonates. There is the description of only 8 patients who attained final height while receiving treatment with pamidronate.<sup>24</sup> In this study, final height, expressed as a percentage of the expected height in untreated patients, was significantly higher than baseline height. This study suggests that an average gain of 7 cm in patients with mild OI, 12 cm



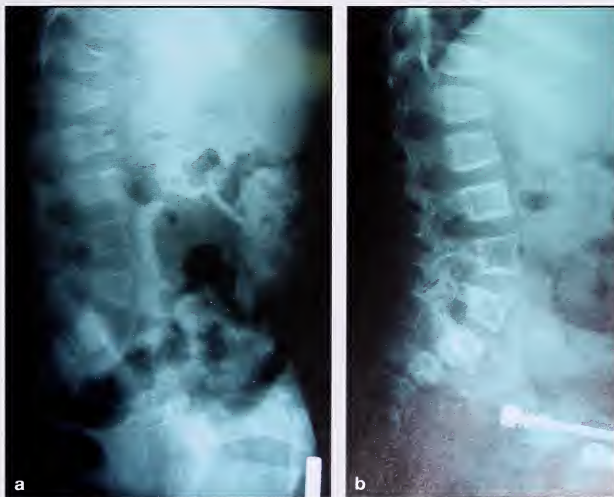
**Figure 4.** Long bones continue growing after treatment with bisphosphonates is stopped (arrow), causing susceptibility to fractures.

in patients with moderate OI, and 9 cm in patients with severe OI can be expected at 15 years of age. These results suggest that acceleration of growth is not just a transitory effect, but rather a lasting outcome on height in children with OI who are receiving pamidronate intravenously. As mentioned above, it is not entirely clear how pamidronate treatment might improve growth. Part of the effects of bisphosphonates on growth in children with moderate and severe OI could be due to prevention of long bone deformity and regeneration of vertebral fractures (Figure 5),<sup>35,62</sup> and to prevention of microfractures affecting growth cartilage.

### EFFECTS OF GROWTH HORMONE IN CHILDREN WITH OI

Growth hormone regulates post-natal bone growth; IGF-I mediates the growth-promoting action of GH, although it has been shown that

GH may have independent, direct effects on growth.<sup>63</sup> Also, IGF-I has mitogenic effects in dividing cells and is closely associated with growth, although plasma levels do not correlate with growth rates. It is known to



**Figure 5.** Lumbar spine x-ray of a child with severe OI before (a) and after 12 months (b) of treatment with low-dose pamidronate (4.5 mg/kg/yr). Treatment was started at 18 months of age. Note increased vertebral height with treatment.



increase 1- $\alpha$  hydroxylase in kidneys, with subsequent increased production of calcitriol (1,25 [OH]<sub>2</sub> vitamin D<sub>3</sub>). As calcitriol is the active form of vitamin D, IGF-I and GH treatment can make calcium more available for bone mineralization,<sup>64</sup> which could add to a possible beneficial effect in patients with OI. Inversely, it has been suggested that vitamin D, calcium, and protein supplements may elicit part of their effect on osteoporosis through increased IGF-I levels.<sup>65</sup>

IGF-I promotes longitudinal bone growth by 'insulin-like' anabolic actions which augment chondrocyte hypertrophy.<sup>66</sup> Chondrocyte differentiation, in turn, leads to cartilage expansion and linear growth. Furthermore, osteoblasts and pre-osteoblasts secrete IGF-I, and bone resorption causes release of stored IGF-I. This hormone appears to be a growth factor for osteoblasts. A homozygous molecular defect in the gene encoding IGF-I caused severe intrauterine growth failure, sensorineural deafness, and mild mental retardation in one individual.<sup>67</sup> Treatment with IGF-I improved linear growth and insulin sensitivity in that patient.<sup>68</sup> There is also some weak evidence that IGF-I has a role in declining BMD with aging. In patients with Laron syndrome, IGF-I treatment increases bone growth in the absence of GH.<sup>69</sup> Low IGF-I concentrations appear to be associated with low BMD in patients with cystic fibrosis.<sup>70</sup>

As discussed above, some children with OI have a blunted response to GH-releasing hormone or fail to double their serum IGF-I in a 5-day somatomedin generation test (13 of 22 had less than a 2-fold stimulation of somatomedin-C by GH).<sup>71</sup> There was no overlap between the group with blunted IGF-I response and the group with decreased GH-releasing hormone response, suggesting that there might be 2 different mechanisms of GH resistance in children with OI. GH is an anabolic hormone and, together with IGF-I, is a potent regulator of muscle mass. As such, there is potential for it to increase bone density. In the absence of trauma, muscles are responsible for the largest loads

and the largest bone strains, and those strains help to control the biological mechanisms that determine whole-bone strength (Figure 6).<sup>72</sup>

There are no large controlled studies of GH treatment in children with OI. Furthermore, there are no data in the literature regarding final height in OI patients treated with GH. An increase of fracture rate during GH therapy has been reported in children with OI by different groups,<sup>73,74</sup> although another group did not find an increase in fracture rate in a small group of children with mild OI who were treated with GH for 1 year.<sup>75</sup> Extending treatment to 2 years did not change the fracture risk either.<sup>76</sup> Like all children who are initially started on GH, children with OI experience an initial acceleration of growth rate,<sup>64,77</sup> but a sustained effect has not been demonstrated. In one study, GH (0.1- 0.2 IU/kg/d for 6 days/wk) was administered for at least 1 year to children with OI of different severity;<sup>78</sup> about one-half of the treated OI children sustained a 50% or more increase in linear growth, compared to their baseline growth rate. It is of note that most responders (10 of 14) did not have a severe form of OI. Incidentally, only the linear growth responders had a significant decrease in long bone fractures. After 1 year of treatment, responders' iliac crest biopsy showed significant increases in cancellous bone volume, trabecular number, and bone formation rate, but no significant increase in cortical thickness. Histomorphometric parameters of bone resorption were not significantly changed in responders, whereas non-responders had an 80% increase in the percentage of bone surface covered by osteoclasts. The incidence of fractures was unchanged in non-responders. Bone formation parameters did not increase with treatment in this group. Although progression of scoliosis was unchanged compared with the National Institute of Child Health and Human Development (NICHD) OI population, data on individual cases are not offered in the report.

Recombinant human IGF-I, complexed with its predominant binding protein IGFBP-3 is currently being tested as a treatment for osteoporosis, alone or in combination with anti-resorptive drugs and GH.<sup>79</sup> There appears to be a correlation between the dose of GH (and the obtained IGF-I plasma levels) and the increase in bone turnover markers and/or BMD in adults,<sup>80</sup> although a different study found that 1 year of IGF-I treatment, at a dose sufficient to elevate circulating IGF-I to young normal values, was not an effective means to alter body composition or blood parameters, nor to improve bone density, strength, mood, or memory in older women.<sup>81</sup>

## CONCLUSION

Bisphosphonate treatment does not appear to have a detrimental effect on linear growth in children and adolescents with OI, regardless of the severity of the condition. Long-term bisphosphonate therapy in children with OI may be associated with a significant height gain, as compared with untreated OI patients with the same

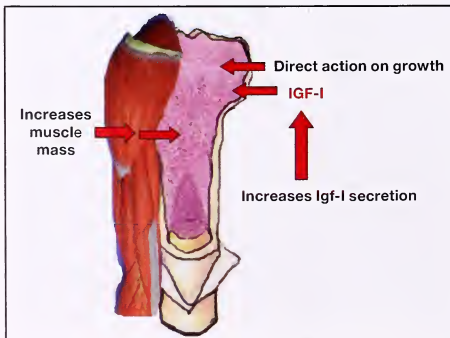


Figure 6. Mechanisms for growth hormone stimulation of bone growth and increase of bone mineral density.

disease severity. The use of GH in this population is still controversial. It has been suggested that GH treatment should probably not be used as a first-line therapy in OI.<sup>82</sup> Combined protocols administering both bisphosphonates and GH are warranted. Other therapeutic options currently used or in research for patients with osteoporosis (PTH, IGF-I, strontium, RANK ligand) may have a role in the treatment of OI in the future.

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## REVIEWS &amp; COMMENTS FROM THE LITERATURE

## Childhood Hypopituitarism after Traumatic Brain Injury

The hypothalamus and pituitary are essential for childhood and adolescent development and are vulnerable to injury and dysfunction following brain trauma. Hypothalamic-pituitary dysfunction has been well recognized after traumatic brain injury (TBI) in adults. However, data regarding hypothalamic-pituitary function in brain-injured children and adolescents are scant. It is necessary for physicians as well as patients and family members to know that onset of hypothalamic-pituitary deficits can occur even after several years following brain injury.

Acerini et al reviewed the available pediatric data, which showed that after both mild and severe TBI, hypopituitarism may occur; growth hormone (GH) and gonadotropin deficiencies appear to be most common. Precocious puberty has also been documented. Detailed investigations of pituitary function have been reported in 20 patients (12 males, 7 females, and 1 sex unspecified). Subjects ranged in age from infancy to 16 years at the time of injury; they were investigated between 1 and 42 years after the initial episodes of TBI. All patients had multiple anterior pituitary hormone deficiencies, except one, who had isolated GH deficiency. The frequencies of deficient hormones were: GH 85%, LH/FSH 80%, TSH 75%, and ACTH 50%. It was notable that in 6 patients, multiple deficiencies were documented after relatively mild head injury without loss of consciousness. Pituitary stalk transection was demonstrated on MRI in several cases. The diagnosis of hypothalamic-pituitary deficiency was made during childhood and adolescence in 17 of the 20 patients and during adult life in the remaining 3. The key presenting symptoms were growth failure, delayed or arrested puberty, secondary amenorrhea or reduced libido. Delay in the diagnosis was extreme in many cases and hypopituitarism was clearly not considered as a possible complication of the TBI until defects of growth or reproductive function became obvious.

Acerini and colleagues urged pediatric endocrinologists, in collaboration with adult endocrinologists, to perform formal prospective research studies in patients suffering from TBI to clarify prevalence, natural history, and response to hormone replacement.

Acerini CL, Tasker RC, Bellone S, Bona G, Thompson CJ, Savage MO. Hypopituitarism in childhood and adolescence following traumatic brain injury: the case for prospective endocrine investigation. *Eur J Endocrinol.* 2006;155:663-9.

**First Editor's Comment:** This is a very interesting report which provides important information for physicians who care for patients with TBI. Traumatic brain injury is a worldwide health problem and a major leading cause of death and disability among young adults. Survivors are

often left with significant neuroendocrine dysfunction and adverse physical and/or psychological problems which are perhaps an even greater risk than previously considered. As well, TBI-induced hypopituitarism has been under-recognized, under-investigated, and untreated. Relatively little attention has been paid to the possibility of TBI-induced hypopituitarism, especially in children. As reported by Acerini et al, it became clear that TBI posed substantial risk to hypothalamic-pituitary function in children; the onset of hypopituitarism can evolve over years following injury.

Road-traffic accidents, falls, sports injuries, and child abuse are the most common etiological factors for pediatric TBI, although the causes are different among age groups. The perinatal brain injury such as difficult forceps delivery at breech delivery is a well-known cause of hypopituitarism. Infants with TBI have primarily suffered from falls or assaults. Toddlers are more frequently injured as passengers in motor vehicle accidents, although falls still account for the majority of injuries. Children and infants have large, heavy heads with weaker cervical ligaments and muscles compared to adults. Given the same deceleration of the body, head trauma is therefore more likely in infants and younger children than adults. Similarly, the resulting brain injury may be more severe due to the thin, pliable skull and unfused sutures of infants and young children. Possible causes of hypopituitarism include hemorrhage, infarction, ischemia, swelling, stalk transection, or direct trauma to the hypothalamus, stalk, and/or pituitary region. Severity of TBI seems to be an important risk factor for developing hypopituitarism; however, even mild trauma may precede hypopituitarism. Accurate evaluation and long-term follow-up of all TBI patients are necessary in order to detect the occurrence of hypopituitarism, regardless of clinical evidence for pituitary dysfunction. The most common endocrine alterations appear to be GH and gonadotropin, followed by ACTH and TSH deficiency. Hyperprolactinemia may also be present. Diabetes insipidus may be frequent in the early, acute phase post-TBI, but it is rarely permanent.

The signs and symptoms of TBI-induced hypopituitarism are often nonspecific and can be additionally masked by what has been assumed to be merely the post-traumatic syndrome. These symptoms are likely to be overlooked if endocrine dysfunction is not actively evaluated. Moreover, hormonal deficits may significantly contribute to the chronic disability and the physical, cognitive, health, and social sequel in patients with TBI. Therefore, regular endocrine evaluation and follow-up should be performed throughout life in patients with TBI. In most instances, patients with



TBI are first seen and treated by trauma surgeons and neurosurgeons, and subsequently by rehabilitation specialists; all physicians must be informed about the risks of TBI-induced hypopituitarism. It is important to increase awareness among physicians, patients, and family members of the risks of hypopituitarism and the need for appropriate endocrinological assessment and adequate hormonal replacement therapy, if necessary. Thorough assessment may make it possible to improve the quality of life and enhance the rehabilitation prospects. Lack of awareness of this problem may result in long-term adverse consequences of untreated hypopituitarism for these patients. A close collaboration among neurosurgeons, neurologists, rehabilitation specialists, and endocrinologists is essential to achieve a coordinated approach to the care of patients with TBI.

Yoshikazu Nishi, MD

**Second Editor's Comment:** The consensus guidelines on screening for hypopituitarism following TBI for adults<sup>1</sup> was published in 2005. These guidelines may also apply to children and adolescents as the data in the paper by Acerni et al on the development of hypopituitarism following TBI are similar to the reported alterations found in adults. A summary of selected studies was presented in a tabular form in the consensus statement. However, the appropriate diagnosis and treatment of the endocrine alterations should always be accompanied by evidence-based cognitive rehabilitation of those patients; these recommendations for clinical practice were published by Cicerone et al.<sup>2</sup>

Fima Lifshitz, MD

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## The Value of Clinical and Radiological Expertise in Mutation Screening

As with many types of genetic disease, it is becoming common for clinical diagnosis of skeletal dysplasias to be confirmed at the DNA level. An issue that often arises is whether or not cases submitted for DNA diagnosis should be evaluated by experts before performing DNA testing. A small study involving multiple epiphyseal dysplasia (MED) reported by Zankl et al suggests that preselection of cases through such evaluation significantly increases the rate of mutation detection. The investigation was carried out under the auspices of the European Skeletal Dysplasia Network (ESDN).

MED is characterized by delayed and irregular ossification of epiphyses and precocious osteoarthritis. It is inherited as a dominant trait in most cases, and mutations have been identified in genes encoding 5 cartilage extracellular matrix proteins including cartilage oligomeric matrix protein (COMP), the 3 chains of type IX collagen (COL9A1, COL9A2, COL9A3), and matrilin 3 (MATN3). Mutations of COMP are most common. MED is occasionally inherited in a recessive fashion with mutations identified in the gene coding for the diastrophic dysplasia sulfate transporter (DTDST, SLC26A2).

The authors first noted that in a recent study COMP mutations were detected in only 36% of 58 families with MED in whom the diagnosis was made by the referring physician, usually a clinical geneticist. Since they expected the rate to be higher they suspected that some of the referral diagnoses were incorrect and that the mutation detection rate could be improved by adding an expert evaluation step between referral and DNA diagnosis.

Between September 2003 and February 2005 a panel of experts in the clinical and radiographical aspects of skeletal dysplasias evaluated, before testing, 35 patients with a diagnosis of MED. Of the 35 patients,

24 were considered to have "classical" MED by the experts, 5 possible MED variants, 2 most likely had type II collagenopathy, and 4 patients were considered "unknowns." Genomic DNA was analyzed from 21 of the 29 patients with classical or possible variant MED. Mutations were detected in 13 of the 16 patients with classical MED and one with a possible MED variant. Of the 14 mutations identified, 13 were COMP mutations and one involved MATN3. A COL2A1 mutation was subsequently identified in the patient with clinical features of type II collagenopathy. No mutation was detected in 3 patients considered to have classical MED.

When the numbers were tallied, the mutation detection rate was 81% for patients with classical MED and 67% if patients with possible MED variant were included, both substantially higher than the 36% reported previously. The authors concluded that review of clinical and radiographical features by experts prior to DNA testing substantially improves the rate of mutation detection since cases misdiagnosed by non-experts are excluded. The results also confirm that mutations of COMP are responsible for most cases of MED.

Zankl A, Jackson GC, Crettol LM, et al. Preselection of cases through expert clinical and radiological review significantly increases mutation detection rate in multiple epiphyseal dysplasia. Eur J Hum Genet. 2007;15:150-4.

**Editor's Comment:** From time to time the skeletal dysplasia community debates the value of clinical and especially radiographical expertise in the diagnosis of skeletal dysplasias. The argument is sometimes made that with DNA diagnosis becoming easily accessible through academic and commercial laboratories and government-sponsored networks such as the ESDN there is no longer a need for special expertise in



*this field. The issue has received special attention in recent years as prominent radiologists with such expertise, ie, the pioneers of this field, have retired faster than young experts have been trained to fill their niche. Accordingly, this paper is very timely since it*

*documents the value of this expertise. Although not discussed in the paper, another issue is the potential cost savings that could be realized from preselection by experts prior to DNA testing.*

William A. Horton, MD

## Major Determinants of Height Development in Turner Syndrome Patients Treated with Growth Hormone: Analysis of 987 Patients from KIGS

It is well known that growth hormone (GH) treatment during childhood can lead to a higher adult height in girls with Turner syndrome (TS). This is a report of 987 girls treated to adulthood or near adult height with recombinant human GH from the KIGS data base. Approximately 5 600 girls with TS have been entered into this registry. Data analyzed for this publication included 908 subjects who had reached near adult height, defined as age >15 years with a height velocity during the last year on GH < 2.0 cm/year. The subjects had all been prepubertal during the first year of treatment and had been treated for at least 4 years with a minimum of 5 injections per week. Puberty was defined at the time when spontaneous breast development occurred (Tanner stage B2) or when estrogen replacement therapy was initiated. The following variables were summarized: 1) status at birth: weight SD score, length SD score, ponderal index; 2) genetic background: mother's height SD score, father's height SD score, midparental height (MPH) SD score, and karyotype; 3) treatment modality: GH dosage per kg of body weight and per kg of ideal body weight (weight for height), frequency of GH injections, and accumulated years of GH treatment; 4) variables at the start of treatment: age, bone age, height SD score, weight SD score, height SD score minus MPH SD score, the peak GH concentration in serum during stimulation tests; and 5) variables at puberty onset: age, bone age, height SD score, weight SD score, height SD score minus MPH SD score. SD scores were calculated as follows: SD score = (patient's measured value minus mean value for age and sex-matched normal subjects) ÷ SD of the value for age- and sex-matched normal subjects. These independent variables were utilized in multiple regression analyses to determine which contributed to height or change in height between the start of treatment and the achievement of near adult height.

Age at the onset of GH treatment averaged 9.7 years. The average predicted adult height was 146.1 cm. In most cases the initial average GH dose was 0.27 mg/kg/wk delivered in 7 daily injections. Initial height was -2.4 SDS and had reached -1.9 SDS after 1 year of GH therapy. The median gain in height velocity during this first year was 7.4 cm. The age of onset of puberty was 13.5 years and height at this age was 141.8 cm (-1.4 SDS). The overall gain in height from time GH therapy was started was 21.2 cm or 1.5 SDS. Height gain from the start of puberty to near adult height was 9.4 cm. The average age of patients near adult height was 16.9 years with a

bone age of 14.5 years. The median height reached was 151 cm, or median gain of 4.9 above the projected height at the time GH treatment was started. The data also showed that height at near adult height was a function of (in order of importance) 1) height at GH start, 2) responsiveness to GH in the first year of treatment, 3) mid-parental height, 4) age at onset of puberty, 5) age at GH start, and 6) mean dose of GH per week. Each of these parameters were significant at a probability level of  $p < 0.01$  and accounted for 67% of the variance in near adult height.

The gain in height between start of therapy and near adult height was found to be a function of (in order of importance) 1) age at GH start, 2) GH responsiveness during the first year, 3) age at puberty, 4) mid-parental height, 5) height at GH start, 6) mean dose of GH per week, and 7) birth weight. These factors explain 90% of the variance in near adult height. Of note, karyotype did not enter into the multiple regression analyses.

The authors remarked that their observations show that responsiveness to GH during the first year of treatment is the foremost factor in response to height gained during subsequent pre-pubertal years. The data also demonstrated that there is no further gain in relative height after the onset of puberty. They stated that their regression equations support the principal concepts of the current treatment recommendations in TS and further suggest that these equations might be suitable to use as guidelines for daily practice. They cautioned however, that use of such equations might mean that the GH dose and timing at the puberty onset should be adapted to individual patient's responsiveness to GH treatment and that in some cases the GH dose might need to be reduced or discontinued.

Ranke MB, Lindberg A, Ferrández Longás A, et al. Major determinants of height development in Turner syndrome (TS) patients treated with GH: analysis of 987 patients from KIGS. *Pediatr Res.* 2007;61:105-10.

**Editor's Comment:** *These data reported from the large KIGS database of girls with TS are both reassuring and disquieting. Clearly GH therapy should be initiated at an early age, regardless of the child's current height. Unfortunately, many girls with TS are not identified until their height falls below the 3rd percentile. The KIGS data suggest that treating these girls at that time while clearly beneficial, is not as beneficial in terms of height gain as treating them shortly after diagnosis regardless of their current height. The data also suggest that higher doses*

of GH, especially during the first year of treatment, may be of significant benefit to achieving greater adult height. Although the authors have carefully developed regression equations for determining near adult height and height gain, between GH start and near adult height, one must

be cautioned that statistically significant contributions to the variance in an outcome variable are meant to be used in populations and may not apply in individual cases and may be inappropriate guides for therapy.

William L. Clarke, MD

## Focusing Illusion: Wealth, Height, and Happiness

If I were a rich man...: be careful what you wish for. I predict my life would be better if I won the lottery. While I'm pretty happy now, I'd be very happy then. To most of us, that statement seems an obvious truth—not to be questioned, much less explored scientifically. Kahneman (psychologist, winner of the 2002 Nobel Prize in Economics) et al questioned this and the related assumptions using creative research methodologies. They propose a "focusing illusion" is responsible for an exaggeration of the benefits of income to happiness. A focusing illusion occurs when people concentrate attention on the influence of any single factor on their global well-being and exaggerate its importance relative to factors contributing to moment-to-moment happiness.

Evidence for the focusing illusion was found in several lines of research. In one study, students were asked "how happy are you with your life in general" and "how many dates did you have last month." When asked in that order, no correlation ( $r = -0.01$ ) was found; when the statements were reversed, a statistically significant correlation ( $r = 0.66$ ) arose—suggesting that asking about dating exaggerated the salience of that single domain when evaluating one's life on the whole. Another study investigated predicted vs actual effects of several variables on the percentage of time spent in a bad mood. Women were asked what percentage of time they spent in a bad mood yesterday, then to estimate percentages of time spent in a bad mood for people having a lower (<\$20k) vs higher (>\$100k) income, being alone vs being married for (women >40 years of age) being micromanaged vs not closely supervised at work, and having no health insurance vs excellent benefits at work. Global estimations of bad mood of participants were compared with their own subjective well-being measured moment-to-moment. Predictions for others' mood were compared with actual reports of respondents. The prevalence of bad mood for oneself was overestimated when compared with subjective well-being measured moment-to-moment. Moreover, the prevalence of bad mood predicted for those with less desirable circumstances was grossly exaggerated.

Kahneman et al catalogued several studies providing similar findings and concluded that false intuitions are likely to arise from failure to recognize that people do not continuously think about their circumstances. While recent significant changes in life circumstances (eg, lottery winnings or becoming disabled) may result in multiple daily reflections, an individual's attention

eventually returns to the routine (eg, having breakfast or watching TV).

Finding an overall weak relationship between income and happiness or global life satisfaction, Kahneman and colleagues proposed that the focusing illusion helps explain why people seek higher income beyond a modest threshold (predictions exaggerate the increase in happiness) and why the long-term effect of increased income becomes relatively small (attention shifts to routine tasks). Another explanation of why high incomes fail to translate to happiness is related to the fact that as income rises, an individual's time use often does not shift toward activities associated with improved affect. Subjective well-being is connected to how people spend their time. The activities in which wealthier people spend relatively more of their time are associated with no greater happiness, on average, but with slightly higher tension and stress. Accordingly, the focusing illusion may be responsible for global judgments of life satisfaction being higher without increasing happiness. When asking people about their well-being, results differ when using a moment-to-moment measure (either collected in the present moment or by asking them to recall feelings during an episode of the previous day) compared with global judgments of life satisfaction or overall happiness or a global report of yesterday's mood.

Despite the weak association between income and experienced happiness, most will work very hard to earn more money. The focusing illusion can lead to misallocations of time if one's objective is increased happiness, for example, accepting lengthy commutes (which are among the worst moments of the day) to sacrificing time spent socializing (which are among the best moments of the day).

Kahneman D, Krueger AB, Schkade D, Schwarz N, Stone AA. Would you be happier if you were richer? A focusing illusion. *Science*. 2006;312:1908-10.

**Editor's Comment:** A 13-year-old boy is referred to a pediatric endocrinologist for an evaluation of short stature. "How are you doing?" the doctor asks. The context of the visit to the growth specialist and extra attention directed toward accurate height measurement makes it clear to the child and his accompanying parent that the doctor is really asking, "How are you doing being short?" At that moment, the child and parent will likely focus on events of height-related name-calling or incidents of being handed a child's menu at the restaurant. The child

hesitates to respond (fairly typical of youth this age in such circumstances), so the mother replies, "He's very upset about his height." The growth chart reveals this young man meets criteria for idiopathic short stature. The physician may conclude he is a good candidate for treatment with growth hormone (GH) because he meets anthropometric criteria and is also suffering from experiences related to his diminutive size.

The analysis of Kahneman and colleagues suggests we would likely arrive at quite a different impression of the child's psychosocial and emotional adaptation if we were to assess these in a manner that does not bias attention toward a single factor: height. Studies which mask "height" as the variable of interest suggest that youths who are markedly shorter than average are, by and large, indistinguishable in their self-reports and in descriptions by their peers from those of average or tall stature with respect to their reputation, the number of reciprocated friendships, and their likeability.<sup>1</sup>

Setting aside these and related findings,<sup>2</sup> there is a reasonable likelihood that this youngster would receive treatment based on complaints of psychosocial stressors and insistent parents. Should we anticipate that treatment will improve this youth's mood state? (Keep in mind that, by asking different questions, we would likely learn this teen's mood is better than our perfunctory evaluation suggests, and that height is far less salient in his life on a moment-to-moment basis than we are led to believe.) Or, based on

this focusing illusion, might we predict that the experience of daily GH injections and regular visits to the pediatric endocrinologist for repeated height measurements and physical exams will increase the likelihood that the youth and parent focus on growth and height not only during the visit but also on a daily basis? If, as suggested elsewhere<sup>3</sup> by the same group of investigators that, "nothing that you focus on will make as much difference as you think," then taking children and their families down this road might be quite counter-productive. An alternative would be to embed a psychosocial component within the medical evaluation and shared decision-making with the family. Independent of whether a decision to initiate GH treatment is made, a psychosocial intervention to address on-going psychosocial stresses associated with short stature would likely result in improved daily function and increased patient happiness and parent satisfaction. Suggestions on how such an interdisciplinary model of care could be implemented have been described.<sup>4</sup>

David E. Sandberg, PhD

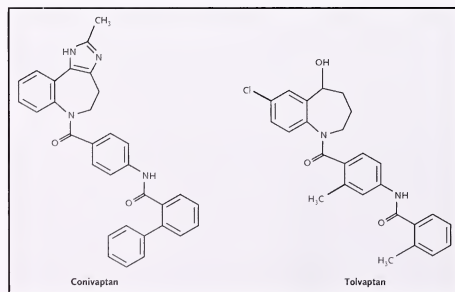
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## Tolvaptan, A Selective Oral Vasopressin V<sub>2</sub>-receptor Antagonist for Hyponatremia

Hyponatremia due to increased secretion of antidiuretic hormone (ADH) may be due to the syndrome of inappropriate secretion of ADH (SIADH) related to an insult to the central nervous secretion (or rarely in children—ectopic secretion of ADH), heart failure, or hepatic cirrhosis. Pathogenetically, it is the result of excessive and inappropriate reabsorption of free water in the renal collecting ducts in response to ADH signaling through the V<sub>2</sub> receptor (OMIM 300538, chromosome

Xq28), a G-protein coupled receptor that stimulates adenylyl cyclase and generation of cyclic AMP. ADH is a cyclical 9 amino acid peptide derived from a larger parent protein that also contains within its structure neurophysin—a carrier of ADH—and a glycoprotein. Parenterally administered non-peptide antagonists to ADH have been developed to block the action of ADH in the renal collecting tubule by binding to the V<sub>2</sub> receptor and increasing the urinary excretion of free water (Figure 1).<sup>1</sup> In a randomized, double-blind, placebo-controlled, outpatient study in which fluid intake was not monitored, the investigators ascertained the efficacy and safety of the oral administration of one such agent, tolvaptan, in 171 adults (>18 years of age) with hyponatremia (120-134 mEq/L), 91 of whom had SIADH. Compared to placebo, tolvaptan rapidly and safely increased and maintained serum sodium concentrations into the low normal range over a 30-day interval of treatment (Figure 2). One week after discontinuation of tolvaptan, serum sodium levels declined to values seen in the group that received the placebo. As anticipated, tolvaptan increased urine output initially. The drug was well tolerated. The authors concluded that orally administered tolvaptan is a clinically effective V<sub>2</sub>-receptor antagonist in adults with hyponatremia of diverse etiology.



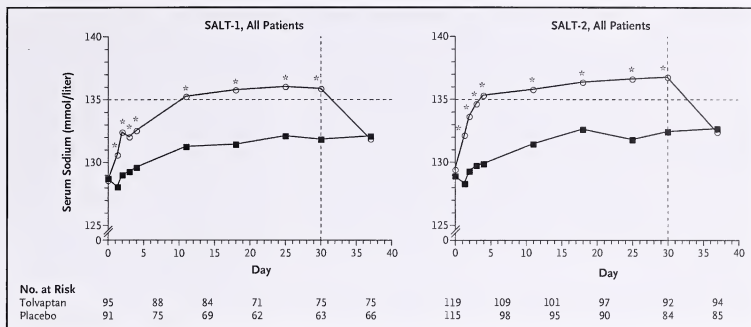
**Figure 1.** Structure of the Oral Vasopressin-Receptor Antagonists Conivaptan and Tolvaptan. Reprinted with permission from: Hays RM. *N Engl J Med*. 2006;355:2146-8. Copyright © MMS. 2006. All rights reserved.



Schrier RW, Gross P, Gheorghiade M, et al. Tolvaptan, a selective oral vasopressin  $V_2$ -receptor antagonist, for hyponatremia. *N Engl J Med*. 2006;355:2099-112.

#### Editor's Comment:

**Management of SIADH in children is primarily accomplished by fluid restriction. In critical situations slow intravenous infusion of 3% saline may be considered in an amount calculated to increase the serum sodium concentration to values that ameliorate symptoms (approximately 125 mEq/L) while carefully monitoring urine output.<sup>2</sup> Very rapid increase in serum sodium concentrations may lead to central pontine myelinolysis. The non-peptide antagonists of the  $V_2$  receptor have not been examined or approved for use in children as yet, but would appear to be promising therapeutic agents that have been termed "aquaretics." In addition to the renal  $V_2$  receptor,**



**Figure 2.** Mean serum sodium concentrations attained with tolvaptan (circles) and placebo (squares). Reprinted with permission from: Schrier RW, et al. *N Engl J Med*. 2006;355:2099-112. Copyright © MMS. 2006. All rights reserved.

there are  $V_{1a}$  and  $V_{1b}$  receptors that mediate the vasoconstrictive and adrenocorticotropin-releasing properties of ADH.

Allen W. Root, MD

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## Growth Hormone Treatment in Cystic Fibrosis

This multi-center study assessed auxological, respiratory, bone health, and quality of life variables in 61 pre-pubertal children with cystic fibrosis (CF) who were randomized to receive either growth hormone (GH [0.3 mg/kg/wk]) or no GH for one year. At the end of one year, there was cross-over and those who received GH stopped therapy and those not on treatment started GH therapy. Both groups were then followed for a second year. Significant improvements in gain in height velocity, weight, lean body mass, bone mineral content, quality of life, and hospitalization rates were demonstrated in the subjects treated with GH. Improvements were also maintained following discontinuation of GH.

Hardin DS, Adams-Huet B, Brown D, et al. Growth hormone treatment improves growth and clinical status in prepubertal children with cystic fibrosis: results of a multicenter randomized controlled trial. *J Clin Endocrinol Metab*. 2006;91:4925-9.

**Editor's Comment:** Hardin is one of the few pediatric endocrinologists who is successfully addressing the problems of chronic severe childhood illness on growth, puberty, bone health, and quality of life. She and her co-authors are to be congratulated on this publication, which not only reports impressive positive results of GH

therapy in children with CF, but can also be considered a notable achievement in terms of interdisciplinary collaboration. A defect in GH action is predictable from the effect of chronic infection and inflammation in children with CF. Insulin-like growth factor (IGF)-I levels have also been shown to be correlated with BMI and disease activity score, which as stated in this paper, may relate to long-term morbidity and mortality.

It is pertinent to ask why convincing results such as these are apparently not changing clinical practice more rapidly. Poor interdisciplinary discussions and interchange must be responsible. Sub- or super-specialization within pediatrics may be considered synonymous with progress, but barriers can be constructed which make interspecialty and joint clinical management difficult. Pediatric endocrinologists are in a position to collaborate with many subspecialists, such as gastroenterologists, rheumatologists, hematologists, etc. Of course, stretched resources may make this difficult. However, as Hardin and her collaborators have demonstrated in this important study, the patient may have a great deal to gain from closer working relationships between colleagues in different pediatric disciplines. Well done!

Martin O. Savage MD



## MC2R Loss in Salt-losing Adrenal Hypoplasia

Familial glucocorticoid deficiency type I (FGD1) is a rare form of primary adrenal insufficiency resulting from mutations in the ACTH receptor (*MC2R*). These children typically have severe neonatal symptoms and signs of cortisol deficiency. The issue of mineralocorticoid deficiency is not well documented in this group and it is widely accepted that classic cases do not require mineralocorticoid replacement. However, hyponatremia has been observed in some patients, not related to hypocortisolism.

Lin et al considered the possibility of alterations in mineralocorticoid control since the *MC2R* is also expressed in the aldosterone producing zona glomerulosa in the adrenal gland. Twenty-two children diagnosed with salt-losing forms of primary adrenal hypoplasia (19 isolated cases, 3 familial) were investigated. All children were negative for the 2 mutations known to be involved in adrenal hypoplasia: DAX1 and SF1. All subjects were investigated for *MC2R* mutations, after amplifying the entire coding region (exon 2).

The *MC2R* mutations were found in 3 kindreds, involving 9 patients; age at presentation ranged from 1 day to 19 months. The initial symptoms were pigmentation, hypoglycemia, jaundice, and failure to thrive. The mutational changes in all 3 families represented disruptive loss-of-function in the G-protein coupled receptor, including the first reported homozygous frameshift mutations. In kindred 1, the patient was diagnosed at 3 months: electrolytes were normal, but aldosterone was low for age with elevated plasma rennin activity (PRA) that improved with prolonged fludrocortisone treatment. Two cases were later diagnosed in family 2. One presented with elevation of PRA, the other sibling had low aldosterone and developed hyponatremia during a severe viral illness. Kindred 3 presented early symptoms. The first child required fludrocortisone because of early salt-losing syndrome. The 2 subsequent siblings were treated before overt sodium imbalance.

Salt-losing forms of adrenal insufficiency are generally clear and occur in well defined conditions. However, if biochemical findings are subtle, the exact biochemical

nature of the condition can be difficult to assess. *MC2R* mutations should be considered in patients with apparent mild disturbances in rennin-sodium homeostasis. These children could be misdiagnosed for primary salt-losing adrenal hypoplasia. The genetic finding has important implications for treatment, counselling and long-term prognosis.

Lin L, Hindmarsh PC, Metherell LA, et al. Severe loss-of-function mutations in the adrenocorticotropin receptor (*ACTHR*, *MC2R*) can be found in patients diagnosed with salt-losing adrenal hypoplasia. *J Clin Endocrinol*. 2007;66:205-10.

**Editor's Comment:** *The authors described important clinical and pathophysiological issues in a rare but often confusing disorder. The diagnosis of isolated cortisol deficiency is suggested by the clinical presentation. Assessment of the mineralocorticoid function in the affected neonate may be difficult. If aldosterone deficiency is partial, as it appears to be in these cases, repeated coupled evaluation of plasma aldosterone and PRA is necessary. Furthermore, severe inactivation of the ACTH receptor may have an impact on the control of aldosterone secretion and suggests a risk of a salt-losing condition. Therefore, it is necessary to identify the genetic defect in these patients, assessing the most severe end of the spectrum of FGD1. These patients paradoxically may require fludrocortisone therapy at critical periods of severe illness. As the authors suggested, these mutations may be found in a significant proportion of children with primary adrenal insufficiency who sometimes have minimal signs of salt loss.*

*In addition, these data confirm the supportive role for ACTH in mineralocorticoid synthesis and secretion, especially in a time of stress. It is possible that, as observed in pseudohypoaldosteronism type 1 due to mineralocorticoid receptor defect, the need for mineralocorticoid replacement weans off during childhood. This requires careful follow-up, as the risk of salt loss may persist at times of stress or potential volume depletion. An ongoing clinical vigilance is necessary.*

Raphaël Rappaport, MD

## Mutant IGF-1R as Cause for Familial Growth Failure

Inagaki et al identified a family in which 2 members had severe growth failure and a mutant type 1 insulin-like growth factor receptor (IGF-1R). A 13.6-year-old girl presented with growth failure (height z-score -5 SD, sitting height z-score -5.2 SD, and weight z-score -2.5 SD), Tanner stage 2 pubertal development, and delayed bone age (9.7 years). She had experienced prenatal growth failure (birth length -4.9 SD, birth weight -3.1 SD), triangular facies, and acromicria. The father's height was -2.2 SD, the mother's height was -5.7 SD, and they

were nonconsanguineous. A maternal aunt's height was -5.7 SD and she seemed otherwise healthy. The patient's 2.5-year-old brother had a height of -1.2 SD. The patient had an elevated basal IGF-I level (404 ng/mL), normal growth hormone (GH) response to clonidine stimulation, and no increase in either her IGF-I level after 4 days of GH treatment or her height z-score after 6 of months GH treatment (0.07 mg/kg/day).

Inagaki et al then performed in vitro studies to ascertain the molecular mechanism of this family's

growth failure. Sequencing revealed substitution of the phylogenetically highly conserved arginine at position 481 to glutamate (R481Q) in the IGF-1R of both the patient and the maternal aunt. This arginine is in the N-terminal fibronectin type III domain, and situated near the first disulfide bond (Cys 514) between the 2  $\alpha$ -subunits. Either wild-type or R481Q IGF-1R was over-expressed in NIH-3T3 fibroblasts to conduct functional assays. R481Q IGF-1R altered neither surface expression nor ligand binding capacity. However, as demonstrated by Western blotting under reducing and non-reducing conditions, the mutant receptor had incomplete dimerization likely related to impairment of that first disulfide bond; the mutant, but not wild-type, IGF-1R showed monomeric forms of the  $\beta$ -subunit under non-reducing conditions. Further, compared to wild-type, R481Q IGF-1R had blunted IGF-I induction of IGF-1R autophosphorylation, p42/44MAPK phosphorylation, Akt phosphorylation, and cellular proliferation.

Thus, the authors concluded that R481Q disturbs the first disulfide bond of IGF-1R, thereby impairing its dimerization and ligand-stimulated conformational change that is required for signal transduction. This translated clinically into IGF-I resistance and growth failure.

Inagaki K, Tiulpakov A, Rubtsov P, et al. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *J Clin Endocrinol Metab.* 2007;92:1542-8.

**Editor's Comment:** The authors astutely recognized the severe pre- and post-natal growth failure of their patient as indicative of reduced IGF-I activity; measurement of basal IGF-I concentration quickly ruled out IGF-I deficiency in favor of IGF-I resistance. The authors are to be commended on their detective work, which led to the discovery of a novel mechanism of IGF-I resistance that joins the short list of previously reported IGF-1R mutations.

This illustrative case also highlights the importance of obtaining a good family history in the evaluation of a poorly growing child. Most often, we ascribe similar multigenerational height z-scores to familial short stature, which is considered a normal variant. However, when the growth failure is severe and affects a subset of relatives, as exemplified by this patient's family, an inherited growth defect should be considered. Another example would be autosomal dominant (type 2) isolated GH deficiency.<sup>1,2</sup> Although the child may be short "like the parent," it is possible that they are sharing an underlying pathologic process.

Adda Grimberg, MD

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1. Binder G, Brown M, Parks JS. *J Clin Endocrinol Metab.* 1996;81:4047-50.
2. Salemi S, Yousefi S, Lochmatter D, et al. *Endocrinology.* 2007;148:45-53.

## Ultimate Height of Growth Hormone Deficient Patients who Normalized Growth Hormone Secretion in Puberty

The objectives of the study by Zucchini et al were to establish the percentage and the characteristics of subjects diagnosed with isolated growth hormone

deficiency (GHD) in childhood who normalized their GH secretion in puberty and discontinued treatment at that time. The final height attained by this group

was compared with that of subjects with persistent GHD who continued on GH therapy after retesting. Sixty-nine subjects (40 males, 29 females) with a diagnosis of isolated GHD before puberty were evaluated by means of arginine and l-dopa testing and were reevaluated after at least 2 years of GH therapy and after the onset of puberty. If peak GH levels were  $>10 \mu\text{g/L}$  therapy was withdrawn.

At retesting, 44 subjects (63.7%; 24 males, 20 females) had a peak GH  $<10 \mu\text{g/L}$ . Patients with confirmed GHD were not different from subjects with normalized GH secretion regarding: height

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deficit at diagnosis, first year growth response to GH, age and height at onset of puberty, or height and insulin-like growth factor (IGF)-I at retesting. Males who continued treatment achieved an adult height of  $165.1 \pm 4.5$  cm, while those who suspended therapy after retesting had an adult height of  $164.0 \pm 3.4$  cm. Final height of females who continued treatment was  $153.2 \pm 4.1$  cm, whereas those who suspended therapy after retesting were  $152.9 \pm 5.2$  cm. Duration of therapy and GH levels at diagnosis and at retesting were found to be unrelated to achieved adult height or to height increments obtained during the period of observation.

Zucchini S, Pirazzoli P, Baronio F, et al. Effect on adult height of pubertal growth hormone retesting and withdrawal of therapy in patients with previously diagnosed growth hormone deficiency. *J Clin Endocrinol Metab.* 2006;91:4271-6.

**First Editor's Comment:** *A significant number of children diagnosed with GHD before entering puberty, particularly those with non-severe GHD not associated with multiple pituitary hormone deficiencies or with alterations of the pituitary anatomy, display normal GH secretion when retested after the completion of puberty. This discrepancy in GH testing before and after puberty could be the result of a transient deficiency which tends to normalize with the secretion of gonadal steroids or could be due to the unreliability of pharmacological tests when repeated over time.*

*Zucchini et al suggested that GH retesting should take place at midpuberty when GH secretion rises and not after the attainment of final height and completion of puberty, thus avoiding unnecessary treatment of subjects who have normal GH secretion when retested at puberty. The final height attained by subjects with normal GH secretion at retesting, who discontinued therapy, was similar to that of individuals with confirmed GHD who continued treatment. Therefore, the withdrawal of GH therapy after retesting did not lead to a reduction in ultimate height. There were no clinical or laboratory parameters that allowed for the differentiation of patients with or without persistent GHD after puberty. In subjects with non-severe GHD it seems advisable to retest GH secretion during puberty and to discontinue treatment in those individuals that are no longer deficient, thus avoiding unnecessary treatment during and beyond puberty in these subjects.*

Roberto Lanes, MD

**Second Editor's Comment:** *Several years ago we described the recovery of patients with isolated suboptimal GH secretion after a short trial of GH releasing hormone (GHRH).<sup>1</sup> Why wait until puberty?*

Fima Lifshitz, MD

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1. Lifshitz F, Lanes R, Pugliese M, et al. *J Clin Endo Metab.* 1992;75:1255-60.



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## DISORDERS OF SEX DEVELOPMENT: MAKING AMBIGUITY LESS AMBIGUOUS

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### INTRODUCTION

The recent consensus conference<sup>1,2</sup> on intersex, subsequently referred to as disorder(s) of sex development (DSD), made several important in-roads towards the establishment of an internationally endorsed intersex management guideline; nevertheless, some very fundamental questions remain.

Some of the most contentious topics simply were not considered for consensus because of a lack of outcome data on which to anchor objective assessment. Other topics, such as how to make management decisions in the absence of objective data, were not approached.

The recent controversy in the medical management of DSD has arisen primarily due to the complaints of adult DSD patients about the lack of full disclosure, absence of participation in treatment decisions, minimal privacy about their condition, and the consequences of genital surgery. There has also been criticism centered on the use of the "optimal gender" concept for gender assignment, an approach that viewed gender as a largely social construct whose predominant influence was sex of

### From The Editor's Desk

Dear Colleague:

The publication of this issue was made possible through an unrestricted medical education grant from Pfizer, Inc. We also received support from Pediatric Sunshine Academics, Inc. and from many individual donations from our loyal readers. This allowed us to close the year with a big bang; the printed issue of Volume 23, Number 3 of GGH was expanded to 28 pages. On behalf of the editorial board and all of our readers I want to express my deep and most sincere appreciation to the sponsors who made possible the publication of this highly regarded journal.

The lead article of this issue was written by Drs. Christopher Houk and Peter Lee—it deals with a subject of great current interest, "Disorders of Sexual Development: Making Ambiguity Less Ambiguous." In addition there are 22 reviews of peer-reviewed papers published in the recent literature, each one of them with erudite editorial comments. Additionally, we will be offering the capability of obtaining CME credits for reading GGH. CMEs will be available online at our website [www.GGHjournal.com](http://www.GGHjournal.com)—click CME.

We will continue to search for the support that is needed to produce a high quality medical education journal for pediatric endocrinologists and for all those interested in the field. The long-term support through a single sponsor that GGH enjoyed in the past is currently not available, thus we will hope to elicit partial funding from multiple sources to be able to provide you with GGH throughout 2008 and thereafter.

Should you wish to contribute to the journal's success please make a special year-end tax-deductible contribution either on line ([www.GGHjournal.com](http://www.GGHjournal.com) or [www.PedsAcademics.org](http://www.PedsAcademics.org)) and click make a donation, or send a check to Pediatric Sunshine Academics, Inc. 1040 Alston Road, Santa Barbara, CA 93108.

Thank you for your interest in and support of GGH.

Fima Lifshitz, MD  
Editor-in-Chief



rearing.<sup>3</sup> Because postnatal factors were felt to carry great weight for the determination of gender identity, and because DSD management was felt to be complex and confusing, parents were given little information and little input in the decision-making process. In this model, sex assignment was based on potential for fertility, penile-vaginal sexual intercourse, and near-normal appearing genitalia—factors felt to be important for adult quality of life. This paradigm held that when sex assignment was followed by genital surgery, a gender identity consistent with the gender assignment would ensue. This was felt to be true in spite of the knowledge that 46,XX individuals exposed to androgens in utero were more likely to exhibit more male-type gender role behavior,<sup>4,5</sup> and, although not well documented, were felt to be more likely to be sexually oriented towards other females.<sup>6,7</sup>

Over recent decades, it has become obvious that some of the core assumptions about the impact of sex of rearing were incorrect. This has been especially true for 46,XY DSD patients<sup>8,9</sup> although issues in virilized 46,XX patients have also emerged.<sup>10,11</sup> While inconsistent gender roles and homosexual orientation are not as imposing issues in current society as they were in the past, gender identity opposite to that assigned during infancy suggests a need for better guidelines. While the relative roles played by the various biological and social determinants of gender remain an enigma, there is accumulating evidence that prenatal influences, particularly those related to androgen exposure during fetal life may influence gender identity.<sup>9,12,13,14</sup> Furthermore, the position that quality of life could be predicted by the extent to which a DSD individual had normal appearing genitalia or ability to participate in traditional sexual activity is too limited in scope to be helpful to an individual patient. In some cases the attempt to create anatomically 'correct' genitals resulted in reduced genital sensitivity and erotic function such that one of the primary goals of surgery—namely satisfying sexual activity—was sacrificed.<sup>15,16</sup> Given the degree of alienation reported by parents of DSD children, it was felt that a new treatment paradigm should be developed—one that allows a more informed, evidence-based approach to the DSD patient.

## REVIEW OF CONSENSUS STATEMENT

The consensus conference (comprised of members of the Lawson Wilkins Pediatric Endocrine Society [LWPES] and the European Society for Paediatric Endocrinology [ESPE]) formed a position when they had reached a collective opinion that there was adequate outcome data to support a position statement and when there was sufficient agreement on the topic. One example of the groups' opinion was seen in the position that genital surgery should be undertaken for the severely ambiguous infant when parents request it, despite the absence of outcome data to show that this approach is superior to its alternative. Although it was agreed that the impact of prenatal androgen exposure is important to gender outcomes, the group was uncomfortable

recommending that treatment be based on an estimate of intrauterine androgen exposure. Another example of this is seen in the groups' reluctance to recommend that a male sex of rearing be considered in a fully virilized (and hence highly androgen exposed) 46,XX infant with congenital adrenal hyperplasia (CAH). A consensus could not be reached for 2 of the more controversial issues: (1) establishing clear indications for sex of rearing for severe gender ambiguity and (2) establishing complete guidelines on when genital surgery should be deferred. However, it was agreed that surgery should not be done in mildly virilized genetic females (Prader stage 1 or 2).

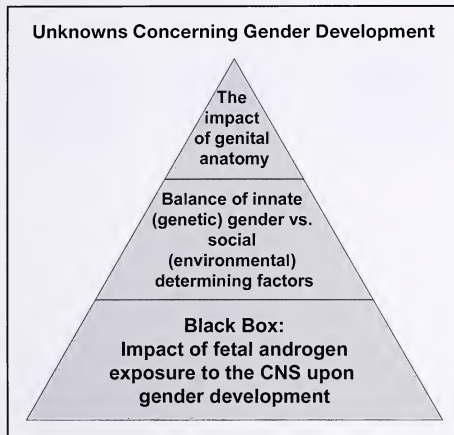
## SEX ASSIGNMENT

Guidelines for gender assignment were addressed only for those DSD patients with substantial outcome data. A review of outcome studies show that: (1) >90% of virilized 46,XX CAH patients identify as females; (2) 100% of 46,XY complete androgen insensitivity syndrome (CAIS) patients identify as female; (3) 100% of 46,XY 5 $\alpha$ -reductase deficient patients assigned male at birth identify as male, while more than half of those assigned female who virilized at puberty live as males;<sup>17,18</sup> and (4) approximately 50% of 46,XY 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ HSD) deficient patients assigned female ultimately switch to the male gender. Accordingly, it was recommended that all 46,XX CAH and 46,XY CAIS patients be assigned female. It is important to note that this recommendation was made in spite of a small number of documented cases of initially undiagnosed extremely virilized 46,XX CAH patients assigned male at birth, who maintained the male gender in adulthood and developed a sexual orientation towards females.<sup>19</sup> A male sex of rearing was also recommended for 46,XY patients with 5 $\alpha$ -reductase deficiency and 17 $\beta$ HSD deficiency (Table 1). The consensus found outcomes for ovotesticular DSD to be so variable that sex assignment recommendations should be based on fertility potential, assuming consistent genitalia.

**Table 1. Current Diagnosis-based Recommendations for Sex of Rearing**

|  |
|--|
| 1. Complete androgen insensitivity syndrome (46,XY CAIS) - female  |
| 2. Partial androgen insensitivity syndrome (46,XY PAIS) - dependent upon judgment of degree of masculinization and parental input  |
| 3. Congenital virilizing adrenal hyperplasia (46,XX CAH) - female, realizing that there are anecdotal reports, but not verified documentation, of those with essentially male external genitalia raised satisfactorily as male |
| 4. 5 $\alpha$ -reductase deficiency - strongly consider male assignment  |
| 5. 17 $\beta$ -hydroxysteroid dehydrogenase-3 deficiency - strongly consider male assignment   |
| 6. Cloacal ectrophy - conflicting outcome data, reports from the US show high rates of self-reassignment to male   |
| 7. Ovotesticular DSD - consider external genital development and fertility potential; given outcome uncertainties potential for fertility (assuming consistent genitalia) is a major factor                                    |

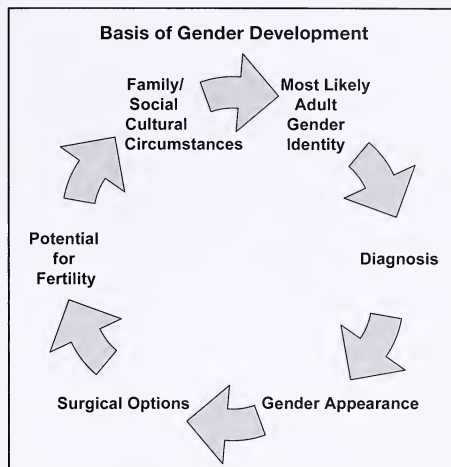
The consensus did not find the outcome studies sufficiently clear to permit a sound recommendation for sex assignment in 46,XY patients with cloacal extrophy or partial AIS (pAIS). The diagnosis of pAIS is particularly problematic, because the criteria used to establish it are vague and the outcomes seen are so variable, resulting in etiologic heterogeneity within this group of DSD patients. Given these uncertainties in all DSD patients, physical



**Figure 1.** It is doubtful whether the unknowns represented in this figure will ever become certain enough to provide precise guidance for sex assignment. All aspects of psychosexual differentiation may not be equally affected by prenatal androgen exposure.

findings alone cannot be used to justify gender assignment. In point, it was felt that the degree of fetal virilization cannot be used as a surrogate of fetal CNS androgen exposure (Figure 1). Accordingly, the use of genital anatomy to guide gender assignment or predict future gender identity should be deemphasized for determining sex of rearing.

It is the authors' opinion that in these cases the DSD team and the parents must decide whether the genitalia (in some cases, only after androgen stimulation), considered alongside other factors (karyotype, social issues, gonadal function, etc) would permit successful male sex of rearing. The basis of gender assignment and the relative weight of the various interrelated factors are shown in Figure 2. Although other factors should not be underestimated, family support is of capital importance in determining a successful sex assignment (Figure 3). In cases of undervirilized 46,XY patients, initial sex assignment should be approached cautiously given the outcomes seen in this group of DSD patients. In particular, the prospect for 46,XY DSD patients assigned female at birth who then later developed disabling gender dysphoria should be discussed with the family. This is especially true of an idiopathic undervirilized 46,XY patient with evidence of testicular function. In patients with micropenis, it was



**Figure 2.** The relative weight of the sex assignment inter-related factors differ in each situation, largely by unknown and non-predictable influences. The magnitude of the impact of each factor upon the others is also variable over time.

the consensus that all should be raised male, based on considerations that include the lack of need for surgery, fertility and adult male gender identity.

In terms of gender re-assignment, the consensus strongly felt that all gender re-assignment—that is, assignment after infancy—should only be undertaken after the gender identity is well established and should be patient initiated. Neither homosexuality nor cross-gender behaviors can be used as support that a gender assignment has failed. Decisions about the need for or utility of gender re-assignment should not be influenced by gender role or sexual orientation and should be solely based on the presence of a durable gender identity.

## SURGICAL ISSUES

The consensus found that the anecdotal reports of DSD individuals dissatisfied with previous genital surgeries was inadequate and insufficiently compelling to recommend that a moratorium be placed on all genital surgeries. The consensus, based upon the recommendations of the surgical subgroup, agreed that the primary goal of genital surgery was to improve functional rather than cosmetic outcome.<sup>1</sup> This represents a shift towards a more conservative treatment approach, but is far short of suggesting that all genital surgery be deferred. For the severely virilized 46,XX infant with CAH—the most frequent cause of severe genital ambiguity—early genital surgery is recommended. For 46,XX infants with mild/moderate clitoromegaly, it was advised that genital surgery be deferred until patients can participate in

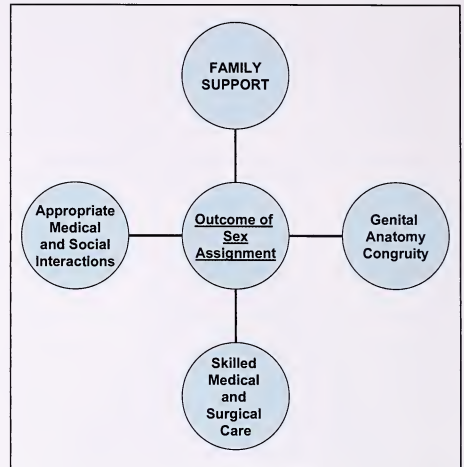
the decision. Given the absence of outcome data showing that it is harmful, it was felt that parental rights and responsibilities as surrogate decision-makers should be respected in cases where informed parents continue to request early genital surgery for their DSD child. This situation further highlights the need for data-driven recommendations.

In addition, outcome data were used to stratify gonadal malignancy risk for specific DSD etiologies and evidence-based recommendations were made on the need for and timing of gonadectomy.<sup>1</sup>

### CURRENT STANDARD

The consensus highlighted several standards of care recommendations: (1) gender assignment for all; (2) avoidance of assignment before expert evaluation; (3) open communication; (4) a multidisciplinary team approach; and (5) confidentiality and attention to patient and family concerns.<sup>1</sup> It was emphasized that patient and family concerns be carefully considered and that adolescent patients should be offered the strictest confidence. This must take into consideration the fact that parents may not be ready to agree with full disclosure to their children. Hence, while respecting this, adolescent patients should be given opportunities to ask questions and discuss their condition confidentially, without their parents being present. The basis of gender assignment (Figure 2 and Table 2) should include: (1) the most likely adult gender identity; (2) diagnosis; (3) genital appearance; (4) surgical options; (5) potential for fertility; and (6) family/social/cultural circumstance. Data are not available to predict outcome in the majority of DSD patients, therefore the consensus felt that decisions based on psychosocial factors, such as parental wishes, should guide management decisions in cases with uncertain

outcomes. This approach recognizes the powerful influence of parental input on outcomes (Figure 3).



**Figure 3.** The primary factor that appears to promote a well-adjusted outcome after sex assignment is family support. Further, the importance of the family's role in assuring supporting medical and social contacts and exchanges, demanding skilled medical and surgical care, and interpreting genital anatomy as appropriate should not be underestimated.

Medical decisions in the DSD patient are usually made in what has been referred to as Category III level of evidence (Category III: Opinions of respected authorities, based on clinical experience, descriptive studies and case reports, or reports of expert committees<sup>20</sup>). Thus, there are major limitations to making recommendations for DSD patients for most issues, particularly those most controversial topics of sex assignment and genital surgery, since the available evidence is considered to be Category III, which is the least compelling.

### UNRESOLVED ISSUES

The effects of the care given to DSD patients are still not well understood, thus it has been difficult to determine which of the traditional and which of the more contemporary treatment approaches provide the optimal chance for a successful outcome. Despite the advances in our understanding of human sexual development, the sheer number of possible etiologies, the high degree of phenotypic heterogeneity and the overlay of psychological/sexual/social/cultural pressures in DSD patients make it difficult to develop comprehensive guidelines for approaching these issues. This difficulty is compounded by the paucity of outcome data for DSD patients. These factors notwithstanding, it remains important to sort out which questions will be answerable with new information versus those (such as which gender assignment should be proposed in all cases) questions which may never be answerable.

**Table 2. Recommendations for Gender Assignment: Factors to Consider**

|  |
|--|
| 1. Most likely adult gender identity based on impression of fetal androgen exposure, parents' expectations and expected impact of sexual differentiation |
| 2. Diagnosis, if specific diagnosis available and outcome data available   |
| 3. Genital appearance (as primarily male, female or intermediate)  |
| 4. Genital surgical options (potential for functional, sensitive genitalia)  |
| 5. Potential for fertility, considering assisted fertility techniques including intracytoplasmic sperm injection (ICSI) and donor ova                    |
| 6. Social and cultural pressures   |
| 7. Family dynamics and social circumstances including parents' desires, expectations, malleability, and reactions to genital ambiguity                   |
| 8. Depending on the degree of unpredictability of outcome, deference given to psychosocial factors when outcome is unpredictable                         |

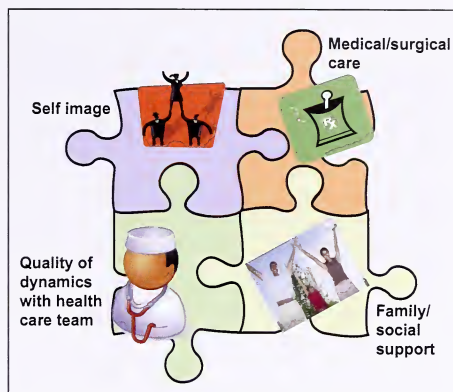


## DECISION MAKING

The consensus conference recognized the role of various entities in decision-making for the DSD child including: the parent, the child, and the medical system (Figure 4). However a practical application of these roles and ways to resolve potential conflicts between decision makers were not addressed. The role of other parties, such as advocacy groups, in decision-making was also not addressed. This pertains to those who have an interest in DSD issues based on ethical, human rights, and legal grounds. However, the potential role that support groups can play to facilitate better understanding of DSD patients or improving their quality of life was recognized. Health care professionals were encouraged to offer participation in support groups, although a practical way to broadly implement this was not addressed.

## NEED FOR OUTCOME DATA

The need for outcome data from a large cohort of randomly selected DSD patients is obvious (Table 3). However, the 1999 North American Task Force on Intersexuality, which was established to address this problem, was unsuccessful for several reasons. These included HIPPA restraints, investigators' hesitation to pool data, and patients' reluctance to participate. Hence, the risk of non-representative sampling, the rarity of the disorders, the variability with which patients are managed, and the lack



**Figure 4.** The puzzle is far from complete. The importance of medical care, support by adults including parents, understanding health care workers, and self-perceptions are obviously key.

of a well-capitalized central data-collection facility may be factors that will continue to preclude an appropriately designed study. Ideally the collection of such data would involve a centralized collection point and be similar to other NIH/federally/foundation funded projects, such as those seen in other pediatric subspecialties relating to malignancies, hemophilia, or cystic fibrosis. It is tempting to think that support groups could serve as a conduit to help capture the additional data needed to answer some of these questions.

**Table 3. Challenges for Future Management and Studies of DSD**

1. How to manage a complex medical condition without outcome data?
  - a. Expert opinion
  - b. Expert consensus
2. How to study DSD in children to assess newer therapies and approaches?
  - a. Case reports (including data from advocacy groups)
  - b. Case series (theoretical concepts extrapolated from existing data)
  - c. Case-control studies
  - d. Cohort studies
  - e. Randomized controlled double-blind studies
  - f. Systematic review/meta-analysis
3. What is the role of early genital surgery?
  - a. Patients psychological and psychosexual adjustment
  - b. Normalcy of appearance of genitalia
  - c. Requirement for subsequent surgery, particularly vaginoplasty
  - d. Genital sensitivity related to adult sexual responsiveness
4. How to best engage support groups?
  - a. Utilize availability at time of presentations and crises
  - b. Collaborate in general and specific educational efforts
  - c. Maintain congenial interactions
5. How to meet the need for psychologists?
  - a. Enhance currently skilled psychologist availability
  - b. Advocate for support via Health and Behavior CPT codes for psychologists involved in counseling of DSD patients and their families, thus providing a mechanism for which counseling can be reimbursed
  - c. Support the education of psychologists interested in the psychosocial and psychosexual factors among DSD individuals

## BARRIER TO BETTER STUDIES

Clear barriers to the study of children with DSD remain. In addition to the relatively poor funding designated to pediatric research, there are inherent issues of recognizing and protecting the rights of children. Furthermore, study of children with DSD presents a unique set of delicate problems given that some of the most important questions revolve around gender identity, genitalia, and sexual function. While the popular literature and the press attempt to be realistic on issues of gender and sexuality, there continues to be a general lack of knowledge and hence a lack of understanding of issues facing the DSD patient. Thus, it would be difficult if not impossible to design, receive approval, and conduct any study tracking the development of human sexuality, particularly the evolution of sexual orientation and sexual function during childhood. It is clear that without answers to these developmental questions, it is much more difficult to anticipate outcomes for most DSD patients with any degree of certainty. It appears, as in most other chronic illness in children, that a good quality of life is largely dependent on a strong family support system (Figure 3). The plan for the care of DSD patients must accommodate cultural and religious sensitivities and incorporate realistic issues regarding DSD patients, such as childhood gender development and sexuality.



Currently, this is the context in which research and patient care concerning DSD must operate. A less contentious approach would be to design a case-control study comparing matched DSD patients with good outcomes to those with less than good outcomes to determine what, if any, management, family, or environmental factors are associated with outcomes.<sup>21,22</sup>

### UNRESOLVED SURGICAL ISSUES

Curiously, the logic used to attack the traditional management paradigms has become one of the greatest hurdles to moving forward; that is, how does one develop a management model in the absence of clear outcome data for the disease/condition in question? The Columbia Court opinion<sup>23</sup> stated that children must be viewed as individuals with dignity and rights who are unable to give consent. This opinion states that while it is unclear whether or not genital surgery is necessary and urgent, it is also unclear whether the alternative of delaying surgery would work in society and would force a type of social experimentation. Given our degree of ignorance in DSD, the Columbia court citation declared that in cases where medical issues were unclear the ultimate decision-making rests with the parents—the “pro dubio in familia” rule—seems particularly wise and relevant.

Foremost in the discussion of modern genital surgery is the need for outcome data on genital sensitivity and function in adulthood following the use of modern, nerve-sparing genital surgery.<sup>24</sup> It is anticipated that function will be greatly improved over previous poor outcomes,<sup>25</sup> but the degree of improvement is at this point only theoretical. Unfortunately, confirmation of this improvement will not be possible for years. One problem with postponing genital surgery until the DSD child is capable of exercising a decision is the absence of a clear definition of the age and stage at which children can be expected to rationally make this decision.

### PSYCHOLOGIST DEFICIT

The LWPES ad hoc Intersex Committee devoted many hours to the completion of the consensus conference to address the continuing need for experienced psychologists who could be part of care teams for DSD patients. However, there has been little progress towards meeting this need in our health care system. Most psychologists receive little training or experience with approaching a gender disordered or DSD patient. The net result has been that most psychologists remain uncomfortable with these patients. There are medical centers which are now supporting obesity centers, using a multidisciplinary approach, in an attempt to demonstrate that a preventative and multi-specialty approach will lead to healthier individuals and ultimately save health care dollars. Perhaps a similar attempt at one or more large medical centers with a functioning DSD team could develop a similar model.

### SCIENTIFIC DEFICIT

The rapid advance of genetic knowledge has resulted in an expansion in diagnostic categories of the DSD patient. This will likely be helpful if it leads to better understanding of the biologic reproductive function in specific DSD types. Little progress has been gained concerning the age-old dilemma of genetic vs. environmental relative influence (nature vs. nurture). The need for funding agencies to support studies that serve to better understand genetic and endocrine factors of physical and psychological sexual differentiation is clear. Such funding should support the total purview impacting DSD issues; basic molecular and genetic research, differentiation using animal models, surgical techniques, physiological, and psychological, including psychosexual domains.

### CONCLUSION

It is important that the caretakers of DSD patients avoid the allure of adopting untested management approaches out of frustration. Regardless of the intent, this sort of approach risks making the same mistake for which the traditional management model stands accused—that is, making medical decisions without a clear understanding of the disorder or most likely outcome in the absence of intervention. An example of this type of thinking would be to advise parents to forego all genital surgeries without providing them a basis on which to frame their own decisions. In this example, one type of prescriptive authority—that is, performing genital surgery on all DSD infants—is exchanged for another—that is, no DSD infant receives genital surgery. Any recommendations must be based either upon clear scientific data or on a synthesis of the effects of the complex interplay between biological, social, and psychological inputs. How parents are expected to make a truly informed decision about sex assignment is unclear. Based on anecdotal experience, it seems that parents often make their decisions in a way that is similar to those championed by the optimal gender approach. A concern about this is that most

**Table 4. Summary: State of Management of DSD**

- |  |
|--|
| 1. Diagnosis and management of DSD continues to be a confusing topic.  |
| 2. All DSD conditions that present major challenges concerning sex of rearing (gender assignment) issues are rare and have received limited scientific study.                |
| 3. As with other congenital or chronic conditions, associated stress of the condition can be greatly alleviated and adjustment enhanced by skilled psychological counseling. |
| 4. In spite of current “enlightened generations”, most people remain uncomfortable discussing human sexuality when it pertains to themselves or their children.              |
| 5. There are minimal outcome data to support existing management of DSD conditions.  |
| 6. Yet, to do nothing is a breach of standard of care.   |

new parents focus upon the issues at infancy and find it difficult to project their thinking in terms of optimal adult outcome. For example, they often chose surgery out of a desire to create normal appearing genitalia even when it is clear that this intervention might compromise genital sensitivity.

Overall, much work and collaboration is needed to address the multiple issues of DSD management. Most DSD infants are not born in close enough proximity to centers with experienced DSD care teams to allow this team aspect of management to be helpful for the majority of affected children. In summary (Table 4), the management of DSD is inherently confusing (Figure 4). It has become clear that outcomes in DSD patients seem to be best characterized with a multi-factorial disease model that presupposes the simultaneous co-alignment of genetic, physical, and environmental risks for disease expression. We have much to learn from DSD patients and it is our hope that this article will help to encourage a genuine discussion of the issues facing these rarely encountered and poorly understood patients.

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**Editor's Comment:** The topic of disorders of sex development is of high scientific and lay public interest. Among pediatric endocrinologists the interest in this topic has long been preeminent. Indeed, one of the most frequently read articles among the subscribers of GGH who have accessed the archives of the journal during the past 4 years is the lead paper by Sheri A. Berenbaum, PhD, entitled "Management of Children with Intersex Conditions: Psychological and Methodological Perspectives."

Among the highlights that stand out of the more recent papers dealing with this subject is the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology publication of their "Consensus Statement for the Management of Intersex Disorders."<sup>2</sup> It is also worth pointing out that at the latest meeting of the LWPES (Toronto, Ontario, May 5, 2007) the State-of-the-Art Lawson Wilkins Lecture was "Ethical and Historical Considerations in Treating Children with Disorders of Sex Development and Idiopathic Short Stature" presented by Alice Dreger, PhD (Fienberg School of Medicine, Northwestern University, Chicago, Illinois). Additional publications that have attempted to bridge the scientific and the public understanding of the issues involved with individuals with disorders of sex development are: a recent book, "Ethics and Intersex" edited by Sharon Sysma<sup>3</sup> and reviewed by Ileana Hughes<sup>4</sup> and the article "What if It's (Sort of) a Boy and (Sort of) a Girl?"<sup>5</sup> The lead paper in this issue of GGH by Drs. Houk and Lee brings forth further clarifications of the intricacies of the management of these patients and the state-of-the-art concepts in this topic to make ambiguity less ambiguous. The ethical and legal implications of genetic testing in androgen insensitivity syndrome are also reviewed in this issue of GGH.<sup>6</sup>

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Androgen Insensitivity Syndrome—Ethical and Legal Implications of Genetic Testing

Berg and colleagues presented a case of a 2-month-old full-term infant with an inguinal hernia. The external genitalia were unambiguously female; however, bilateral hernias with solid structures having internal blood flow but no follicles or Fallopian structures were detected. Hernia repair revealed seminiferous tubules with germ cell hyperplasia, no vas deferens, and presence of round ligament tissue. The karyotype was 46,XY and MRI of the pelvis revealed absence of the uterus, ovaries, and a blind vaginal pouch. The diagnosis of androgen insensitivity syndrome (AIS) was confirmed by identification of a novel homozygous nonsense mutation predicted to negatively impact androgen receptor (AR) gene function. The authors then provided an informative discussion of the syndrome and its clinical management.

In the context of counseling this particular family about the heritability of AIS, testing the proband's sisters was recommended. The patient's 22-month-old sister was diagnosed with AIS; and the 9-year-old maternal half-sister had a 46,XX karyotype. A four-generation pedigree detected a significant bias toward female offspring in previous generations; 10 of the 11 individuals in the great-great-grandmother's generation were female, 5 of whom were infertile and some of whom were known to have absent ovaries, uterus, or both. There were also women in more recent generations. The authors assumed many of these women were at risk for being previously undiagnosed 46,XY females or 46,XX heterozygous carriers of the familial AR mutation who could have affected children with future pregnancies. Due to the possible health risks associated with AIS (an increased risk of testicular neoplasms, which is reportedly greatest after puberty but can occur even in the elderly, and increased risk for osteopenia), provision of genetic testing for other at-risk family members could be considered an ethical responsibility of the health care team.

The ethical aspects of diagnostic disclosure elucidated were: (1) the history of withholding information from patients with disorders of sex development (DSD) based on the assumption that physicians were better able to determine what was in the patient's best interest; (2) the principle of informed consent asserts an ethical imperative to disclose such a diagnosis to the patient; in the case of minors, participation in decision-making is guided by the concept of "assent" commensurate with developmental capacity; and (3) the extent to which a physician has the dual responsibility to maintain confidentiality and to inform other members of the family that they may be at risk for being affected by a condition or for transmitting it to their offspring. The best resolution to the latter issue is to request that the parents of the affected child disclose their child's condition to other

members of the family and ask those members to contact a physician. The authors advised, in the case of refusal to disclose information to other family members, that the clinician should carefully document discussions held with the family and to continue to encourage them to disclose information to those at risk.

Among the considerations of offering genetic testing to other at-risk family members in this pedigree, are the potential legal ramifications of a diagnosis of AIS. In a 1999 case (*Littleton vs. Prange*) involving a wrongful death suit, a court in Texas ruled that the transsexual woman's marriage to her deceased husband was invalid because of her 46,XY karyotype. This sort of ruling could extend to many areas of the law in which sex is a central issue (eg, discrimination, choice in marriage, participation in sports, housing in higher education and the penal system) and could conceivably affect individuals with common sex chromosome aneuploidy, ie, Turner syndrome and Klinefelter syndrome.

The gender medicine team involved in the present case discussed AIS extensively with the parents. Details of both physical and psychosexual development, specifically gender identity and gender role, were reviewed. Questions were answered and additional information was provided pertaining to child rearing, the rationale for gonadectomy to prevent testicular malignancy, future hormone replacement, infertility issues, and the potential for legal complications to arise. The medical team conducted an assessment of the family's understanding of the condition and its future implications. The family was also made aware of psychologists' availability for support and assistance in discussing the diagnosis with the affected girl at an appropriate age. The parents were also strongly encouraged to disclose the information about AIS to extended family members so that they could seek genetic counseling and testing, if desired.

With regard to DSD, more generally, the authors noted that in the context of current knowledge regarding the process of sex determination and differentiation, unidimensional definitions of "sex" are inherently problematic. They suggested that clinicians be prepared to advocate on behalf of affected patients when caught in legal predicaments, perhaps in the form of an amicus curiae from the American Academy of Pediatrics.

Berg JS, French SL, McCullough LB, et al. Ethical and legal implications of genetic testing in Androgen Insensitivity Syndrome. *J Pediatr*. 2007;150:434-8.

**Editor's Comment:** *The reader can find additional guidance regarding the friction between the principles of confidentiality and disclosure of genetic information*



in 2 recent reports.<sup>1, 2</sup> The authors of the current case report do not inform us whether the family in question gave consent to disclose the patient's diagnosis to at-risk extended family members. Regardless of whether they did or not, the "gender team" should be commended for delivering care to the family in a manner consistent with the recent Consensus Statement of Management of Intersex Disorders.<sup>3</sup> The process of disclosing all aspects of the DSD and its clinical care should be collaborative, on-going, and planned with the parents from the time of diagnosis. But, what if the family in this case refuses to allow disclosure to other, potentially affected family members? The 1983 President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research<sup>4</sup> provides some valuable guidance. For example, it states that when the patient refuses, a health care professional's disclosure to at-risk family members should take place only when: (1) reasonable efforts to elicit voluntary consent to disclosure have failed; (2) there is a high probability that harm will occur if the information is withheld, and the disclosed information will actually be used to avert harm; (3) the harm that would result to identifiable individuals

would be serious; and (4) appropriate precautions are taken to ensure that only the genetic information needed for diagnosis and/or treatment of the disease in question is disclosed. Approximately 10 years later, the Committee on Assessing Genetic Risks of the Institute of Medicine<sup>5</sup> added an additional criterion: that there is no other reasonable way to avert harm. Neither group implied that the clinician has a legal duty to inform relatives, instead arguing for an ethical duty and legal permission to inform in certain cases.

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## CAH Women: Sexual and Reproductive Outcomes

Gastaud et al performed a cross-sectional study using face to face interviews, written questionnaires, the Female Sexual Function Index (FSFI), a brief self-report measure of female sexual function, and a gynecological examination in 35 women aged 18 to 43 years with congenital adrenal hyperplasia (CAH), presenting Prader stages I-V at birth who had been treated from birth to adolescence in the same pediatric endocrine clinic. The objectives of the study were to obtain a detailed description of sexual and reproductive outcomes in adult women with CAH and to compare these outcomes among CAH subtypes and with non-CAH controls. Fourteen of the CAH patients had presented with severe masculinization of their external genitalia at birth (11 with Prader IV and 3 with Prader V stages).

None of the patients expressed doubts about their gender assignment. At gynecological examination cosmetic and anatomic outcomes were considered good by both the patients and the examiner, and 65% of the subjects presented with a satisfactory clitoris, introitus and vagina. However, 9 of 35 patients (26%) were diagnosed with vaginal stenosis, 6 of these belonging to the Prader IV-V group at birth. Seven subjects (20%) reported homosexual inclinations, compared with 5.7% in the control group and 6.6% in a large survey of age-matched women in France (ACSF) and these tendencies were present in 43% (6 of 14) of the Prader IV-V women. A decrease in sexual function was noted when the 35 CAH patients were compared with the 69 healthy controls utilizing the FSFI questionnaire, thus 37% (13 of 35) reported

never having sexual intercourse with vaginal penetration by their partners compared with 5% in the ACSF survey. Of these women, 8 attributed their lack of sexual intercourse to the anatomy of their genitalia, 2 believed intercourse would be painful and/or 7 had no partner; the 3 patients born Prader V were among this group. Some degree of pain during vaginal penetration was experienced by 56%, 9 of them presented with moderate or marked stenosis of their introitus. Eight patients cohabited with their partner or were married and 77% wished to be pregnant in the near future or at a later time. Eight subjects became pregnant, only one in the Prader IV-V group; however, only 17% (6 of 35) had children compared to 71% of French women in the ACSF survey. The authors concluded that despite the expert medical and surgical care received by these patients, women with CAH suffer major limitations in their sexual function and their reproductive life.

Gastaud F, Bouvattier L, Duranteau L, et al. Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2007;92:1391-6.

**Editor's Comment:** Female neonates with CAH may present with some degree of masculinization of their external genitalia at birth and those with severe virilization (Prader stages IV-V) may require extensive surgery to correct for different degrees of clitoral enlargement and labio-scrotal fusion. In addition, many may develop chronic masculinization as a consequence of being exposed to an excess of adrenal androgens postnatally with the development of hirsutism, acne, muscle



hypertrophy and stature, all of which may affect their sexuality and their physical attractiveness.

A number of studies have shown that 46,XX CAH women develop female gender identities,<sup>1,2</sup> but while earlier studies suggested that they had mostly satisfactory sexual intercourse,<sup>3</sup> more recent reports have suggested that they may present with an increased incidence of sexual dysfunction, which seems to be largely related to difficulties in vaginal penetration.<sup>4,5</sup> This seems to be true mainly for those with the most virilized external genitalia at birth, whereas CAH women with a lesser degree of sexual ambiguity at birth seem to have nearly normal sexual outcomes.

While cosmetic and anatomic outcomes of surgery were generally satisfactory to most patients and medical examiners, CAH women, particularly those with Prader IV-V stages, expressed an increased homosexual orientation and a decreased frequency

of sexual intercourse. This report and previous studies seem to show that while a large percentage of women with CAH are satisfied with their physical and genital appearance, sexual dysfunction and impaired reproductive outcomes are frequent in this population and will require better medical and particularly surgical care, longer and more detailed follow up, and the transmission of more comprehensive information to parents and/or patients of the risks to sexual function following reconstructive surgery.

Roberto Lanes, MD

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## Height in Survivors of Childhood Acute Lymphoblastic Leukemia

This paper describes adult height in a Childhood Cancer Survivor Study (CCSS) cohort of 2434 subjects who were at least 5-year survivors of acute lymphoblastic leukemia (ALL) and were diagnosed between 1970 and 1986. Their data were compared to that of 3009 siblings selected for being the closest in age to the proband. Only those over 18 years of age were included. Survivors were excluded from this analysis if they were diagnosed after 17 years of age or if they had a recurrence of their primary leukemia, a secondary malignant neoplasm, or underwent stem-cell transplant before 18 years of age. Cumulative chemotherapy doses were categorized into none, low, medium, or high based on tertiles from previously published end-cut points. For some of the agents dosage information was not available and exposure was recorded as yes or no. Central nervous system (CNS) radiotherapy doses were abstracted in 5-Gy increments. Of the survivors who received cranial radiotherapy, 95% were treated with doses of 15 to 29 Gy and as a result, radiotherapy was characterized into <20 Gy and >20 Gy. Height was expressed in absolute terms as well as SDS. Pubertal status was not always recorded, therefore this variable was dichotomized at age 8 for girls and 10 for boys.

The median age of the study cohort was 27 years, and 51% were female. Median age of the siblings was 31 and 52.7% were female. All survivor treatment groups, including those treated with chemotherapy alone, had decreased adult height and height SDS compared with siblings ( $p < 0.001$ ). Effects of radiotherapy on adult height SDS differed between those who were prepubertal versus postpubertal at diagnosis. The height SDS was decreased at all doses of cranial and craniospinal radiotherapy in survivors diagnosed before puberty, compared with those treated with chemotherapy alone. Those survivors who had received >20 Gy of cranial radiotherapy were on average

shorter with height SDS scores on average 0.88 lower than those treated with cranial radiotherapy alone. Among survivors diagnosed after pubertal onset, significant negative impact on height SDS was not seen on any cranial radiotherapy dose as compared with chemotherapy alone. On average, the adult height SDS of survivors treated after pubertal onset remained shorter than their siblings. All survivor exposure groups were at significant greater risk of adult short stature (that is height SDS < -2) as compared with siblings. No chemotherapeutic agent analyzed had a consistent dose effect on adult height SDS analyzed individually or in combination. There was an increased proportion of female survivors with adult short stature (12.5%) as compared with male survivors (5.5%).

The authors stated that this report represents the largest cohort of adult ALL survivors evaluated for adult height to date. Significant differences in height outcomes between survivors treated with high doses of cranial radiotherapy as well as those treated with lower dose cranial radiotherapy versus chemotherapy alone were demonstrated. Survivors who received any spinal radiotherapy had the shortest adult heights.

Mechanisms by which cranial radiotherapy affects short stature remain uncertain. It is speculated that at higher doses of radiation there may have been some degree of growth hormone deficiency, especially as it relates to the pubertal growth spurt and peak growth velocity. The second possibility is that cranial radiotherapy exerts its effects on pubertal timing. It would appear that early puberty occurs, especially in females, when treated at an early age. A combination of growth hormone insufficiency and early puberty is certainly associated with short adult stature. Findings in the current study are consistent with this hypothesis, since the risk of adult short stature was greater in those diagnosed at a

younger age, and girls were more affected than boys. The authors pointed out that the limitations of the study included the use of self or proxy reported height data, lack of longitudinal growth information, and the specific time of documentation of pubertal status. However, the large size of the study and the use of sibling controls helped to validate the significance of the differences found. Finally, the authors stated that most patients with ALL were currently treated with chemotherapy alone. Therefore the relationship between chemotherapy agents and linear growth velocity should be available in the future.

Chow E, Friedman D, Yasui Y, et al. Decreased adult height in survivors of childhood acute lymphoblastic leukemia (ALL): A report from the Childhood Cancer Survivor Study. *J Pediatr*. 2007;150:370-5.

**Editor's Comment:** This paper is accompanied by a thoughtful editorial by Oberfield.<sup>1</sup> Her comments included a discussion of previous reports from the CCSS regarding morbidity among childhood cancer survivors

and specifically those who were survivors of childhood brain cancers and were subsequently treated with growth hormone. Oberfield points out shortcomings with regard to self reported or proxy reported height and the definition of prepubertal and pubertal based on age, but affirms the uniqueness of the study because of its large size and the fact that even with chemotherapy alone there was a greater than threefold increased risk of decreased stature.

The data in this study involved survivors of ALL who were treated with a treatment regimen which differs from that currently in use. It clearly demonstrated that previous treatment regimens were associated with reduced adult height. It is hoped that oncologists will continue to carefully record auxologic and pubertal data on their patients so that similar long-term outcomes can be examined from a different therapeutic era in the future.

William L. Clarke, MD

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## Congenital Hypothyroidism—Outcome of Early Treatment

Previous research conducted by Kempers and colleagues, in a cohort born and screened in 1981-1982, demonstrated persistent cognitive and motor deficits associated with congenital hypothyroidism despite initiating  $T_4$  replacement at a median age of 28 days after birth. In the present study, the same investigators examined potential benefits of commencing  $T_4$  replacement at an earlier age (median = 20 days) for a cohort born in 1992 and 1993. During this time, Dutch pediatricians were advised to start with 6-8  $\mu\text{g}$   $T_4$ /kg/day with  $T_4$  dose adjustments based on thyroid function labs obtained at regular outpatient follow-up visits.

Participants included 82 Dutch children (mean age 10.5 years, range 9.6 to 11.4 years) diagnosed with thyroidal congenital hypothyroidism (CH-T). An additional 5 participants were diagnosed with central congenital hypothyroidism (CH-C); results were

analyzed separately for these due to differing etiology, treatment regimen, and sequelae.

Intelligence was assessed with the Dutch version of the Wechsler Intelligence Scale for Children, third edition (WISC-III), except for the first 10 patients for whom the WISC-R was used (and recalculated into WISC-III scores according to recommended guidelines). Three IQ scores were derived for each participant: full-scale IQ (FSIQ), verbal IQ (VIQ), and performance IQ (PIQ). General population IQ scores for each domain have a mean of 100 ( $\pm 15$ ). Motor skills were assessed with the Movement Assessment Battery for Children (MABC), designed to identify motor function impairments in children aged 4-12 years, including subscales for manual dexterity, ball skills, and balance; higher scores indicating more motor problems. For the 1981-1982 cohort, motor skills were assessed using a

**IQ scores of the CH-T group**

|                             | FSIQ                           | P (t)       | Verbal IQ                    | P (t)       | Performance                    | P (t)       |
|-----------------------------|--------------------------------|-------------|------------------------------|-------------|--------------------------------|-------------|
| <b>Severe CH-T (n=41)</b>   | 93.7(89.5-97.9) <sup>1,3</sup> | 0.004(-3.0) | 94.9(90.1-99.7) <sup>2</sup> | 0.039(-2.1) | 93.9(90.9-97.8) <sup>1,3</sup> | 0.003(-3.1) |
| <b>Moderate CH-T (n=19)</b> | 96.2(88.9-103.5)               | 0.290(-1.1) | 95.4(87.9-102.9)             | 0.210(-1.3) | 98.0(91.1-104.9)               | 0.550(-0.6) |
| <b>Mild CH-T (n=22)</b>     | 105.0(99.5-110.4)              | 0.73(1.9)   | 103.6(98.2-109.1)            | 0.182(1.4)  | 105.3(99.3-111.3)              | 0.082(1.8)  |
| <b>Total (n=82)</b>         | 97.3(94.2-100.4)               | 0.088(-1.7) | 97.4(94.1-100.6)             | 0.113(-1.6) | 97.9(94.8-100.9)               | 0.172(-1.4) |
| <b>Range</b>                | 57-129                         |             | 65-138                       |             | 58-134                         |             |

IQ scores, expressed as mean (confidence interval), are presented for the total CH-T group and the three severity subgroups.

P values (with t value in parentheses) refer to the comparison with the normative population.

<sup>1</sup> P < 0.01 compared to the population mean

<sup>2</sup> P < 0.05 compared to the population mean

<sup>3</sup> P < 0.01 compared to mild CH-T

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forerunner of the MABC: the Test of Motor Impairment (TOMI). IQ and motor scores were compared among the following subgroups: severe vs. moderate vs. mild CH-T (based on pretreatment free  $T_4$  concentration) and early-treated vs. late-treated patients (ie, before or after the mean starting day of treatment) with severe, moderate, or mild CH-T.

Although mean FSIQ, VIQ, and PIQ scores for the total 1992-1993 cohort were not significantly different from population norms, those in the severe CH-T subgroup received lower scores in all 3 areas (Table). In contrast, IQ scores were not significantly different from the population means for the moderate or mild CH-T subgroups. With regard to motor development, the mean total MABC was significantly poorer than that of the normative population; and a significantly higher proportion of all CH-T severity subgroups received "subnormal" scores. Patients with severe CH-T had significantly worse total MABC and manual dexterity scores than patients with moderate CH-T.

In the severe CH-T group, IQ and motor scores did not differ in patients treated before or later than 19 days after birth. Moreover, IQ and motor scores were not different in the moderate and mild CH-T group when treatment was initiated either before or after 19 and 31 days, respectively. Only the severity of CH-T appeared to be a significant predictor of FSIQ when a multiple regression analysis was conducted using severity of CH-T and starting day of treatment as predictor variables.

Compared to patients from the earlier cohort, those from the 1992-1993 cohort with mild or severe CH-T had initiated  $T_4$  supplementation at a significantly younger age (days 31 and 19 vs. days 68 and 29, respectively). The initial  $T_4$  dose and the FSIQ scores of the subgroups were not significantly different between the 2 cohorts. In patients with mild CH-T, the percentage of patients with a subnormal total motor score was significantly higher in the 1992-1993 cohort; differences were not significant for severe and moderate CH-T. The authors speculated the reason for the increased motor problems scores in the latter cohort may be a result of selecting a measurement tool (the MABC vs. TOMI) exhibiting enhanced sensitivity.

In summary, patients with severe CH-T, whose treatment with  $T_4$  was initiated at a mean age of 19 days after birth, exhibit significant cognitive and motor deficits. Those with mild or moderate CH-T (initiated at a mean age of 31 and 19 days, respectively) had a better

prognosis for IQ, but still showed substantial motor deficits. Based on the observed deficits, despite earlier initiation of  $T_4$  treatment, the authors speculated that intellectual and motor development deficits may be the consequence of the hypothyroid prenatal state.

Kempers MJE, van der Sluijs Veer L, Nijhuis-van der Sanden R, et al. Neonatal screening for congenital hypothyroidism in the Netherlands: cognitive and motor outcome at 10 years of age. *J Clin Endocrinol Metab.* 2007;92:919-24.

**Editor's Comment:** In a review of the earlier paper by Kempers and colleagues in GGH,<sup>1</sup> Lanes noted 2 other recent reports of cognitive deficits among those born with severe CH.<sup>2,3</sup> In the current report, IQ deficits were evident among those with severe CH and motor deficits were discernable across all 3 severity subgroups. The American Academy of Pediatrics and other professional societies recently published a clinical report "Update of Newborn Screening and Therapy for Congenital Hypothyroidism,"<sup>4</sup> in which it was acknowledged that those showing signs of prenatal hypothyroidism may evidence more marked cognitive and other impairments; whether these differences, which were characterized as "minor" are preventable by further optimizing postnatal therapy was considered an open question.

In consideration of the potentially increased vulnerability of children with severe CH despite early and adequate  $T_4$  supplementation, this subgroup should receive particular scrutiny with regard to neurocognitive function. Parental reports of adequate school performance in early years obviously do not rule out specific learning disabilities that, if left undetected, could result in suboptimal academic achievement misattributed to other factors. Finally, when neurocognitive capacity is the clinical outcome of interest, do not assume good adherence to

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recommended  $T_4$  supplementation; in a psychometric and school achievement study of 14-year-olds with CH, identified by newborn screen, approximately 45% had poorly controlled hypothyroidism.<sup>5</sup> Of particular relevance to the issue of cognitive function in these youths, improved hormonal values were accompanied by significant improvements in test results.

David E. Sandberg, PhD

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## Failure to Thrive: Terminology and Anthropometry

In the February 2007 issue of the *Archives of Diseases in Childhood*, there are 6 articles or perspectives pertaining to one form of aberrant infant growth termed "failure to thrive" (FTT). As Hughes<sup>1</sup> commented—except for infants with obvious disease (eg, cystic fibrosis, celiac disease), the operative definition of "non-organic" FTT in developed societies is not agreed upon, resulting in difficulty in establishing a clear diagnosis and in blurring the divide between a normal extreme and clinical illness; the latter perhaps associated with impaired development. However, a suboptimal nutritional state is usually recognized as one of the hallmarks of this entity.<sup>2</sup> Olsen et al evaluated growth data from 6090 Danish children examined between 1 to 5 weeks of age, 2 to 6 months of age, and 6 to 11 months of age in an effort to establish the prevalence of this growth pattern. Utilizing 7 anthropometric criteria of FTT (Table), they examined the concurrence of these criteria in establishing its presence. In this population of infants, 27% met one or more of the anthropometric criteria at either the earlier (3-6 months) or later (6-11 months) examinations. Only 1.3% of infants met the criterion "weight <80% of median weight for length," and they were a good deal longer than other infants. Twenty-two percent of infants crossed 2 major weight percentiles downward, but they were substantially heavier at birth and

throughout the study than were other children with FTT. None of the infants in this study were concordant for all 7 criteria, and approximately 70% of subjects with FTT met only one criterion. Significant under-nutrition, defined as BMI <5th percentile for chronological age, was present in only 2% of children screened. Olsen et al concluded that "... no single measurement ... is adequate to identify nutritional growth delay ... (or) to predict outcomes such as neurodevelopmental or behavioral outcomes." Spencer reached the same conclusion; indeed this investigator stated unequivocally "weight monitoring is not a good screening test for FTT."<sup>2</sup>

Emond and co-workers previously examined family, socioeconomic, and prenatal factors that were epidemiologically related to FTT and found that only higher parity (infants born in a 4th or subsequent pregnancy) and small maternal stature (<160 cm) were associated with poor infantile weight gain during the first 9 months of life.<sup>3</sup> They reported that parental postnatal factors associated with FTT as assessed by conditional weight gain of the offspring are maternal age >32 years, height <160 cm, and parity >3; infant characteristics are prolonged breast feeding (>7 months), slow feeding, and ingesting only small amounts of solid food after 6 months of age.

Lucas et al reviewed the literature reporting lay (primarily maternal) views on infant growth and well being. In this population, infant size was primarily utilized as an index of the health and the quality of care provided by the parent(s). While supranormal growth is not of concern, subnormal growth evokes anxiety and fear about the infant and self-recrimination. Wright and Weaver<sup>4</sup> commented that it is essential to differentiate between size (a static measurement) and growth (a dynamic change) when assessing the likelihood of underlying illness in an infant with FTT and that aggressive intervention in the short, thin, normally growing and developing infant is unnecessary.

Olsen EM, Petersen J, Skovgaard AM, Weile B, Jorgensen T, Wright CM. Failure to thrive: the prevalence and concurrence of anthropometric criteria in a general infant population. *Arch Dis Child*. 2007;92:109-14.

Emond A, Drewett R, Blair P, Emmett P. Post natal factors associated with failure to thrive in term infants in the Avon Longitudinal Study of Parents and Children. *Arch Dis Child*. 2007;92:115-9.

### Anthropometric Criteria of Failure to Thrive

- Weight <75% of median weight for chronological age (Gomez criterion)
- Weight <80% of median weight for length (Waterlow criterion)
- Body mass index for chronological age <5th centile
- Weight for chronological age <5th centile
- Length for chronological age <5th centile
- Weight deceleration crossing more than two centile lines; centile lines used: 5, 10, 25, 50, 75, 90, 95, from birth until weight within the given age group
- Conditional weight gain—lowest 5%, adjusted for regression towards the mean from birth until weight within the given age group\*

\* Conditional weight gain was determined by the "thrive index"—the change in weight z-scores between 2 points, from birth to the later age, adjusted for regression to the mean.

Adapted from Olsen EM, et al. *Arch Dis Child*. 2007;92:109-14.



Lucas P, Arai L, Baird J, Kleijnen J, Law C, Roberts H. A systematic review of lay views about infant size and growth. *Arch Dis Child* 2007;92:120-7.

**Editor's Comment:** Given the many auxologic criteria (Table) for the identification of an infant with FTT and the observation that one criterion is little better than another, it appears that this diagnosis falls into those typified by "I can't define it, but I know it when I see it." The critically essential finding in most of these subjects is that despite a poor appetite, relatively restricted caloric intake, and low weight for stature, linear growth rate remains normal. (Indeed, this pattern of growth is the diametric opposite of the voracious infant/child who steadily gains weight and crosses weight and height percentiles!) It is particularly important not to designate the normal, slowly growing or small child as abnormal, both because of the need to avoid unnecessary diagnostic and therapeutic interventions as well as to support the parents' confidence and sense of competence to care for their child and to avoid a misplaced charge of negligence.

Clearly, the clinician needs to know not only her/his patient but also the child's parents. The criterion for FTT of downward crossing of weight percentiles certainly reflects in most subjects normal variations of growth as such changes are indeed quite frequent.<sup>5</sup> It is of interest that the term FTT has been adopted by the geriatricians to denote "an elderly patient who undergoes a process of functional decline, progressive apathy and a loss of willingness to eat and drink that culminates in death."<sup>1</sup> Were there such a precise definition for the pediatric population, the identification and management of such children would be far more precise.

Allen W. Root, MD

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## GH and GnRHa Therapy for Short Stature

This study assessed the final height (FH) and adverse effects of combined growth hormone (GH) and gonadotropin-releasing hormone agonist (GnRHa) treatment in short adolescents with relatively early puberty. Van Gool et al studied 32 adolescents born small for gestational age or with normal birth size, in Tanner stage 2-3, with age and bone age of <12 years for girls and <13 years for boys. Subjects had a height SDS of either  $\leq -2$  SDS or between  $-1$  and  $-2$  SDS and a predicted adult height (PAH) of  $\leq -2$  SDS. Patients were randomly allocated to receive GH and GnRHa (n=17) or no treatment (n=15) for 3 years; FH was determined at the age of 18 years or older in girls and 19 years or older in boys.

The FH was not different between treated and untreated subjects. However, treated patients had a greater height gain (FH minus PAH at the beginning of treatment) than the untreated patients ( $4.4 \pm 4.9$  vs.  $-0.5 \pm 6.4$  cm, respectively;  $p < 0.05$ ). Of the treated and control subjects, 76 (60%) had a FH that was greater than the PAH. A significant gain in PAH of 9.3 cm after 3 years of combined therapy was noted in the treated group compared with a 1.2 cm gain in the untreated group. However, during the period of time between treatment discontinuation and FH, 50% of the PAH gain during treatment was lost, resulting in a mean height gain of 4.9 cm (range of  $-4$  to 12.3 cm). Although, treatment did not seem to affect BMI or hip bone mineral density (BMD), the mean lumbar spine BMD and the bone mineral apparent density (BMAD) tended to be lower in treated males. The authors concluded that given the expensive and intensive treatment regimen and the modest height gain attained, as well as the possible adverse effect of therapy on bone mineralization in males, GH and GnRHa treatment cannot be considered

for routine treatment of short stature in children entering into early puberty. However, treatment could be considered in children, particularly females, with extremely low adult height prediction, early pubertal onset, and considerable psychosocial problems.

Van Gool SA, Kamp GA, Visser-van Balen H, et al. Final height outcome after three years of growth hormone and gonadotropin-releasing hormone agonist treatment in short adolescents with relatively early puberty. *J Clin Endocrinol Metab*. 2007;92:1402-8.

**Editor's Comment:** The final height of short children entering into puberty at an early age may be quite limited due to premature epiphyseal fusion induced by the early secretion of gonadal steroids. Treatment with GnRHa to delay or halt pubertal onset has been attempted in this group of patients, but the growth velocity of some of them has been noted to decrease to levels below the normal pubertal velocity, possibly as a result of accelerated growth plate senescence induced by previous estrogen exposure. GH treatment in short children with idiopathic short stature or born small for gestational age has been shown to increase final height, particularly if begun at an early age. Combined GH and GnRHa therapy in short children entering into puberty at an early age has been attempted in several studies with a height gain of between 1 to 10 cm and the effectiveness of this form of therapy remains controversial. Treatment response has been generally analyzed by comparing treated patients to patients treated only with GH, to an untreated group not randomly assigned, or to no controls at all; most studies included only females.

Treatment of short, but otherwise healthy children with medications that require parenteral administration, close

supervision, frequent laboratory testing, and are extremely expensive, should only be considered if the height gain obtained is significant and if the medications are proven to be safe. As clearly stated by the authors, the costs of this form of therapy seem to overshadow the modest benefit in height gain obtained; therefore this form of therapy should not be recommended for routine use in short but otherwise healthy patients who enter into puberty at an early age.

Roberto Lanes, MD

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## GH Inhibition of IGF-I in STAT5b Expression

Ligand binding of the growth hormone (GH) receptor activates, via the Jak2 tyrosine kinase, the Stat transcription factors and the MAP kinase and PI3 kinase/Akt pathways. As is well known to the readers of GGH, GH-stimulated transcription of the insulin-like growth factor (IGF)-I gene requires the Jak2/Stat5b mechanism. However, GH signaling also leads to transcriptional repression of a cohort of genes, including the IGF binding protein (IGFBP)-1. Ono et al sought to elucidate the mechanism of this facet of GH action.

Hypophysectomized Sprague-Dawley rats were given a single systemic pulse of GH, and hepatic RNA was isolated 30, 60 or 120 minutes afterwards. By both microarray and RT-PCR methods, GH acutely increased the mRNA levels of IGF-I and Socs-2 while decreasing that of IGFBP-1. GH also acutely induced the nuclear accumulation of phosphorylated Stat5b. Adenoviral-mediated delivery of a constitutively active Stat5b construct to livers of GH-deficient rats similarly increased IGF-I and Socs-2 expression while decreasing IGFBP-1.

To further examine the transcriptional regulation of IGFBP-1, Cos-7 cells were transiently transfected with a rat IGFBP-1 promoter-luciferase reporter construct as well as an expression vector for mouse GH receptor. Cotransfection with wild-type or constitutively activated FoxO1, a transcription factor important for *IGFBP-1* expression, stimulated promoter activity. GH treatment altered neither IGFBP-1 promoter activity nor the abundance of the FoxO1 proteins. In contrast, when wild type Stat5b was also co-transfected, GH treatment led to a 35%-50% reduction of IGFBP-1 promoter activity with either type of FoxO1; GH stimulated phosphorylation of the wild-type but not constitutively activated FoxO1, and abundance of the FoxO1 proteins again were not altered. Thus, GH-induced IGFBP-1 repression is mediated by Stat5b and not Akt (the constitutively activated FoxO1 is Akt resistant.)

Because IGFBP-1 expression is also repressed by insulin, which acts via Akt inhibition of FoxO1, the authors sought to further examine the interactions between Akt, Stat5b and FoxO1. A tamoxifen-inducible Akt fusion protein, iAkt, repressed IGFBP-1 promoter activity in the presence of wild type, but not a constitutively activated, FoxO1; the former form of FoxO1 was phosphorylated by

Akt while the latter cannot be. In contrast, a constitutively activated Stat5b did not phosphorylate FoxO1.

Further experiments were performed to mechanistically examine Stat5b inhibition of FoxO1. Using a luciferase reporter construct driven by a minimal promoter containing 3 copies of IRSA (one of the tandem FoxO1 binding sequences found in the IGFBP-1 promoter), the FoxO1 binding site was shown sufficient for GH and Stat5b inhibition of FoxO1-stimulated gene transcription. To examine the possibility of reciprocal inhibition, a luciferase reporter construct driven by the Stat5b-dependent HS7 response element (found in the IGF-I gene) was examined. It increased activity in response to GH in the absence of FoxO1, and increased further still when wild type or constitutively activated FoxO1 were cotransfected, even though there were no FoxO1 binding sites in the HS7-promoter sequences. Thus, competition for transcriptional co-factors does not seem to be the mechanism of Stat5b's inhibition of FoxO1 activity. A dominant-negative Stat5b was shown to lose the ability to mediate GH inhibition of IGFBP-1 promoter activity, in both co-transfected Cos-7 cells in vitro and in GH-treated hypophysectomized rats in vivo. Co-transfected Cos-7 cells further showed that GH induced nuclear accumulation of Stat5b, but neither nuclear levels of FoxO1 protein nor its DNA-binding ability were reduced by activated Stat5b. Direct protein-protein interactions between FoxO1 and Stat5b from Cos-7 nuclear extracts were not detected by co-immunoprecipitation assays or avidin-biotin complex DNA binding assay.

Finally, the authors returned to their hepatic microarray results from GH-stimulated hypophysectomized rats. They compared the list of GH-repressed genes to genes repressed by adenovirally introduced constitutively activated Stat5b. Eighty-nine gene transcripts were similarly reduced by both mechanisms. *In silico* search for FoxO1 binding sites within phylogenetically conserved (rat and human) regions of these genes revealed 19 hits, or 21% of the repressed genes. Of 322 randomly selected genes not regulated by GH or Stat5b 19% were also found to contain FoxO1 binding sites. Thus, FoxO1 inhibition accounts for only a subset of transcriptional repression by GH/Stat5b.

Ono M, Chia DJ, Merino-Martinez R, Flores-Morales A, Unterman TG, Rotwein P. Signal transducer and activator of transcription (stat) 5b-mediated inhibition of insulin-like growth factor binding protein-1 gene transcription: a mechanism for repression of gene expression by growth hormone. *Mol Endocrinol*. 2007;21:1443-57.

**Editor's Comment:** *Through a well constructed series of experiments, Ono et al clearly showed that GH inhibits IGFBP-1 expression via activated Stat5b and FoxO1. However, the exact mechanism of FoxO1 inhibition by Stat5b remains elusive; FoxO1 protein degradation, nuclear exclusion and impaired DNA binding ability were all ruled out, as was direct protein-protein interaction between Stat5b and FoxO1. Nonetheless, this paper expands our thinking along 2 lines. First, GH, via activated Stat5b, not only induces gene expression (eg. IGF-I), but also represses transcription of other genes, such*

*as IGFBP-1. Thus, the genetic response to GH/Stat5b signaling is a richer compilation of coordinated alterations than previously appreciated. Second, the mechanism whereby IGFBP-1 expression is repressed by GH is clearly distinct from that of insulin (activated Akt phosphorylating FoxO1, thereby sequestering it out of the nucleus and impairing its ability to transcribe IGFBP-1'). Although we are used to thinking of GH as counter-regulatory to insulin, in certain circumstances, like IGFBP-1 expression as shown here, the two hormones can act synergistically because they effect the same molecular change through separate pathways.*

Adda Grimberg, MD

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## GH Neurosecretory Dysfunction and Cranial Irradiation

The group of Shalet in Manchester, UK has made fundamental contributions to the understanding of the broad range of endocrinopathies which may follow cancer therapy in children. In terms of clinical practice, deficiency of growth hormone (GH) following cranial irradiation constitutes an important entity of which all pediatric endocrinologists need to be aware. Prophylactic cranial irradiation for leukemia has been largely replaced by use of intrathecal cytotoxic agents. However, targeted high-dose radiotherapy (RT) for brain tumors outside the hypothalamic pituitary region, such as medulloblastomas, remains an essential and potentially life-saving therapy.

The relationship between the dose of RT and the frequency of subsequent GH deficiency has been clearly established. This article critically considers whether patients who have normal GH responses to pharmacological testing may have a more subtle defect of physiological pulsatile GH release, ie, so-called GH neurosecretory dysfunction. The presence of this 'defect' of probably hypothalamic origin was assumed when subnormal pulsatile secretion was reported

during adolescence, particularly after low-dose RT in several studies.

Darzy KH, Pezzoli SS, Thorner M, Shalet SM. Cranial irradiation and growth hormone neurosecretory dysfunction: A critical appraisal. *J Clin Endocrinol Metab*. 2007;92:1666-72.

**Editor's Comment:** *The combined groups of Shalet and Thorner have performed extremely detailed assessments of physiological GH secretion (cluster analysis) in adult patients, most of whom received RT during childhood, and in normal controls. Such a study would have been impossible in pediatric subjects. The hallmarks of neurosecretory dysfunction, ie, normal GH secretion, after provocation compared with decreased spontaneous secretion were not seen. This helpful finding effectively dismisses this abnormality from potential sequelae of cranial RT in childhood. The peak GH concentration after a pharmacological provocation test can be taken as a realistic index of somatotrope secretory capacity. Performing physiological studies is unlikely to add further clinically relevant information.*

Martin O. Savage, MD

## Growth in Treated Classical Galactosemia Patients

Paris and co-workers studied height and weight growth over a period of 2 years in a group of 40 Dutch children and adolescents with classical galactosemia. These subjects (13 boys, 27 girls, median age 7.8 years, range 3 to 17 years) had the diagnosis established in the neonatal period by galactose-1-phosphate-uridylyltransferase (GALT) and enzymatic studies in erythrocytes. Of the 40 subjects, 31 were prepubertal, and 5 had reached Tanner stage 5. Urinary galactose and galactitol concentrations and GALT levels in the erythrocytes were measured during the study and all were within the

range of treated patients. Prenatal growth was evaluated by obtaining length, weight, and head circumference data from infant welfare centers or from parents. The results, corrected for gestational age, were within normal limits for the Dutch population. Yearly, for 2 successive years, postnatal growth was evaluated by z-scores and corrected for target age. Mean height growth velocity was  $0.87 \pm 1.2$  (range  $-0.4$  to  $3.6$ ) for boys and  $-0.89 \pm 2.1$  (range  $-2.5$  to  $3.7$ ,  $p=0.047$ ) for girls. Weight growth velocity in z-scores was  $0.91 \pm 1.6$  (range  $-0.8$  to  $4.2$ ) for boys and  $-0.74 \pm 1.3$  (range  $-3.1$  to  $2.3$ ,  $p=0.008$ ) for girls. Mean



height in z-scores corrected for target height z-scores was decreased in both genders with girls being more affected than boys. Height velocities were correlated with insulin like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 z-scores and with the height z-scores corrected for target.

The authors affirmed normal prenatal growth in boys and girls with galactosemia, but decreased height and weight growth velocities. In addition they stated that predicted final height was less than target height in most patients after birth. The authors' review of the literature suggested a variety of variable findings in at least 3 other studies, some showed decreased height-for-age but final height within normal limits,<sup>1</sup> microcephaly,<sup>2</sup> and reduced birth weight in affected neonates.<sup>3</sup> The authors speculated that possible risk factors for abnormal growth include either intrinsic or diet-related factors, decreased mean IGF-I and IGFBP-3 concentrations and/or hormonal factors.

Panis B, Gerver W, Rubio-Gozalbo ME. Growth in treated classical galactosemia patients. *Eur J Pediatr*. 2007;166:443-6.

**Editor's Comment:** *Galactosemia may be a more common finding in genetics clinics than in endocrine*

*clinics. The growth data which Panis reported in a large group of children with classical galactosemia would not usually result in a referral to a pediatric endocrinologist for evaluation. It would have been interesting had these investigators provided a little more information especially in regard to how they determined predicted adult height. There is no mention of bone ages being performed in these individuals. It is easy to speculate that girls with galactosemia and ovarian dysfunction would most likely have lower height z-scores than the normal population. Despite its shortcomings, this paper presented important information which suggests when children with classical galactosemia are evaluated in either genetics or metabolic clinics, there should not be an expectation for short stature or failure to thrive, at least when the diet is followed consistently. Thus short children with classical galactosemia should be evaluated thoroughly for other hormonal causes of growth failure.*

William L. Clarke, MD

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## Height Screening During the Primary School Years: Evidence Behind Practice?

Height and weight monitoring has long been a fundamental aspect of pediatric care as indicators of both health and possible underlying pathology. Unfortunately, delays in diagnosis and treatment of underlying growth problems are frequently observed. The optimal strategy remains elusive, as the standard cut-offs between normal and abnormal and the recommended growth screening practices vary widely. For example, the Child Health Subcommittee of the UK National Screening Committee recommended a cut-off of 0.4<sup>th</sup> centile and a single height and weight measurement at or around the time of school entry for screening.<sup>1</sup>

Fayter et al performed a systematic review of the effectiveness and economic modeling of height screening in primary school aged children to identify height-related conditions (focusing on stature, not obesity). They collected all studies from database inception (1974) to July 2005 that measured child height as part of a population-level assessment of children aged 4 to 11 years in Western Europe, North America, Australia and New Zealand (excluding aboriginal populations). All study designs, except case reports, were accepted.

Effectiveness was assessed from the number of cases of all conditions detected. Meta-analysis of diagnostic yield data was precluded by the heterogeneity of child age, reference charts and screening methods used; thus, effectiveness data were limited to descriptive summaries. Twelve studies of height screening programs provided diagnostic yields of new cases and measured 45% to 90%

of eligible children. A single measurement at school entry identified new cases of underlying growth conditions at rates of 0.54 to 0.56 per 1000 children screened.

Economic modeling was based on pooled raw data from 12 diagnostic yield studies, providing probability distributions for new case detection of each included condition. Lifetime costs and outcomes were modeled, following NICE guidelines, and included screening, referral, and treatment costs related to 2006 values. A cost/QALY analysis (a QALY = a year of life, adjusted for its quality or perceived value) compared height screening at school entry (age 5 years) versus no screening (diagnoses found later in clinical practice). QALY estimates, based on the literature and an expert clinical panel, assumed early detection and screening would provide double the QALY gains than later detection from no screening. Using the number of 5-year-old children in England and Wales, the model found an incremental cost-utility of height screening at £9,900 (~\$19,800 US) per QALY. Probabilistic sensitivity analysis found that all of the model's data distributions fell below the UK willingness to pay thresholds of £30,000 per QALY. Thus, the authors concluded that height screening in primary school aged children is diagnostically useful and economically justifiable.

Fayter DA, Nixon J, Hartley S, et al. Effectiveness and cost-effectiveness of height screening programmes during the primary school years: a systematic review. *Arch Dis Child*. 2007 May 2. [Epub ahead of print]



**Editor's Comment:** It is striking that such financial analyses are now needed to justify growth screening, a fundamental tenet of pediatric care. However, as highlighted by this paper, many of the considerations remain elusive. What is the optimal height cut-off to identify likely pathology? What is the optimal screening paradigm? Serial height measurements will capture cases of growth deceleration before they become severe enough to cross the single height cut-off for pathology, but how frequent and how many are needed to balance improved sensitivity with increased cost? What is the actual cost of missed or delayed diagnoses and how are QALYs estimated, especially since the impact of short stature on quality of life remains so controversial? And what about the cost of height monitoring itself? Height

measurements in the United States are performed as part of routine pediatric well child care,<sup>2</sup> and the cost of a stadiometer spread across the patient population is so negligible that it seems virtually free. The only real cost is the time to accurately measure the child and plot the measurements on the appropriate growth chart. With the increasing pressures to expedite patient flow faster and faster, time may be the most expensive aspect of growth screening.

Adda Grimberg, MD

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## Histrelin Subdermal Implant in Central Precocious Puberty

This important article describes efficacy and safety data related to the use of a single annual subcutaneous implantation of a gonadotropin-releasing hormone analogue (GnRHa) to induce pituitary gonadotropin suppression in children with central precocious puberty. Histrelin provides a continuous slow release at an average rate of 65 µg/d of GnRHa. Its use as a single yearly implant has previously been shown to effectively suppress LH, FSH and testosterone secretion in adult males with prostate cancer.<sup>1,2</sup> This report is the first in children with precocious puberty.

The procedure of implantation will require more detailed examination with wider clinical use. A pediatric surgeon is required to perform this procedure and in this study, local or general anesthetic or sedation was used. There is no comment about any practical difficulties with the implantation in terms of interference with daily activities such as sports and recreation, or whether the implant became dislodged in some patients.

Eugster EA, Clarke W, Kletter GB, et al. Efficacy and safety of histrelin subdermal implant in children with central precocious puberty: A multicenter trial. J Clin Endocrinol Metab. 2007;92:1697-704.

**Editor's Comment:** The data on sex steroid, LH and FSH evaluation are impressive and clearly show that effective suppression of gonadotrope function occurs for 12 months after a single subcutaneous implantation. The choice of patients may need to be individualized and an implantation technique which avoids general anesthetic would clearly be preferable. Longer term studies to assess recovery of the pituitary-gonadal axis following discontinuation of treatment are important. This first report in children is encouraging and may eliminate the discomfort of monthly or three-monthly injections as currently practiced.

Martin O. Savage, MD

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## Hypogonadotropic Hypogonadism—Mutations and Phenotypes

Isolated hypogonadotropic hypogonadism (IHH) has been associated with mutations in 7 genes to date (Table). The products of the genes encoded by *KAL1*, *FGFR1*, *PROK2*, *PROKR2*, and *NELF* assist in the regulation of neural movement within the CNS—particularly the migration of olfactory and gonadotropin releasing hormone (GnRH)-containing neurons from the olfactory placode during early embryogenesis. Mutations in these genes result in abnormalities of GnRH secretion and the reproductive endocrine system (delayed adolescence, hypogonadotropism) and the sense of smell (hyposmia, anosmia), and those afflicted often display other neurologic (bimanual synkinesia) and somatic (renal agenesis) anomalies. These traits

are transmitted in an autosomal dominant manner often with incomplete penetrance and substantial inter- and intrafamilial variability in clinical manifestations. Mutations of *GPR54* limit release of GnRH while those of the gonadotropin-releasing hormone receptor (*GNRHR*) impair its function at the gonadotroph membrane. These disorders are transmitted in an autosomal recessive manner and are not associated with other specific clinical or anatomic abnormalities.

Intrigued by the variable clinical manifestations of IHH, Pitteloud and colleagues examined the genotype of 2 families in which single gene defects thought to have resulted in IHH had been previously identified. In pedigree #1, a 21-year-old male with IHH and

Table: Genetic causes of isolated hypogonadotropic hypogonadism.

| Gene   | Locus        | Gene product   | OMIM   |
|--------|--------------|--|--------|
| KAL1   | Xp22.2       | Anosmin (KAL1)                                       | 308700 |
| FGFR1  | 8p11.2-p11.1 | Fibroblast growth factor receptor 1 (KAL2)           | 136350 |
| PROK2  | 3p21.1       | Prokineticin 2 (KAL4)                                | 607002 |
| PROKR2 | 20p13        | Prokineticin receptor 2 (KAL3)                       | 607123 |
| NELF   | 9q34.3       | Nasal embryonic luteinizing hormone-releasing factor | 608137 |
| GPR54  | 19p13.3      | G-protein coupled receptor 54                        | 604161 |
| GNRHR  | 4q21.1       | Gonadotropin-releasing hormone receptor              | 138850 |

hyposmia was initially found to have a heterozygous mutation in *FGFR1* (Ser342Leu—chromosome 8p11.2-p11.1); the proband's father and sister had the same *FGFR1* mutation; the father had delayed onset and the sister normal timing of puberty. In vitro studies demonstrated that the Ser342Leu mutant of *FGFR1* acted in a dominant-negative manner. A heterozygous 8 bp deletion in the negative elongation factor (*NELF*) resulting in a truncated product was later identified in the proband, his mother and his brother; the latter 2 subjects underwent normal puberty. The authors suggested that loss of a single copy of *FGFR1* resulted in a less severe phenotype than did loss of a single copy (allele) of both *FGFR1* and *NELF*. In pedigree #2, two sisters with IHH (no evident spontaneous ovarian function) were found to have inactivating mutations in both *GNRHR* alleles (Gln106Arg, Arg262Gln—chromosome 4q21.2) ie, the sisters had compound heterozygosity. Their father had a history of delayed puberty and carried the Arg262Ser mutation, while

their normal mother carried the Gln106Arg mutation. Further studies revealed that the sisters also had a loss-of-function heterozygous mutation in *FGFR1* (Leu470Arg), an allele that they had inherited each from their father. Thus, these sisters with a severe IHH phenotype were triallelic for this trait. Why the father who was heterozygous for mutations in

both *GNRHR* and *FGFR1* manifested only delayed puberty is uncertain. The investigators concluded that disorders thought to be monogenic in origin and that manifest variable degrees of clinical involvement may actually be oligogenic due to the involvement of 2 (possibly even more) different genes whose mutations sum to produce the clinical phenotype.

Pitteloud N, Quinton R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest.* 2007;117:457-63.

**Editor's Comment:** A gene mutation has been found in only 30% of patients with IHH. Other genes that regulate migration of GnRH neurons and synthesis and release of or response to GnRH await identification. Clearly the concept of digenic inheritance of disease is one that may well be applicable to many disorders of the endocrine and other systems.

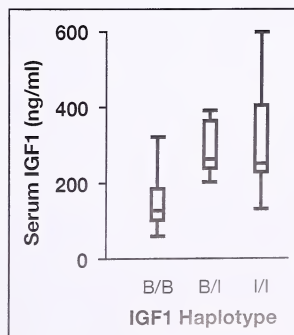
Allen W. Root, MD

## IGF-I Allele in Small Size Dogs

Intrigued by the great diversity in size among the dog family (Canidae), these investigators first identified by genome-wide scan a skeletal size-related quantitative trait locus (QTL) on chromosome 15 within a single breed—the Portuguese water dog (PWD), a breed with great inter-individual variation in size. They next examined the relationship of single-nucleotide polymorphisms (SNPs) within this QTL to skeletal size in large and small Portuguese water dogs. They found one such SNP in this QTL to be associated with size that was near the gene encoding insulin-like growth factor-I (*IGF1*). Designating the haplotypes I and B, the investigators found that Portuguese water dogs homozygous for haplotype I were larger in size and had higher serum IGF-I concentrations than did dogs that were homozygous for haplotype B; they calculated that 15% of the variability of skeletal size within this breed could be accounted for by this *IGF1* haplotype (Figure 1). Performing the same SNP analyses in more size-homogeneous small ( $n=23$ , <9 kg) and giant ( $n=20$ , >30 kg) canid breeds, the authors found skeletal size to be related to an *IGF1* haplotype characterized

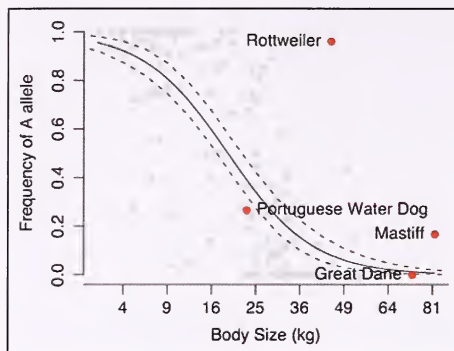
by 20 SNPs that was shared by all small breed dogs (and one in particular designated SNP 5 A) (Figure 2). Sequencing of *IGF1* revealed a SNP in exon 3 and several

**Figure 1.** Serum levels of IGF1 protein (ng/ml) as a function of haplotype. Serum levels of IGF1 protein were assayed in 31 PWDs carrying haplotypes B and I. Box plots show the median (center line in box), first and third quartile (box ends), and maximum and minimum values (whiskers) obtained for each category: homozygous B/B ( $n=15$ ), heterozygous B/I ( $n=7$ ), and homozygous I/I ( $n=9$ ).



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SNPs in flanking genomic sequences (promoter region) and introns that were unique to small breeds but no specific variant related to size was definitely identified.



**Figure 2.** Association of body size and frequency of the SNP 5 A allele. Binomial regression of allele frequency on square root of mean breed mass. Dashed lines indicate the 95% confidence interval on the predicted equation line as estimated from nonparametric bootstrap resampling. Between 5 and 109 (median = 22) dogs were genotyped for each of 143 breeds. The PWD is highlighted in red along with three giant breeds that have larger breed average masses than is predicted by their SNP 5 allele frequency. Reprinted with permission from Sutter NB, et al. *Science*. 2007;316:112-5. Copyright © AAAS 2007. All rights reserved.

The authors concluded that “a narrow ... genomic region holds the variant ... (in *IGF1*) ... responsible for ... size in a disparate set of small ... (and giant) ... dog breeds ...”

Sutter NB, Bustamante CD, Chase K, et al. A single *IGF1* allele is a major determinant of small size in dogs. *Science*. 2007;316:112-15.

**Editor's Comment:** Although previous studies have identified a relationship between serum levels of *IGF-I* in various dog breeds and have been related to growth in humans, the fact that it is tissue and not serum *IGF-I* values that determine growth must be remembered.<sup>1</sup> The findings in this report should in no way be construed or utilized to support the use of recombinant human (*rhIGF-I*) in the treatment of children with idiopathic short stature, a contentious practice.<sup>2</sup> The use of *rhIGF-I* is of limited value in patients with severe *IGF-I* deficiency due to growth hormone (*GH*) resistance due to inactivating mutations of the genes encoding the *GH* receptor or *STAT5* or due to development of neutralizing antibodies to *rhGH*; it is not indicated nor particularly efficacious in other short stature children while exposing them to significant risks.

Allen W. Root, MD

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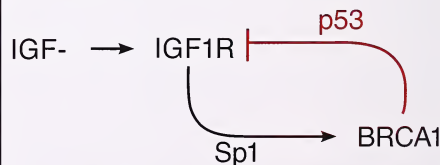
## IGF-I and BRCA1: A New Feedback Loop?

The growth hormone (*GH*)/insulin-like growth factor (*IGF*) system plays an important role in normal breast physiology and carcinogenesis. *GH* receptor (*GH-R*),<sup>1</sup> *IGF-I* and type 1 *IGF* receptor (*IGF1R*) knock-out mice show impaired mammary ductal development from reduced proliferation in the terminal end buds.<sup>2</sup> Conversely, transgenic mice over-expressing human (*h*)*IGF-I* or *hIGF-II* have reduced apoptosis and hence, delayed breast involution that normally occurs with the cessation of suckling and lactation.<sup>2</sup> Further, dysregulated *GH/IGF* signaling has been implicated in breast cancer, a subject extensively reviewed elsewhere.<sup>3,4</sup>

Maor et al therefore sought to investigate the regulatory relationship between gene expression of *IGF1R* and the breast and ovarian cancer susceptibility gene (*BRCA1*), a major tumor suppressor in breast carcinogenesis. As indicated by Western immunoblotting and RT-PCR, *BRCA1* expression was induced by treating MCF-7 breast cancer cells in vitro with *IGF-I* or *IGF-II*. Using *BRCA1* promoter-luciferase reporter constructs, *IGF-I* treatment of MCF-7 and *BRCA1*-null HCC1937 breast cancer cells significantly enhanced promoter activity of the full-length *BRCA1* promoter but not a minimal *BRCA1* promoter deletion construct that lacks binding sites of the transcription factor *Sp1*. *Drosophila*-derived, *Sp1*-null Schneider cells were then co-transfected with the *BRCA1*

reporter construct and an *Sp1* expression vector, which led to an almost 12-fold increase in *BRCA1* promoter activity. Conversely, Mithramycin A, an *Sp1*-inhibitor, inhibited the *IGF-I*-stimulated *BRCA1* expression and promoter activity in MCF-7 cells. Likewise, siRNA against *Sp1* markedly reduced *BRCA1* protein levels in MCF-7 cells. Binding of *Sp1* to the *BRCA1* promoter, as indicated by chromatin immunoprecipitation (*ChIP*) assay, was enhanced by *IGF-I* treatment of the MCF-7 cells. Finally, transfection of an anti-*BRCA1* siRNA, versus a scrambled siRNA, increased the proportion of MCF-7 cells arrested at *SubG0* and reduced those at the *G2/M* phase in response to *IGF-I* treatment.

### A new feedback loop for IGF signaling?



As shown by this paper (black), *IGF1R* signaling induces *BRCA1* gene expression via *Sp1*. As previously shown (red), *BRCA1* represses *IGF1R* via *p53*.



The authors concluded that BRCA1 is a novel downstream target of IGF1R signaling. IGF1R signaling induces BRCA1 gene expression via the Sp1 transcription factor, and BRCA1 gene silencing stunted IGF-stimulated cell cycle progression. Thus, they inferred that aberrant IGF signaling may lead to dysregulated BRCA1 expression during breast cancer pathogenesis.

Maor S, Papa MZ, Yarden RI, et al. Insulin-like growth factor-I controls BRCA1 gene expression through activation of transcription factor Sp1. *Horm Metab Res*. 2007;39:179-85.

**Editor's Comment:** *BRCA1 is major tumor suppressor involved in breast carcinogenesis, including both somatic dysfunction and increased familial cancer risk due to germline inactivating mutations. Normally, BRCA1 plays a role in genomic stabilization, inducing cell cycle arrest and DNA repair in response to DNA damage.<sup>6</sup> BRCA1 acts as transcription factor, interacting with co-repressors and co-activators, to inhibit expression of growth-promoting*

*genes and stimulate expression of cell cycle arrest and DNA repair genes, DNA damage inducible genes and interferon inducible genes.<sup>6</sup> As shown by the same authors as the current paper, one of the genes whose transcription is repressed by BRCA1 is IGF1R.<sup>7</sup> Thus, their 2 findings may form a feedback loop (Figure), whereby IGF1R signaling induces BRCA1 transcription which in turn represses IGF1R transcription.*

Adda Grimberg, MD

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## Intrauterine Growth Retardation and Pituitary Gonadal Function

Low birth weight as a consequence of intrauterine growth retardation (IUGR) is associated with an increased risk of disease in adult life. It has been reported to have a detrimental effect on gonadal development in boys, including cryptorchidism and hypospadias. Little is known on the male pituitary-gonadal axis functioning in adulthood. Small for gestational age (SGA) is a result of IUGR during variable periods of gestation, hence a consequence of different adverse events occurring during gestation. This study focused on fetal growth restraint occurring during the third trimester of pregnancy; the authors hypothesized that IUGR in the third trimester of pregnancy would determine the ultimate male reproductive function. Jensen also evaluated the influence of birth weight in relation to gestational age on the pituitary-testicular axis. Participants were recruited from a large prospective study of pregnant women who provided third trimester fetal growth velocity and birth weight. Fifty-two adolescent males participated in the follow-up study and were divided into appropriate for gestational age (AGA),  $n=32$  and SGA ( $n=20$ ). The authors were careful to avoid major selection bias. Pubertal stage, testicular size, and reproductive hormones were determined, including overnight LH and FSH profiles.

No significant differences were found in testosterone levels, inhibin B levels and LH/testosterone ratio between AGA and SGA. Neither difference was observed between both groups for testicular size and morphology (determined by ultrasonography and overnight secretory patterns of gonadotropins). Median basal LH secretory rates were two-fold higher in men born AGA but the difference did not reach statistical significance. Fetal growth during the third trimester of pregnancy did not influence any of the reproductive

hormone levels nor their secretory pattern as estimated by deconvolution analysis.

This is the first study to explore the influence of the third trimester fetal growth rate on subsequent adult gonadal function. These results do not rule out the gonadal damage in relation to genital malformations as cryptorchidism and hypospadias which also occur in relation with SGA. The testicular function was not impaired in adolescent males born after compromised fetal growth hormones.

Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *J Clin Endocrinol Metab*. 2007;92:1353-7.

**Editor's Comment:** *Most IUGR studies have focused on female reproductive function and have suggested that young women born SGA have reduced ovarian volume, decreased ovarian hormones, and increased number of anovulatory cycles.<sup>1,2</sup> Hyperinsulinemic insulin resistance occurring in these girls is also considered a setting for subsequent development of PCOS in adult women. The rise in FSH levels is greater during infancy in both boys and girls born SGA, whereas inhibin B levels are similar to those in infants born AGA. In adolescent males there is only limited information suggesting impaired spermatogenesis. In only one clinical study<sup>3</sup> of males, a significantly decreased testosterone secretion and elevated LH levels were reported, suggesting primary testicular failure in men born SGA. In 54% of those subjects, a mean testicular volume  $>2$  SD below the control mean, with reduced inhibin B was detected; the authors considered that their data supported a link between low birth weight and reduced fertility in males born SGA. The*



presence of cryptorchidism in several cases might have played a role in the data they presented.<sup>3</sup> The present study provided no evidence for impaired testicular function. It may mean that whatever its cause, late fetal growth restraint is not associated with testicular dysfunction, hence there is a risk of subfertility. In a recent review<sup>4</sup> the limitation of information in this area has been stressed, yet many reports have dealt with connected issues such as cryptorchidism, testicular cancer, and hypospadias.

A working hypothesis would be that males with early fetal growth restraint, generally resulting in symmetric SGA, would be at greater risk. Developmental factors would play

a role at this early phase of fetal growth. It would require new prospective studies in a setting similar to that reported in this paper to elucidate this hypothesis.

Raphaël Rappaport, MD

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## Jeune Syndrome: Defective Intracellular Flagellar Transport

Major advancements have been made in recent years in identifying gene loci that harbor mutations responsible for human genetic disease. In many, if not most instances, the studies have begun with delineating the disease, then progressing to linkage analysis and other approaches, which eventually lead to the relevant gene locus and the mutations. In the paper discussed here, however, the authors began with a disturbance of gene function and used a bioinformatics approach to find the disease.

More specifically, Beales et al were interested in disturbances of ciliary function, the ciliopathies. Several disorders including Bardet-Biedl, oral-facial-digital type 2, Joubert, Senior-Löken, and Meckel-Gruber syndromes have recently been assigned to this group. The authors questioned if a set of minimum clinical criteria could be used to predict additional ciliopathies. After compiling a list of overlapping features, they queried the London Dysmorphology Database, which yielded a list of 10 features that would potentially predict a ciliopathy. The features included retinitis pigmentosa, polydactyly, renal cystic disease, and situs inversus. When these were ranked and used to query the database again, 25 conditions were identified as possible ciliopathies, among which was Jeune syndrome, often referred to as asphyxiating thoracic dysplasia (ATD), OMIM 208500.

ATD is an autosomal recessive bone dysplasia characterized by limb shortening, constricted thoracic cage and respiratory insufficiency in infancy. Other features often include polydactyly, cystic renal disease, and retinal degeneration. ATD is known to be genetically

heterogeneous with one locus at chromosome 15q13. The authors ascertained and studied 3 families with linkage to a second locus at chromosome 3q24-3q26. One of the candidate genes in this region encodes WDR56, a protein that has been identified originally as expressed in *C. elegans* ciliary cells. Mutation analysis revealed a single amino acid deletion and 2 missense mutations in the 3 ATD families. Additional mutations were not detected in other patients with ATD and none of the patients with ATD who had WDR56 mutations exhibited extraskelletal manifestations of ATD.

WDR56 is conserved across species and has been renamed IFT80. It encodes a component of intraflagellar transport complex B and is essential for development and maintenance of motile and sensory cilia. To investigate its function further, the authors "knocked down" its expression in developing zebrafish. The treatment disturbed tail, kidney, and heart development and was consistent with a disturbance of hedgehog signaling in the developing fish. The authors suggested that their bioinformatics approach may lead to identification of other ciliopathies.

Beales PL, Bland E, Tobin JL, et al. IFT80, which encodes a conserved intraflagellar transport protein, is mutated in Jeune asphyxiating thoracic dystrophy. *Nat Genet.* 2007;39:727-9.

**Editor's Comment:** Clinicians value the London Dysmorphology Database for its diagnostic utility. This paper demonstrates another use that could be applied to other clinical phenotypes.

William A. Horton, MD

## Natural History of Noonan Syndrome

One-hundred and fifty-one subjects with Noonan syndrome from 123 families were recruited into the Noonan Syndrome Research Group at St George's University of London Hospital between 1989 and 1991. Between 2001 and 2003 all families were invited to participate in a follow-up assessment which included clinical examination, echocardiography, three-dimensional

digital facial photography and analysis of the *PTPN11* gene. Of the 151 patients, 34 dropped out of the study and 10 (6.6%) died. The final study cohort comprised 112 individuals (57 males) from 92 families. Seventy of these were fully assessed and 32 partially assessed. The mean age at assessment was 25.3 years and the mean interval for follow-up was 12 years.

*PTPN* analysis was done in 79 individuals; mutations were found in 35%. The mutations were more likely to be found in familial cases (50%).

Height SDS at entry into the study was  $-2.184$  and at follow-up was  $-1.755$ . Twelve individuals received growth hormone (GH) treatment. There were no statistical differences in the height SDS between those who received and those who had not received GH therapy. Those with *PTPN* mutations had similar mean height SDS. Final adult height was 167.4 cm (males) and 152.7 cm (females). When the individuals who received GH treatment were excluded, the mean final height increased to 169.8 cm (males) and 153.3 cm (females) as those who received GH were shorter. The final height in those with the *PTPN11* mutation was about 4 cm less than the others. Pulmonary stenosis was present in 65% and more prevalent in those with the mutation. No intervention was required in 58% of subjects. Hypertrophic cardiomyopathy was present in 19%; 9 of these subjects also had pulmonary stenosis. Five individuals died from complications related to hypertrophic cardiomyopathy and one person had a cardiac transplant. Feeding difficulties at ascertainment were common; some were associated with developmental speech delay. Approximately 73% of these individuals attended mainstream schools while 27% attended schools for children with learning disabilities (the mutation was equally distributed between the 2 groups); 16% had achieved higher education (this compares with 25% of the UK population). Sixty percent were full-time employed, while 26% were registered as disabled. Orthodontic work had been performed on 51%. Six percent of the individuals had required hormone injections to induce puberty. Puberty was somewhat delayed, starting at 14.5 years (males) and 14 years (females). Of those individuals who attempted to have children, 67% experienced no problems. Easy bruising or bleeding was seen in about 79%, but not associated with any known coagulopathy; prevalence of the *PTPN* mutation was higher in those with a history of easy bruising. Refractive errors were seen in 71% of the individuals. Lymphedema affected the lower limbs of 2, and scoliosis was present in 13%. Approximately 13% had recurrent seizures; *PTPN11* mutations were identified in 2 of those. All subjects, with the exception of 4, had normal hearing at follow-up.

The authors stated that their longitudinal follow-up was one of the largest databases on well-characterized Noonan syndrome. However, they have some potential bias in follow-up data because of the individuals who had dropped out. They also pointed out that they could not correlate the *PTPN* mutations with short stature as others have demonstrated. They found that about 10% of the subjects with mutations had hypertrophic cardiomyopathy.

Shaw AC, Kalidas K, Crosby AH, Jeffery S, Patton MA. The natural history of Noonan syndrome: a long-term follow-up study. *Arch Dis Child*. 2007;92:128-32.

**Editor's Comment:** Shaw and colleagues are to be congratulated in following a large group of individuals with Noonan syndrome from childhood through adulthood and final height. Their data do not confirm the data of others with regard to mutations and short stature. Studies from France, Brazil, and Germany have demonstrated different findings with regard to Noonan syndrome. Limal et al<sup>1</sup> showed that individuals with the *PTPN11* mutation have poor growth and do not respond to GH administration as well as those without the mutation. Ferreira et al<sup>2</sup> have also shown reduced GH response to long-term GH treatment. Finally, Binder et al<sup>3</sup> have shown that those with *SHP-2* mutation have mild GH resistance and also poor GH response. Thus it is not surprising that the individuals treated with GH in the current longitudinal study, although shorter, also ended up shorter than those who had not been treated. Other studies will be needed in order to determine whether higher doses of GH or insulin like growth-factor (IGF)-I treatment<sup>4</sup> may enhance final height of those children with Noonan syndrome who have the *PTPN11* mutation.

William L. Clarke, MD

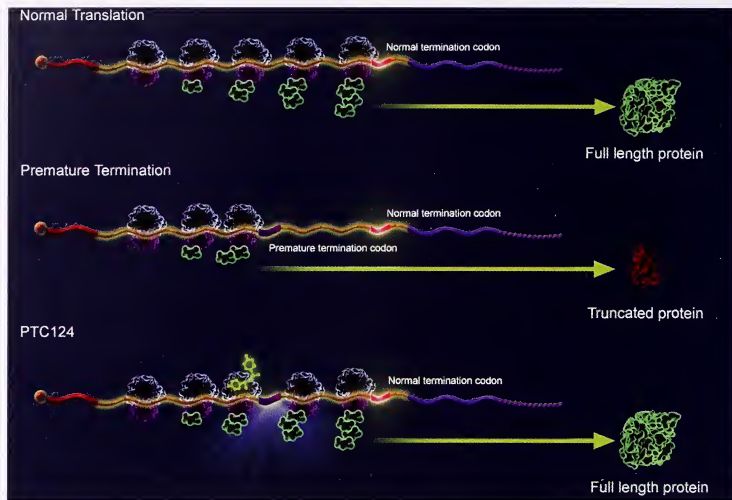
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## Nonsense Mutations in Genetic Disease—A Novel Treatment

Nonsense mutations are a common cause of human genetic disease. They give rise to in-frame premature translation termination or stop codons within the coding regions of genes and lead to truncated protein translation products that are typically nonfunctional and also promote mRNA destruction by so-called nonsense-mediated mRNA decay (NMD). The idea of developing pharmacologic means to induce a cell's translation machinery to readthrough premature termination

codons (PTCs) has been around for some time, and there is evidence that the antibiotic gentamicin prompts ribosomes to readthrough PTCs to generate full-length proteins (Figure). In fact, gentamicin has received attention in this context and preliminary trials have been carried out in patients with Duchenne muscular dystrophy (DMD) and cystic fibrosis due to mutations that introduce PTCs. However, the high doses that are required, potential for renal and otic toxicity and



Translation of an mRNA into protein: comparison of normal translation, premature translation termination, and treatment with PTC124 restoring synthesis of full-length protein. Reprinted with permission from PTC Therapeutics.

need for intravenous or intramuscular administration of gentamicin have limited its potential usefulness for treatment of human diseases. A new compound has now been identified that appears to suppress PTCs with fewer potential problems.

Welch et al utilized high-throughput screening of ~800,000 compounds to identify small molecules that would suppress PTCs. One compound designated PTC124 promoted dose-dependent readthrough of PTCs, including human and mouse nonsense alleles of the dystrophin gene. Compared to gentamicin, PTC124 was effective at much lower doses and it could be delivered orally. After documenting an increase in dystrophin protein levels in primary muscle cell cultures, they then treated *mdx* mice, a mouse model of DMD due to a mutation-induced PTC in the dystrophin gene.

PTC124 treatment led to partial rescue of the muscle disturbance in the *mdx* mice. The most notable functional result was protection against susceptibility to contraction-induced injury. This injury, which is a typical feature of the *mdx* mouse and most likely occurs in DMD patients, involves repeated cycles of degeneration—regeneration, inflammation, and necrosis that eventually leads to muscle destruction. Susceptibility to this injury for mice treated with PTC124 was no different than for wild-type mice.

*Mdx* mice treated with PTC124 for 8 weeks showed significant decreases in serum creatinine kinase levels compared to untreated controls. Their muscle tissues displayed increased levels of dystrophin protein as well as  $\gamma$ -sarcoglycan consistent with production and stabilization of the dystrophin-associated membrane complex

that is missing in the absence of dystrophin. Drug treatment also led to a partial restoration of dystrophin to the membrane of skeletal muscles, although the relative amount appeared to be less than in wild type mice.

The authors concluded that PTC124 is a more potent nonsense mutation suppressing agent than gentamicin. They attribute its effect to directly suppressing premature termination during translation rather than to interference with NMD. Importantly, they also provided evidence that it does not affect the function of normal

termination codons. The authors suggested that through increasing the efficiency of translation, PTC124 may benefit patients with genetic diseases due to nonsense mutations. An accompanying news and views comment indicated that Phase II clinical trials are underway for PTC124 in DMD and cystic fibrosis.

Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature*. 2007;447:87-93.

Schmitz A, Famulok M. Chemical biology: ignore the nonsense. *Nature*. 2007;447:42-3.

**Editor's Comment:** The research described in this paper could have significant impact on the treatment of a subset of genetic disease. It reflects a marriage between so called chemical biology, which seeks to identify small molecules that produce desired therapeutic effects on disease processes, and continued efforts to understand the molecular consequences of mutations. It underscores an importance of DNA diagnoses.

The paper raises the concern that suppressing PTCs would lead to synthesis of mutant proteins. In many instances such as enzymopathies and disorders in which structural proteins serve as platforms for or link together cellular machinery, such as in DMD, having more protein even if it harbors a mutation, would seem beneficial. However, there may also be instances where having no protein is better for a cell or a tissue than having a mutant protein that adversely affects other normal molecules.

William A. Horton, MD



## Premature Menopause in Survivors of Childhood Cancer

Female childhood cancer survivors (CCS) who retain ovarian function after completing cancer treatment are at increased risk of developing premature menopause, defined as cessation of menses before age 40 years. However, the data regarding premature menopause in female CCS are scanty, although particular attention should be also paid to other endocrine alterations, and neurocognitive and neurobehavioral problems.

Sklar and colleagues assessed the incidence of and risk factors for premature menopause in 2819 CCS females older than 18 years of age who continued to menstruate for at least 5 years after their cancer diagnosis. The group was composed of control participants in the multicenter Childhood Cancer Survivor Study (CCSS), including 1065 female siblings of participants in the CCSS. Female CCS patients who received more than 30 Gy of radiation to the brain and/or had a primary tumor in the region of the hypothalamus-pituitary gland (known to cause hypogonadotropic hypogonadism) were excluded. Of 2819 subjects, 1025 had leukemia, 404 Hodgkin's lymphomas, 324 bone tumors, 297 kidney tumors, 271 sarcomas, 207 neuroblastomas, 154 non-Hodgkin's lymphomas, and 137 brain tumors. The comparison group was 1065 female siblings of participants in the CCSS. A total of 126 CCS and 32 control siblings developed nonsurgical premature menopause. The cumulative incidence of nonsurgical premature menopause was higher for CCS than control siblings (8% vs 0.8%; RR=13.21, 95% CI=3.26 to 53.51;  $P<0.001$ ). A multiple Poisson regression model showed that risk factors for nonsurgical premature menopause included attained age, exposure to increasing dose of radiation to the ovaries, increasing alkylating agent score (based on number of alkylating agents and cumulative dose), and a diagnosis of Hodgkin's lymphoma. For female CCS subjects who were treated with alkylating agents plus abdominopelvic radiation, the cumulative incidence of nonsurgical menopause approached 30%. The results of this study will facilitate counseling current female CCS about their future risk of premature menopause and aid in designing new regimens that seek to diminish late ovarian toxicity.

Sklar CA, Mertens AC, Mitby P, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. *J Natl Cancer Inst.* 2006;98:890-6.

**Editor's Comment:** This is a very interesting observational study which provides important information for physicians who care for female CCS patients. Because survival rates of cancer patients have improved markedly in recent years, the long-term complications and late effects, such as endocrine impairments and neuropsychological problems, have become increasingly important even after many years following the conclusion of treatment. The interest in the late effects of ovarian function, especially of acute ovarian failure, premature menopause and fertility,

has increased over time.

Acute ovarian failure (AOF), defined as never menstruating or premature menopause within 5 years of diagnosis of childhood cancer, is known to develop in female CCS. Chemaitilly et al<sup>1</sup> studied AOF in 3390 eligible female CCS in the CCSS. In this group, 215 patients (6.3%) developed AOF. Survivors who received cranial irradiation at doses of more than 30 Gy, those with hypothalamic/pituitary tumors, and survivors who underwent bilateral oophorectomy were excluded. Survivors with AOF were older at diagnosis and more likely to have been diagnosed with Hodgkin's lymphoma or to have received abdominal or pelvic radiotherapy than survivors without AOF. Among survivors with AOF, 116 (54%) had received at least 10 Gy ovarian irradiation. In a multivariable logistic regression model, increasing doses of ovarian irradiation, exposure to procarbazine, and exposure to cyclophosphamide at ages 13 to 20 years were independent risk factors for AOF.

Concerning premature menopause in CCS, Sklar and colleagues studied a total of 2819 subjects. Median age at cancer diagnosis was 7 years (range 0 to 20), and median age at study was 29 years (range 18 to 50), 69% of survivors had reached age 25 years, 47% reached age 30 years, 26% attained age 35 years, 10% were age 40 years, and 8% were older than 40 years of age. The results of their study indicated that the risk of developing nonsurgical premature menopause was 13-fold higher than that of siblings, with cumulative incidence of 8% by 40 years of age. The risk factors for nonsurgical premature menopause are: attained age, diagnosis of Hodgkin's lymphoma, and exposure to increasing doses of both alkylating agents and radiation to the ovaries.

Premature menopause and AOF leads to the early and often unexpected loss of reproduction potential as well as the cessation of ovarian sex hormone production. Thus, survivors who experience AOF or premature menopause are at increased risk of developing a variety of adverse health outcomes, including osteoporosis, cardiovascular disease, and psychosexual dysfunction.

Their results have confirmed that treatment of female childhood cancer is associated with a considerable risk of developing AOF and premature menopause. Therefore, it is necessary to inform young adult female CCS patients who are still menstruating, about the risk factors of premature menopause (ie, attained age, Hodgkin's lymphoma, chemotherapy with alkylating agents, and radiation to the ovaries) to facilitate family planning and timing of future pregnancies. It is also necessary for physicians as well as patients and family members to know that premature menopause can occur even after several years following childhood cancer treatment.

Yoshikazu Nishi, MD

### Reference

1. Chemaitilly W, Mertens AC, Mitby P, et al. *J Clin Endocrinol Metab.* 2006;91:1723-8.



## SHOX Haploinsufficiency—Clinical Indicators

Mutations in the short stature homeobox-containing gene (*SHOX*) are one of the more frequent genetic causes of growth retardation in individuals with short stature. *SHOX* is therefore an important mediator of linear growth, presenting with marked disorganization of chondrocyte proliferation. There is also a dose dependent association between the number of copies of this gene and height. In addition, *SHOX* deficiency includes a continuum of clinical conditions spanning from the severe Langer syndrome with no functional copy of the *SHOX* gene to the Leri-Weill syndrome (LWS) and isolated idiopathic short stature (ISS) without dysmorphic features and haploinsufficiency of the *SHOX* gene. The aim of this study was to determine the phenotypical spectrum of *SHOX* deficiency in a large cohort of children with short stature and to propose a scoring system to select patients who qualify for diagnostic *SHOX* molecular testing.

A cohort of 1608 unrelated prepubertal children with short stature was studied. Investigators were requested to report the presence or absence of a number of dysmorphic signs often observed in LWS. In addition, for sake of comparison, a group of Turner syndrome patients ( $n=33$ ) was included as this condition also presents one absent *SHOX* allele. *SHOX* defects were identified in 68 short stature participants (4.2%) and in 34 ISS patients (2.2%). LWS was reported in 55 participants (3.4%).

For LWS patients, the presence of a *SHOX* defect did not induce a greater frequency of dysmorphic signs. For ISS children, a number of physical signs (shortening or bowing of the forearm, Madelung deformity [dinner fork-like deformity of the wrist], dislocation of the ulna of the elbow, high-arched palate, bowing of the tibia and appearance of muscular hypertrophy) were clinical indicators for *SHOX* deficiency. In addition, ISS patients presented a significantly greater BMI SDS than those without the molecular defect. They also they had disproportion and appearance of muscular hypertrophy; ISS with *SHOX* deficiency had a significantly greater sitting height and forearm, upper arm and upper leg circumference adjusted for standing height.

The variability of the phenotype makes it difficult to decide molecular testing. Therefore various logistic regression models were developed. The diagnosis of LWS or the presence of Madelung deformity resulted in very high odds ratios. Therefore the study focused on non-syndromic ISS, and these strong indicators were excluded from the models. A scoring system was presented (Table) using rounded odds ratios as weighted score points to help identify patients who qualify for *SHOX* gene testing based on clinical criteria. These criteria included 3 anthropomorphic variables (arm span/height, sitting height/height, and BMI) and 5 dysmorphic signs (cubitus

valgus, short forearm, bowing of forearm, appearance of muscular hypertrophy, and dislocation of the ulna at the elbow). Testing was recommended for patients having a score greater than 4 or 7 out of a total possible score of 24, a range that allows a better sensitivity.

This study provided clinical guidelines for testing of the *SHOX* gene. The most reliable clinical indicators of *SHOX* deficiency were related to disproportionate growth. The clinical findings were present in a significant number of ISS phenotype patients as well as in those with LWS. These diagnoses represent a continuum rather than discrete entities. However, there was no clear correlation between the specific *SHOX* gene defect and the clinical features.

Rappold G, Blum WF, Shavrikova EP, et al. Genotypes and phenotypes in children with short stature: clinical indicators of *SHOX* haploinsufficiency. *J Med Genet.* 2007;44:306-13.

**Editor's Comment:** *SHOX* haploinsufficiency is an important genetic cause of short stature associated with well recognized dysmorphic signs. Several studies have already reported on this clinical phenotype. The present report provided new data prospectively established from a large group of patients with short stature. This analysis should help to identify the large continuum of phenotypes related to *SHOX* haploinsufficiency among the various types of short stature. The gene-phenotype relationship is not a simple one, as the authors found no difference in the degree of short stature between the children with or without an identifiable *SHOX* defect (according to the molecular methods applied in this protocol). Furthermore, among short stature patients with a clinical diagnosis of LWS the frequency of dysmorphic signs was not different between children with a *SHOX* defect and those without an intragenic mutation of the *SHOX* gene. Other genetic defects, not investigated in this study, might have been missed: intragenic mutations located in regulatory regions of

**Scoring system for identifying patients that qualify for short-stature homeobox containing gene (*SHOX*) testing based on clinical criteria**

| Score item                         | Criterion        | Score points |
|------------------------------------|------------------|--------------|
| Arm span/height ratio              | <96.5%           | 2            |
| Sitting height/height ratio        | >55.5%           | 2            |
| Body-mass index                    | >50th percentile | 4            |
| Cubitus valgus                     | Yes              | 2            |
| Short forearm                      | Yes              | 3            |
| Bowing of forearm                  | Yes              | 3            |
| Appearance of muscular hypertrophy | Yes              | 3            |
| Dislocation of ulna (at elbow)     | Yes              | 5            |
| Total                              |                  | 24           |

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the gene, or defects at a different, but related, genetic locus. It is of interest that, for an unknown reason, BMI is increased in those patients with muscular hypertrophy. This finding is in contrast with the frequency of low to low-normal BMI values in children with ISS. A recent report<sup>1</sup> showed that similar stimulation of growth was obtained in ISS with SHOX insufficiency and in a group of girls with Turner syndrome who received growth

hormone treatment of 50 µg/kg/day. This study also showed the importance of carefully analyzed familial histories using clinical scoring and radiographic examination of the forearm and hand.

Raphaël Rappaport, MD

## Reference

1. Blum WF, Crowe BJ, Quigley CA, et al. J Clin Endocrinol Metab. 2007;92:219-28.

## Stem Cells: A New Kind of Breakthrough

Stem cells have received much attention in recent years because of their potential to regenerate damaged and diseased tissues. Two types of stem cells have been distinguished historically—embryonic stem cells (ESC) and adult stem cells. Because of the potential to differentiate into any cell type, pluripotentiality, the former have more potential in regenerative medicine than the latter. In fact, this principle is illustrated well in knock-in and knock-out mice in which new mouse strains are generated from ESC into which mutations have been introduced. However, serious ethical issues are raised in human ESC research since until now, they could only be obtained from human embryos. Moreover, if this technology is to be applied to adult disease, sometimes referred to as custom transplantation therapy, means must be developed to produce cells equivalent to ESC from the patient needing treatment, which has raised controversial issues of human cloning with its own set of ethical concerns. Attempts to convert easily accessible cells such as fibroblasts to ESC-like cells have been unsuccessful until now. But 3 papers have recently been published which signal a major breakthrough in the field.

The recent story starts with the realization that converting somatic cells to ESC-like cells requires nuclear or epigenetic reprogramming of the cells, ie, resetting of DNA methylation, histone modification and chromatin structure, to that of ESC. Last year a Japanese group headed by Yamanaka<sup>1</sup> generated ESC-like cells from mouse embryonic fibroblasts by expressing 4 transcription factors (Oct3/4, Sox2, c-myc and Klf 4) and subsequently selecting cells that expressed another transcription factor, Fbx15. The concept was that the 4 transcription factors would trigger expression of genes highlighted by Fbx15 that induced the pluripotent state, and the cells were termed induced pluripotent stem cells or iPS cells. While these cells exhibited many stem cell properties, they were not fully reprogrammed epigenetically and did not produce chimeras when injected into mouse embryos. Chimeras are mice containing cells from the recipient mouse embryo and cells from a donor source—iPS cells in this case.

Technical modifications have now led to a second generation of mouse iPS cells with properties that more closely approximate those of ESC, including the ability to produce chimeras in the next generation of mice. The work was reported by Okita et al, Wernig et al and

Maherali et al. Although each group differed in certain methodologic details, their common protocol started as before, but utilized different transcription factors—Nanog and Oct4—to identify and isolate iPS cells.

The second generation iPS cells possessed an epigenetic signature remarkably similar to ESC; they differentiated into cells of different lineages and germ layers and all 3 groups were able to establish chimeras and in 2 cases germ-line transmission in the next generation, the most stringent evidence of developmental potency.

Two concerns were raised relative to the potential use of this technology in humans. One is the development of tumors in nearly 15% of mice derived from iPS cells. This risk was attributed to expression of c-myc, which is a known oncogene and possibly reactivation of its expression at a later time. The second and related issue is the use of retroviral vectors to introduce the transcription factors that trigger nuclear reprogramming. They may predispose to oncogenesis through insertional mutagenesis as well. However, one of the conclusions from the studies is that the triggering mechanism may require only transient expression of the transcription factors, ie, once initiated, reprogramming may drive itself. If so, then transient expression, perhaps by using adenoviral vectors with less risk of problems, may suffice. All groups acknowledge that application of this technology to humans is still some time away.

Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007 [epub ahead of print].

Wernig M, Meissner A, Foreman R, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 2007 [epub ahead of print].

Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*. 2007;1:55-70.

**Editor's Comment:** *These reports are very encouraging and give regenerative medicine a major boost.<sup>2</sup> But as stated repeatedly, more research will be needed to translate this breakthrough to the clinic. Nevertheless, the findings and especially that the three groups are able to confirm each other's results are very exciting.*

William A. Horton, MD

## References

1. Takahashi K, Yamanaka S. *Cell*. 2006;126:663-76.
2. Cyranoski D. *Nature*. 2007;447:618-9.

## Transition to Adulthood of Growth Hormone Deficient Children

This "Approach to the Patient" review presented a discussion on continuing care of growth hormone deficient (GHD) patients who have attained final adult height and have low bone mineral density, abnormal lipids, and impaired cardiac function. Radovick and DiVall delineated when to retest, how to test, when to treat, and how to treat. In their conclusion they recommended the establishment of specialized clinics "to improve compliance and follow-up during the transition to adult services."

Radovick S, DiVall S. Approach to the growth hormone deficient child during transition to adulthood. *J Clin Endocrinol Metab.* 2007;92:1195-200.

**Editor's Comment:** *This is an extremely detailed article addressing the important issues of transitional care of the GHD deficient patient following completion of linear growth. I cannot fault the careful objective analysis of the published literature and the recommendations are sensible and logically argued. However, this article emphasizes the very wide transatlantic divide in the approach to this subject.*

*The authors described the clinical situation of the care of a GHD 17-year-old patient as being "relatively new to pediatric endocrinologists." The entity of the adult GHD syndrome and evidence of beneficial GH replacement therapy in adult hypopituitary patients was established in the late 1980s. That is nearly 20 years ago. Active transitional care programs have been operating in Europe, admittedly more so in northern rather than*

*southern parts of the continent, for at least 10 years.*

*The challenge is to get pediatric and adult endocrinologists to work together to improve care of the patient with hypopituitarism who has completed growth. With this aim, a consensus meeting, organized jointly by ESPE, LWPES, and the GRS (Manchester, UK, December 2003) and its consensus statement<sup>1</sup> was published in 2005. This statement, authored by an equal number of pediatric and adult endocrinologists discussed all the questions raised in the present review. However the Clayton et al paper is not even referenced!*

*The Radovick and DiVall review is written very much for the practicing pediatric endocrinologist. This again points to a difference in approach to the care of the young adult with GHD. The consensus view, as previously published,<sup>1</sup> is that care should be transferred, ie, the patient should be transitioned to the adult endocrine service. In this way, GH retesting using an insulin tolerance test (ITT), which is recognized to be the best test of adult GHD, can be performed in a safe adult environment and the decision whether to continue GH therapy in a young adult, can be taken, logically, by an adult endocrinologist.*

*I make a plea for transatlantic co-operation. We have a great deal to learn from each other.*

Martin O. Savage, MD

### Reference

1. Clayton PE, Cuneo RC, Juul A, et al. *Eur J Endocrinol.* 2005;152:165-70.

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## SF1 MUTATION IN HUMANS

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### INTRODUCTION

The purpose of this lead article is to bring readers up to date on the phenotypes, genotypes, and pathogenesis of the steroidogenic factor (SF)1 mutation that pediatric endocrinologists encounter in their practices and to provide new insights into SF1 function in humans. Steroidogenic factor 1 (Sf1 in mice or SF1 in humans), also called Ad4BP or NR5A1, is a nuclear transcriptional factor that binds to target gene promoters as a monomer and recognizes a canonical half-site motif. Structurally, both Sf1 and SF1 have characteristic domains

of nuclear transcriptional factors. These consist of a zinc finger DNA-binding domain, a ligand-binding domain, and an activation function-2 domain. There is also an accessory DNA-binding domain that confers binding site stability and specificity.

Originally, SF1 was isolated as a global regulator for P450 steroid hydroxylases.<sup>1,2</sup> SF1 was thought to be responsible for tissue-specific expression of these enzymes in the adrenals and gonads. Subsequent studies in vitro have shown that Sf1 and SF1 regulate a lot of genes involved in adrenal and gonadal development, sex differentiation, steroidogenesis, reproduction, and many other metabolic functions.<sup>2,3</sup> Thus, Sf1 and SF1 play pivotal roles in the development and function of multiple endocrine organs.

### From The Editor's Desk

This issue of GGH Volume 24, Number 1 is only available on-line and will be not be printed and mailed due to budgetary constraints. However this issue is available either as a PDF file or a web page so you can file it and/or print it and keep it for your enjoyment and as a reference resource.

The current issue includes an excellent and timely review of the "SF1 Mutations in Humans" by Dr. Tomonobu Hasegawa, plus 19 reviews of current papers in the literature with comments by the editorial board. There are four reviews pertaining to growth hormone treatment including the consensus guidelines of adult growth hormone deficiency, two addressing growth of celiac patients, three pertaining to height related issues on quality of life, the in vitro fertilization children or the genetics of stature. There are also two reviews regarding the aortic dilatation and the uterine development of Turner patients. In addition the late effects of cancer survivors, hypopituitarism following traumatic brain injury, and diabetes and stroke in hypopituitarism are also reviewed. I also want to bring to your attention the reviews on the FTO gene in obesity and the monoallelic expression of autosomal genes. Finally there are two reviews of papers dealing with two frequent alterations in pediatric endocrine practices, namely metabolic syndrome in brothers of PCOS women and the ventricular function of congenital hypothyroidism in neonates.

The economic situation in the country is being reflected in our journal. The reduced funding for continuous medical education will only allow us to publish two electronic issues in 2008, unless there is a renewed commitment for sponsorships that will allow us to provide our readers with a high quality journal more frequently. We will continue to search for means and will appreciate your tax deductible contributions. You may do so on line ([www.GGHjournal.com](http://www.GGHjournal.com) or [PedsAcademics.org](http://PedsAcademics.org)) and click *make a donation*, or you may send a check to Pediatric Sunshine Academics, 1040 Alston Rd., Santa Barbara, CA 93108.

Thank you for your support,  
Fima Lifshitz, MD  
Editor-in-Chief



The murine Sf1 also orchestrates the development and function of multiple endocrine organs *in vivo*, judging from the striking, but complex phenotypes of its knockout mice. The Sf1 knockout mice showed adrenal and gonadal agenesis, impaired function of pituitary gonadotropes, and structural abnormalities of ventromedial hypothalamic nucleus (VMH).<sup>2,4</sup> All knockout mice died within 2 weeks due to adrenal insufficiency. Moreover, recently established tissue- or cell-specific Sf1 knockout mice clearly demonstrated *in vivo* the direct and pivotal function of Sf1 in Leydig cells, granulosa cells, pituitary gonadotropes, and VMH.<sup>5-7</sup>

### PHENOTYPES, GENOTYPES, AND PATHOGENESIS OF SF1 MUTATION IN HUMANS

The critical role of murine Sf1 *in vivo* strongly suggests the importance of SF1 in humans, prompting endocrinologists to identify patients with SF1 mutations. Initially, the rare 46,XY patients that showed severe gonadal dysgenesis together with primary adrenal failure were the main focus to identify SF1 mutations. These alterations were analogous to the phenotypes of the knockout mice. Indeed, the first described human patient with SF1 mutation (a heterozygous G35E) was a 46,XY female who presented with primary adrenal failure in the first 2 weeks of life; she had a vascular collapse at 17 days of age. The phenotype of this patient was similar to those seen in Sf1 knockout mice, albeit less severe. This patient's serum cortisol was 1.2 g/dL and aldosterone was 5.0 ng/dL, both of which were quite low considering the clinical condition, together with a high plasma ACTH (1,165 pg/mL). She had been treated with glucocorticoids

and mineralocorticoids. Before the induction of puberty as female, her pituitary gonadotropins responded to GnRH stimulation test: LH (1.2 → 8.6 mIU/mL) and FSH (17.8 → 38.0 mIU/mL). No response of testosterone was observed by hCG stimulation test. At laparotomy, normal Mullerian structures and streak-like gonads were found. Histological examination of the gonads showed poorly differentiated tubules and connective tissue. Mutation analysis of SF1 revealed a heterozygous mutation in the proximal box (P-box) of the first zinc finger of SF1. The P-box is important for the recognition of DNA binding and confers specificity to nuclear receptors in the regulation of target genes. The mutant SF1 protein did not bind to a canonical SF1 binding site, did not transactivate the SF1 responsible gene, and did not exhibit dominant-negative effects.<sup>8</sup>

The next reported 46,XY patient with SF1 mutation (homozygous R92Q) was a normal female baby who presented one day after birth with a hypoglycemic convulsion due to primary adrenal failure.<sup>9</sup> Thereafter, the phenotypic spectrum of the SF1 mutation in humans has been strikingly expanded.<sup>10-18</sup> The phenotypes, genotypes, and pathogenesis of SF1 mutation in humans reported to date are summarized in Tables 1-3. A number of "milder form" 46,XY patients have also been reported. These patients had 46,XY disorders of sex development (DSD), namely, testicular dysgenesis (or impaired androgen production) with normal adrenal function. Six 46,XX subjects have been reported, all of whom had seemingly normal ovarian development and function; one out of the 6 had primary adrenal failure.

**Table 1. Reported Cases of SF1 Mutation in Humans**

| Case                                      | 1                     | 2             | 3                              | 4                              | 5                     | 6             |
|---|-----------------------|---------------|--------------------------------|--------------------------------|-----------------------|---------------|
| Age (years)                               | 20                    | newborn       | 31                             | 6                              | 27                    | newborn       |
| Karyotype                                 | 46,XY                 | 46,XY         | 46,XY                          | 46,XY                          | 46,XY                 | 46,XY         |
| Legal sex                                 | Female                | Female        | Female                         | Female                         | Female                | Female        |
| Mutation                                  | G35E/wild             | R92/R92       | 1058-1065del/wild              | C16x/wild                      | 18delC/wild           | V15M/wild     |
| <b>Clinical Features</b>                  |                       |               |                                |                                |                       |               |
| External genitalia                        | Normal female         | Normal female | Clitoromegaly urogenital sinus | Clitoromegaly urogenital sinus | Clitoromegaly         | Normal female |
| Gonadal histology                         | Testicular dysgenesis | Not described | Testicular regression          | Testicular dysgenesis          | Testicular dysgenesis | Testis        |
| Adrenal failure                           | Yes                   | Yes           | No                             | No                             | No                    | No            |
| Obesity                                   | Yes                   | NA            | Yes                            | Yes                            | Yes                   | NA            |
| Sf-1 function of mutant allele (%)        | 0                     | 0-50          | 0                              | 0                              | 0                     | 0             |
| Dominant negative effect of mutant allele | No                    | Not described | Yes                            | No                             | No                    | No            |
| Total SF-1 function <i>in vivo</i> (%)    | 50                    | <50           | 0-50                           | 50                             | 50                    | 50            |
| Reference                                 | 8                     | 9             | 10                             | 11                             | 12                    | 13            |

These phenotypes in 46,XX subjects suggested sexual dimorphism in SF1 function in gonads.

### THE IMPORTANT ROLE OF SF1 GENE DOSAGE

Heterozygous mutation of SF1 causing human disease has established the concept of a dose-dependent action of SF1 *in vivo*. In contrast, heterozygous SF1 knockout mice show no variations in phenotype, although latent adrenal insufficiency has been unmasked under stressful conditions.<sup>19</sup> Nineteen identified patients are listed in Tables 1-3. All reported patients except case 2 had a heterozygous mutation of the SF1 gene. In cases 1 and also in cases 4 to 11, the mutant SF1 had null function without dominant negative effects. Thus, all of these 9 patients had 50% of total SF1 function *in vivo*. In cases 12 to 15, the mutant SF1 had null to 20% function. These 4 patients had 50% to 60% of total SF1 function. In case 18, the mutant SF1 only had a 55% function without dominant negative effects, suggesting that this patient had 77.5% of total SF1 function. It was of note that case 2 had a homozygous mutation of SF1. This patient had less than 50% of total SF-1 function *in vivo*, judging from the mutant SF-1 with 0% to 50% of total SF1 function. On the other hand, her parents and a brother had heterozygous mutations, thus these 3 members of the patient's family were phenotypically normal and had more than 50% of total SF1 function. Case 3 had heterozygous mutation. This mutation had null function together with dominant-negative effect. Therefore, case 3 had less than 50% of total SF1 function *in vivo*. Taken together, these

reported patients have established the importance of dosage-dependent action of SF1 in humans.

### "MILDER FORM" OF 46,XY PATIENTS

Sixteen out of 18 of the 46,XY patients reported were reared as female from birth (case 18 was suspected to have 46,XY although the karyotype was not described). Androgen production in fetal testis must therefore be insufficient. Four patients showed testicular dysgenesis or regression on macroscopic or microscopic examination. Conversely, only 2 patients (cases 1 and 2) showed adrenal failure. This suggests that in humans the testis might be more sensitive to a partial loss of SF1 function than the adrenal gland.

We described a 27-year-old Japanese woman with testicular dysgenesis without adrenal failure.<sup>12</sup> This woman never had an adrenal crisis, even at the time of infection. She had clitoromegaly, advanced virilization during pubertal age (such as voice breakage and hirsutism), and primary amenorrhea. Her karyotype was 46,XY. Small masses were palpable bilaterally in the inguinal regions. Skin pigmentation was not observed and her plasma ACTH (21 pg/mL) and serum cortisol (13.4 g/dL) were normal. An ACTH stimulation test showed a normal response of cortisol (25.3 g/dL). Urine steroid profile by a gas liquid chromatograph/mass spectrometry indicated normal steroidogenic enzyme activities. Bilateral gonadectomy was performed, and histological examination of the gonads showed dysgenetic testes,

**Table 2. Reported Cases of SF1 Mutation in Humans**

| Case                                      | 7             | 8                              | 9                                 | 10                             | 11                             | 12   |
|---|---------------|--------------------------------|-----------------------------------|--------------------------------|--------------------------------|--|
| Age (years)                               | newborn       | newborn                        | newborn                           | 2                              | 2                              | 22   |
| Karyotype                                 | 46,XY         | 46,XY                          | 46,XY                             | 46,XY                          | 46,XY                          | 46,XY  |
| Legal sex                                 | Female        | Female                         | Male                              | Female                         | Female                         | Female                                       |
| Mutation                                  | M78I/wild     | G91S/wild                      | L437Q/wild                        | C55/wild                       | Delta395E/wild                 | R84C/wild                                    |
| <b>Clinical Features</b>                  |               |                                |                                   |                                |                                |  |
| External genitalia                        | Normal female | Clitoromegaly urogenital sinus | Small phallus hypospadias chordee | Clitoromegaly urogenital sinus | Clitoromegaly urogenital sinus | Slight clitoromegaly posterior labial fusion |
| Gonadal histology                         | Testis        | Testis                         | Testis                            | Testis                         | Testis                         | Testis                                       |
| Adrenal failure                           | No            | No                             | No                                | No                             | No                             | No   |
| Obesity                                   | NA            | NA                             | NA                                | NA                             | NA                             | NA   |
| SF-1 function of mutant allele (%)        | 0             | 0                              | 0                                 | 0                              | 0                              | 10   |
| Dominant negative effect of mutant allele | No            | No                             | No                                | No                             | No                             | NA   |
| Total SF-1 function <i>in vivo</i> (%)    | 50            | 50                             | 50                                | 50                             | 50                             | 55   |
| Reference                                 | 13            | 13                             | 13                                | 14                             | 14                             | 15   |

#### Remarks

Case 7 Mother has M78I/wild  
Case 8 Mother has G91S/wild

severely hyalinized seminiferous tubules containing a few Sertoli cells, and loose interstitium containing a few Leydig cells. Molecular analysis of SF1 revealed a heterozygous single base pair deletion (18delC), theoretically leading to frameshift and early termination. Indeed, mutant SF1 failed to activate the target gene in transactivation analysis and did not have a dominant-negative effect.

The presence of "milder form" of 46,XY patients were again in contrast with XY heterozygous mice, the Sf1 knockout allele showed normal external genitalia, normal fertility, but latent adrenal insufficiency under stressful conditions.<sup>19</sup> Thus, species differences between mice and humans exist in terms of phenotypes due to loss of function of Sf1 or SF1.

### SEEMINGLY NORMAL OVARIAN DEVELOPMENT AND FUNCTION IN 46,XX PATIENTS

In humans, there have been 6 cases of 46,XX reported with SF1 mutation. All 6 had seemingly normal ovarian development and function. Only one had primary adrenal failure. Case 20 was the first reported 46,XX patient with SF1 mutation.<sup>18</sup> This phenotypically normal 14-month-old girl developed adrenal insufficiency and seizures after otitis and tonsillitis. At that time, hyponatremia (serum Na 104 mmol/L), hyperkalemia (serum K 8.0 mmol/L), elevated plasma ACTH (2,200 pg/mL), and inappropriately

low cortisol (165 nmol/L) indicated primary adrenal insufficiency. Serum LH and FSH were 0.5 mIU/mL and 2.8 mIU/mL at the age of 14 and 27 months, respectively. Imaging studies using pelvic ultrasonography and MRI confirmed the presence of bilateral ovaries of normal size. Thus, no evidence of abnormality of ovarian development and function were found.

Recently, the mothers of cases 7, 8, 12, 16, and 18 were reported to have the same mutation that was detected in the patients, indicating the ovarian development and function of these mothers were completely normal. Moreover, none of the mothers showed adrenal insufficiency. These 5 families suggested a sex-limited autosomal dominant inheritance of the SF1 mutation.

### OBESITY IN ADULT PATIENTS

Four out of 5 of the 46,XY adult patients with an SF1 mutation had obesity. Thus, obesity might be part of the phenotype of SF1 mutation in humans. A partial loss of SF1 function in the VMH in humans may lead to obesity. The presence of obesity was consistent with mice studies. Majdic et al<sup>20</sup> rescued Sf1 knockout mice with corticosteroid injections, followed by adrenal gland transplantation. These transplanted mice had indistinguishable ACTH and corticosterone levels to wild-type mice, indicating restoration of hypothalamic-pituitary-adrenal axis. With gonadectomy, at earlier ages

**Table 3. Reported Cases of SF1 Mutation in Humans**

| Case                                      | 13                             | 14                             | 15                             | 16                             | 17                   | 18                   | 19            |
|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|----------------------|----------------------|---------------|
| Age (years)                               | 4                              | 14                             | 10                             | 8                              | 22                   | NA                   | 1             |
| Karyotype                                 | 46,XY                          | 46,XY                          | 46,XY                          | 46,XY                          | 46,XY                | NA                   | 46,XX         |
| Legal sex                                 | Female                         | Female                         | Female                         | Female                         | Female               | Male                 | Female        |
| Mutation                                  | C33S/wild                      | R84H/wild                      | Y138X/wild                     | c1277dupT/wild                 | C424_427dupCCCA/wild | V333M/wild           | R255L/wild    |
| <b>Clinical Features</b>                  |                                |                                |                                |                                |                      |                      |               |
| External genitalia                        | Clitoromegaly urogenital sinus | Clitoromegaly urogenital sinus | Clitoromegaly urogenital sinus | Clitoromegaly urogenital sinus | Normal female        | Micro-penis anorchia | Normal female |
| Gonadal histology                         | Testis                         | Testis                         | Testis                         | Testis                         | Streak               | Fibrous tissue       | Ovary         |
| Adrenal failure                           | No                             | No                             | No                             | No                             | No                   | No                   | Yes           |
| Obesity                                   | NA                             | NA                             | NA                             | NA                             | NA                   | NA                   | NA            |
| Sf-1 function of mutant allele (%)        | 0-20                           | 0-20                           | 0-20                           | NA                             | NA                   | 55                   | 0             |
| Dominant negative effect of mutant allele | No                             | No                             | No                             | NA                             | NA                   | No                   | No            |
| Total SF-1 function in vivo (%)           | 50-60                          | 50-60                          | 50-60                          | NA                             | NA                   | 77.5                 | 50            |
| Reference                                 | 16                             | 16                             | 16                             | 16                             | 16                   | 17                   | 18            |

#### Remarks

Case 16 Mother has c1277dupT/wild

Case 18 Mother has V355M/wild Phenotypically normal dizygotic twin brother has V355M/wild

the weights of transplanted mice did not differ significantly from the wild-type mice. Later in life, adrenal-transplanted Sf1 knockout mice developed obesity due to decreased spontaneous locomotor activity, rather than increased appetite. It was of note that obesity was considerably more severe in females, although the reason for this sexual difference was unknown. Sf1 and the VMH nucleus in the hypothalamus were thought to play important roles in metabolism rather than in appetite regulation.

Increased weight also occurred in CNS-specific Sf1 knockout mice fed a high-fat diet.<sup>7</sup> Considering the Sf1 expression in CNS, the responsible region of obesity must be VMH. CNS-specific Sf1 knockout mice showed decreased wheel running capacity before becoming obese, indicating that obesity was due to decreased spontaneous locomotor activity.

Brain-derived neurotrophic factor (Bdnf), which stimulates growth of neurons via TrkB receptor, is expressed in the hypothalamus including the VMH. CNS-specific Bdnf knockout mice also became obese. This raised the question of whether Bdnf was a direct target gene of Sf1 in VMH. However, CNS-specific Sf1 knockout mice developed obesity only when ingesting a high-fat diet, while adrenal-transplanted Sf1 knockout mice showed obesity when fed a regular diet. Some plausible explanations are possible for these differences. Subtle abnormalities in function of adrenal transplants in original Sf1 knockout mice may result in glucocorticoid excess. The presence of gonads in CNS-specific Sf1 knockout mice may ameliorate the effects of sex steroid deficiency. It should also be kept in mind that Cre-mediated disruption of Sf1 in CNS-specific Sf1 knockout mice at ~E14 may permit certain developmental events to occur before inactivation, in contrast to original Sf1 knockout mice. Most patients with Sf1 mutation were children at the time of the study, thus long-term follow-up is necessary to ascertain if they develop obesity as adults.

### UNSOLVED ISSUES OF SF1 MUTATION IN HUMANS

Some important issues regarding SF1 mutation in humans remain unsolved. First, it is not known if any "milder form" of 46,XY patients with DSD, and seemingly normal adrenal function, would eventually develop late-onset adrenal insufficiency. Thus, longitudinal follow-up of these patients is mandatory. Second, if "milder form" 46,XY patients with DSD persist with normal adrenal function even on long-term follow-up, why does the SF1 mutation cause testicular dysgenesis or impaired androgen production, but not adrenal insufficiency? Third, the full phenotypic spectrum of 46,XY SF1 mutation has not been documented. Previous publications have shown that most of the patients had 46,XY DSD without adrenal insufficiency. Additionally, one patient with SF1 mutation was described to have bilateral anorchia and micropenis.<sup>17</sup> He had a mild partial loss of SF1

function. Thus, 46,XY SF1 mutation may result in a wide spectrum of male reproductive phenotypes. Fourth, the molecular mechanism of sexual dimorphism of the SF1 phenotypes regarding gonads is not clear. The 46,XY patients showed testicular dysgenesis or impaired androgen production, whereas 46,XX patients showed an apparent normal ovarian development and function. SF1 might have different target gene(s) depending on developing fetal testis or ovary. Fifth, the molecular mechanism of the development of obesity in SF1 needs further clarification, although mice studies suggested the loss of VMH function.

### SUMMARY

The identified patients with SF1 mutation definitively showed a critical role of SF1 function in vivo. There is a different functional importance of Sf1 (SF1) between mice and humans. In summary, in humans: (1) the important role of SF1 gene dosage has been elucidated; (2) a number of "milder form" of 46,XY patients have been reported with DSD and normal adrenal function; (3) 46,XX patients had seemingly normal ovarian development and function; and (4) adult patients might develop obesity.

### Acknowledgements

The author thanks Dr. Tomohiro Ishii (Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan) for fruitful discussion and critical comments.

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## Letter to the Editor

### Caucasian or White Phenotype?

The term Caucasian is frequently employed to describe a white individual. Caucasian is used as a synonym for a group of people that share the common character of whiteness. It is frequently used by distinguished researchers when they analyze the differences between various ethnic groups in relation to a phenomenon they have studied. According to the definitions given by *The American Heritage Dictionary of the English Language*, "Caucasus or Caucasia is a region between the Black and Caspian seas that includes Russia, Georgia, Azerbaijan, and Armenia." Caucasian relates to the Caucasus region or its peoples, languages or cultures. It also refers to a major human racial division traditionally distinguished by physical characteristics such as very light to brown skin pigmentation and straight to wavy or curly hair, and including peoples indigenous to Europe, Northern Africa, Western Asia, and India. Thus there are dark and curly haired Caucasians, as there could be very white, light-eyed Latinos or very light to dark skin in other groups. I believe that what scientists who use the term Caucasian are trying to say is that the term refers to a Caucasian, especially of Nordic type or, at least, as defined by the dictionary, "White: A member of a racial group of people having light skin coloration, especially one of European origin." If so, why abandon the term white?

The term Caucasian may intend to reduce a great number of phenotypes into a group that shares other characteristics as well. In a superb, well documented article the significance of the term phenotype is discussed.<sup>1</sup> The current definition of phenotype is: "the complete observable characteristics of an organism or group, including anatomic, physiological,

biochemical, and behavioral traits, as determined by the interaction of both genetic makeup and environmental factors." One realizes that an external character cannot imply, by itself, a necessary similarity between two or more individuals or groups. As the authors state, "The interaction of genes and the environment has the potential to produce a myriad of phenotypes." For example, is it not true that among the Caucasian population in the world there are those who differ greatly in skin hues, ethnicity, and genetic factors? On the other hand a white skinned, light-eyed Latino (or Hispanic as the US Census classifies) may have the appearance, genetic background, behavioral traits, and environmental influences of a Caucasian. If it is melanin that determines the grouping, why not just use the term white?

Cesar Chavarria, MD  
Mexico City, Mexico

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**Editor's Response:** *Dr. Chavarria makes a good case to cease using the term Caucasian in describing white patients. The AMA Manual of Style states, "Racial categories should not be used automatically. Authors should explain and justify racial designators. Caucasian is occasionally used to indicate white but is technically specific for people from Caucasus region and thus should be avoided." For several years GGH has used the term white. Unfortunately, the classification of Hispanic and Latino is far more complicated and controversial.*

Fima Lifshitz, MD

## REVIEWS & COMMENTS FROM THE LITERATURE

### Dosing of Growth Hormone Therapy According to IGF Levels

Cohen and colleagues conducted a 2-year, open-label, randomized, insulin-like growth factor (IGF)-I concentration-controlled trial, administering varying doses of growth hormone (GH) to test whether IGF-I levels achieved during GH therapy are determinants of the growth responses to GH treatment. The 172 subjects (77% male) were pre-pubertal children (mean age 7.53 years) with short stature (mean height SDS -2.64, mean IGF-I SDS -3.56). Subjects were randomized to receive GH treatment following one of 3 regimens: (1)

conventional GH dosing based on the patient's weight (40 mcg/kg/d, n=34); (2) regularly adjusted GH doses to achieve an IGF-I SDS of -0.5 to +0.5 (IGF<sub>low</sub> group, n=70) or; (3) regularly adjusted GH doses to achieve an IGF-I SDS of +1.5 to +2.5 (IGF<sub>high</sub> group, n=68). Groups did not differ significantly on demographic or baseline variables such as height, IGF-I levels, peak GH, or bone age.

Baseline data collected included concomitant illness and medications, physical examination, funduscopy, height, weight, determination of IGF-I, pubertal staging,

checks for scoliosis and slipped capital femoral epiphysis (SCFE), blood sampling, and urinalysis. Study visits occurred at months 0, 1, 3, and every 3 months thereafter until 2 years. Adverse event reporting, height, weight, IGF-I, funduscopy, vital signs, and physical examinations for SCFE and SCFE were conducted at all repeat visits. Laboratory evaluations performed at baseline were repeated annually, and bone age x-rays were obtained at baseline and year 2. Analysis of covariance was used to test for treatment effects, using baseline height-SDS (HT-SDS) as a covariate. Of the 172 enrolled participants, 147 completed the study. An intent-to-treat statistical analysis was performed including all randomized patients who received GH and at least one post-baseline height and IGF-I assessment.

**Dosage and Growth.** All 3 treatment groups demonstrated increased HT-SDS scores at the end of the study (median of 24 months), with the IGF<sub>(high)</sub> group showing the greatest increase (1.58 SDS) compared with the IGF<sub>(low)</sub> group (1.08 SDS) and the conventional dosing group (1.00 SDS). Annualized growth velocities for the IGF<sub>(low)</sub>, IGF<sub>(high)</sub>, and conventional groups were 9.71, 11.20, and 9.01 cm/year at 12 months, and 8.38, 10.03, and 8.16 cm/year at 24 months, respectively. Mean IGF-I SDS showed a rapid increase in all 3 groups during the first month after initiation of GH treatment; the target IGF-I values were generally reached within 6 to 9 months. The IGF<sub>(high)</sub> group had a target IGF-I SDS value of 2.0 (1.5–2.5) and the IGF<sub>(low)</sub> 0 (–0.5 to 0.5). IGF-I SDS values for the IGF<sub>(high)</sub> group were significantly higher than for the IGF<sub>(low)</sub> and the conventional groups from 6 months onward; no differences were found between mean IGF-I SDS for IGF<sub>(low)</sub> and conventional groups. Mean daily GH doses for the 3 treatment groups were 110 (median 98, range 20 to 346) mcg/kg/day for the IGF<sub>(high)</sub> group, 33 (median 28, range 9 to 114) mcg/kg/d for the IGF<sub>(low)</sub> group, and 41 (median 41, range 34 to 45) mcg/kg/day for the weight-based GH dosing comparison group. The IGF<sub>(high)</sub> group received a substantially larger mean GH dose than the other 2 groups, but no significant differences in the mean dose between the IGF<sub>(low)</sub> group and the comparison group were found. For all participants, the change in HT-SDS from baseline was positively correlated with both the IGF-I SDS change from baseline and with the cumulative GH dose. Multivariate analysis revealed that height outcome was significantly related to treatment group (accounting for 42% of the variance), inversely related to baseline peak GH level (39%), and inversely related to baseline IGF-I SDS (15%).

**Safety.** Over the 2-years, treatment-emergent adverse events were reported in 95.7% of participants in the IGF<sub>(low)</sub> group, 86.6% of patients in the IGF<sub>(high)</sub> group, and 82.4% in the conventional treatment group; most commonly, upper respiratory tract infection, headache, fever, coughing, and injection site hematomas. There was no occurrence of intracranial hypertension or malignancy. There was one case of SCFE in the IGF<sub>(high)</sub> group and 11 cases of

worsening scoliosis (3 in the conventional, 4 in the IGF<sub>(low)</sub> group, and 4 in the IGF<sub>(high)</sub> group). Change in fasting serum insulin levels from baseline in the IGF<sub>(high)</sub> group was significantly greater than in the other groups, although mean serum insulin remained within the normal range for all groups. Bone age was delayed by approximately 2 years in all 3 groups at baseline, and after 2 years of treatment, bone age showed an increase of 2.45 to 2.82 years with no differences identified among the 3 groups.

The authors concluded that the IGF<sub>(high)</sub> group, titrated to the upper portion of the normal range, demonstrated significantly greater height gains than the IGF<sub>(low)</sub> and conventional groups. Expressed in height benefit, the IGF<sub>(high)</sub> group gained approximately 3 cm more in height than the comparison groups after 24 months of GH treatment. The study lacked sufficient power to detect the safety of IGF-based dosing in terms of rare side effects. No information regarding the long-term safety of such a regimen, especially in terms of cancer risk, was provided.

Cohen P, Rogol AD, Howard CP, et al. Insulin growth factor-based dosing of growth hormone therapy in children: A randomized, controlled study. *J Clin Endocrinol Metab.* 2007;92:2480-6.

**Editor's Comment:** This study provides evidence for the feasibility of IGF-based GH dose titration; however, the increased height gains compared to the conventional treatment dosing were only significant for the IGF<sub>(high)</sub> group. The authors were circumspect by restricting interpretation of the findings to a demonstration of the feasibility of IGF-I GH dose titration and not as a recommendation for clinical practice. Important considerations to explore before implementing such a strategy in regular practice include: (1) GH doses administered to this group were as high as 346 mcg/kg/day (mean 110), compared to the mean conventional weight-based dose of 41 mcg/kg/day; this represents as high as a 9-fold increase compared to previously studied values; (2) given the lack of safety data beyond the length of 2-year study, movement toward increasing GH above the conventional dosing should be discouraged. An editorial by Baron accompanying this paper stressed that the principle of *primum non nocere* dictates that weight-based dosing remain the standard of care.<sup>1</sup>

Although there is a dearth of information to inform us about the possible negative side effects that may be associated with prolonged treatment with high doses of GH, there is certainly a theoretical basis for concern. A growing body of epidemiological data suggests that high levels of circulating IGF-I constitute a risk factor for the development of breast, prostate, colon, and lung cancer.<sup>2</sup> This study by Cohen and colleagues demonstrated a height gain of 3 cm for the IGF<sub>(high)</sub> group compared to the IGF<sub>(low)</sub> and conventional weight-based dosing groups. Even if the substantial excess cost of the additional GH administration of higher dosages is not considered, does the potential (not guarantee) for taller adult height justify potentially increasing a child's risk of developing cancer?

Baron reminds the reader that "risk must be weighed against benefit" and states that "although short stature may be quite unpleasant for some individuals and carry social disadvantages, it generally does not cause death, serious physical dysfunction, or probably even serious psychological dysfunction."<sup>1</sup> This opinion is grounded in empirical evidence.<sup>3</sup>

Baron also encourages careful evaluation of the etiology of short stature before prescribing a costly and invasive procedure to which greater than 80% of children experienced some adverse side effects. Although Cohen *et al* used GH therapy in children with GH deficiency as well as in children with other categories of non-GH deficient short stature, the situation may be more complex and different among the various types of patients. As an example, it is well known that decreased IGF-I levels reflect nutritional status, not necessarily GH deficits,<sup>4</sup> yet no attempts were made to distinguish patients who

may have had nutritional growth retardation, nor were the body weights of the patients defined. It has been shown that a subgroup of children with idiopathic short stature show decreased weight for height,<sup>5</sup> which is not typical of GH deficiency, suggesting their decreased growth and IGF-I may reflect insufficient nutrition. In such cases, lifestyle and dietary changes would be a more expedient, safer, and cost-effective treatment for the child.<sup>6</sup>

David E. Sandberg, PhD

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## IGFs and Cytokines in Celiac Disease

The interesting study reported in this paper is the result of one of the few productive collaborations between pediatric endocrinologists and their gastroenterologist colleagues. This endocrine group from Parma, Italy has already published papers on the interaction of the cytokine and insulin-like growth factor (IGF) systems in Crohn's disease and cystic fibrosis. Growth failure is a well known feature of childhood celiac disease, however the precise mechanisms are not established and the possible influences of pro-inflammatory cytokines have not been well explored. The patients studied had "atypical" celiac disease, ie, they presented after the classical period of infancy. These patients were not extremely short at diagnosis but BMI SDS was decreased and both height and BMI increased significantly after treatment with a gluten-free diet.

Baseline values of IGF-I were reduced compared to controls ( $P < 0.05$ ) and interleukin (IL)-6 and tumor-necrosis factor (TNF)- $\alpha$  values were significantly elevated. IGF binding protein (IGFBP)-2 acts as an acute phase protein and, as reported in inflammatory bowel disease and childhood malignancy, values were elevated in affected subjects compared to controls. On treatment with a gluten-free diet, IGF-I and IGFBP-3 normalized and IL-6

and TNF- $\alpha$  decreased significantly. This study provides indirect evidence that cytokines may be involved in the abnormalities in the IGF system and when mucosal inflammation is suppressed, as occurs with treatment of celiac disease, and leads to the increases of IGFs and IGFBP-3 which facilitate normalization of linear growth.

Street ME, Volta C, Ziveri MA, *et al*. Changes and relationships of IGFs and IGFBPs and cytokines in coeliac disease at diagnosis and on gluten-free diet. *Clin Endocrinol (Oxf)*. 2008;68:22-8.

**Editor's Comment:** *The celiac disease debate remains as to whether it is improvement in nutrition or suppression of inflammation which drives the recovery of growth. Both factors probably contribute, however as shown in Crohn's disease,<sup>1</sup> suppression of inflammation can independently result in increase of serum IGF-I, therefore the contribution of active inflammation may be subtle, but should not be discounted.*

Martin O. Savage, MD

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## Aortic Dilatation and Dissection in Turner Syndrome

The cardiovascular phenotype in Turner syndrome (TS) is largely defined on clinical signs such as aortic valve abnormalities and aortic coarctation. Investigation in asymptomatic patients has revealed a far more complex phenotype. Combined echocardiography and MRI have shown that up to 75% of adult women with TS have significant cardiovascular abnormalities. In parallel there have been reports of a high rate of aortic dissection in

TS and dilation of ascending aorta could be among predisposing factors. It is still unknown whether aortic dilatation precedes dissection in these patients and what specific diameter predicts impending deterioration.

The purpose of this study by Matura *et al* was to reliably identify girls and women at risk for such acute aortic events. This study included 166 adult volunteers with TS, aged more than 18 years, who



were not selected for cardiovascular disease and a group of healthy females. Ascending and descending aorta diameters were measured by MRI at the right pulmonary artery. Average diameters were identical in both groups; however results needed to take into account a mean 20 cm difference in height between both groups. When normalized to body surface area (aortic size index) the ascending aortic diameters were significantly greater in the TS group, and close to 32% of the TS women had values >95<sup>th</sup> percentile of 2.0 cm/m<sup>2</sup>. Ascending/descending aorta diameters ratios were significantly greater in the TS group. During 3 years of follow-up aortic dissection occurred in 3 women with TS. Their ascending aortic diameters ranged from 3.7 to 4.8 cm and the aortic size indices were >2.5 cm/m<sup>2</sup>. This rate is almost 100 fold higher than that of normal women who are usually affected at a much later age. Unfortunately there are no prospective data to know whether dilatation of the ascending aorta preceded dissection or elongation of the transverse aortic arch—a feature more recently described in TS.

The risk for aortic dissection is greatly increased in young women with TS. Because of their small stature, ascending aorta diameters of >5 cm may represent significant dilatation. The use of an aortic size index is therefore recommended. Individuals with a dilated ascending aorta defined as aortic size index >2.0 cm/m<sup>2</sup> require close cardiovascular surveillance, and values >2.5 cm/m<sup>2</sup> indicate a high risk for aortic dissection. The authors suggested that haploinsufficiency for a pseudoautosomal gene is responsible for the linked cardiovascular and lymphatic defects in TS. In addition, it is acknowledged that this study did not provide evidence-based recommendations for the follow-up of these patients

with aortic dilatation. Further studies are also needed, like those in Marfan syndrome, to determine whether beta-blocker or rennin-angiotensin system blockade may prevent or retard aortic dilatation and if prophylactic surgery is appropriate.

Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. *Circulation*. 2007;116:1663-70.

**Editor's Comment:** Recently published clinical guidelines<sup>1</sup> for care of girls and women with TS recommended that magnetic resonance angiography be used, in addition to echocardiography to evaluate the cardiovascular system. It was suggested that patients with defined defects be cautioned in regard to pregnancy. The present study of Matura et al provided an interesting addition of a new tool with appropriate reference data, which should help to evaluate the vital risk of aortic dissection in TS. However, prospective studies are needed which should include adolescent girls as well. The handling of the infertility issues is critical. The patients with spontaneous puberty and apparent ovarian activity should be evaluated for additional risk factors, such as systemic hypertension. The large group of infertile TS patients who have been told that assisted pregnancy can be considered in adulthood should keep in mind there is a risk of fatal aortic dissection during pregnancy. The aortic diameter should be monitored and be part of the follow-up and be taken into account in the reproductive life during adulthood.

Raphaël Rappaport, MD

## Reference

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## The Late Effects of Childhood Cancer Survivors

Modern therapies and supportive care have increased the number of the childhood cancer survivors (CCS); as well, there has also been an increase in the late effects such as endocrine impairments and neuropsychological problems. These late effects often do not become clinically apparent until decades after cancer therapy. Unfortunately, over time the likelihood of medical follow-up decreases. Therefore, it is important for physicians to be aware of the late effects facing this population over their lifetime and the need to recall CCS patients for follow-up. However, where and by whom the follow-up of CCS can best be done is still a question that remains to be answered. Dickerman has set forth the recommendations for monitoring the late effects of CCS. He listed in a table both radiation-therapy site and chemotherapeutic agents along with the late effects that result from their use. These include: hypopituitarism, growth problems, hypogonadism, neurocognitive

defects, coronary artery disease, cardiomyopathy, lung fibrosis, interstitial pneumonitis, breast cancer, nephropathy, muscle atrophy, osteoporosis, and second cancers. He recommended that in addition to being followed by a primary care physician, all CCS patients should also attend a specialized late-effects clinic on a yearly basis. At that specialized clinic, CCS patients would be evaluated by a member of the oncology team and subspecialists such as an endocrinologist, psychologist and neurologist. Ideally, such clinics should be located in the same center in which the patient was initially treated and be available on or near the site of residence.

Dickerman JD. The late effects of childhood cancer therapy. *Pediatrics*. 2007;119:554-68.

**Editor's Comment:** This is a very special review article which provides important information for



physicians who care for CCS patients. The survival rate of childhood cancer patients has markedly improved, thus the long-term late effects, such as endocrine impairments and neuropsychological problems, have become increasingly important. These alterations may result many years after conclusion of the cancer treatment. Currently, 10 million individuals in the US are living with a cancer diagnosis, 3 times the number of survivors in decades past. In the near future 1 of 450 individuals in the population will be a long-term CCS. The 5-year survival rate of children with cancer is 80% to 85%; presently 1 in 640 individuals between 20 and 39 years of age is a CCS. Approximately 270,000 in the US present long-term morbidity of CCS.

In another paper, Oeffinger et al<sup>1</sup> recently reported the chronic health conditions (late effects) in adults following the treatment of childhood cancer. Their retrospective cohort study tracked the health status of adults who received a diagnosis of childhood cancer between 1970 and 1986 and compared the results with those of siblings of the patients. They calculated the frequencies of chronic conditions in 10,397 survivors and 3034 siblings (mean ages 26.6 years and 29.2 years, respectively, at the time of the study). In 62.3% of the cancer survivors there was at least one chronic condition; 27.5% had a severe or life-threatening condition. The adjusted relative risk of a chronic condition in a survivor, as compared with siblings, was 3.3 (95% CI, 3.0 to 3.5); for a severe or life-threatening condition, the risk was 8.2 (95% CI, 6.9 to 9.7). Among survivors, the cumulative incidence of a chronic health condition reached 73.4% (95% CI, 69.0 to 77.9) 30 years after the cancer diagnosis, with a cumulative incidence of 42.4% (95% CI, 33.7 to 51.2) for severe, disabling, or life-threatening conditions or death due to a chronic condition (Table). Thus, CCS have a high rate of illness owing to chronic health conditions that occurred long after the cancer was treated. There are many long-term CCS who were treated in the last 50 years, and these patients still need monitoring.

The late effects resulting from current treatment will likely decrease with improved radiotherapy being delivered with newer equipment in better fractionation schedules, along with the replacement of, or the use of, reduced doses of second-cancer-inducing chemotherapy. However, new cancer therapies used now or in the future will, in all likelihood, be associated with their own late effects. The patients who are treated with these new therapies must also be monitored closely to assess the magnitude of any late effects. It is necessary for physicians, as well as patients and family

**Relative risk of selected severe (grade 3) or life-threatening or disabling (grade 4) health conditions among cancer survivors, as compared with siblings.**

| Condition                         | Survivors<br>(N=10,397)<br>percent | Siblings<br>(N=3034)<br>percent | Relative Risk<br>(95% CI) |
|-----------------------------------|------------------------------------|---------------------------------|---------------------------|
| Major joint replacement*          | 1.61                               | 0.03                            | 54.0 (7.6–386.3)          |
| Congressive heart failure         | 1.24                               | 0.10                            | 15.1 (4.8–47.9)           |
| Second malignant neoplasm†        | 2.38                               | 0.33                            | 14.8 (7.2–30.4)           |
| Cognitive dysfunction, severe     | 0.65                               | 0.10                            | 10.5 (2.6–43.0)           |
| Coronary artery disease           | 1.11                               | 0.20                            | 10.4 (4.1–25.9)           |
| Cerebrovascular accident          | 1.56                               | 0.20                            | 9.3 (4.1–21.2)            |
| Renal failure or dialysis         | 0.52                               | 0.07                            | 8.9 (2.2–36.6)            |
| Hearing loss not corrected by aid | 1.96                               | 0.36                            | 6.3 (3.3–11.8)            |
| Legally blind or loss of an eye   | 2.92                               | 0.69                            | 5.8 (3.5–9.5)             |
| Ovarian failure‡                  | 2.79                               | 0.99                            | 3.5 (2.7–5.2)             |

\* For survivors, major joint replacement was not included if it was part of cancer therapy.

† For both groups, this category excludes basal-cell and squamous-cell carcinoma (grade 2). For siblings, this category includes a first cancer.

‡ Values are for women only.

Reprinted with permission Oeffinger KC, et al. *New Engl J Med*. 2006;355:1572-82. Copyright © Massachusetts Medical Society. 2006. All rights reserved.

members, to know that late effects of a cancer survivor can occur even after many years following cancer treatment. The signs and symptoms of late effects of CCS are often nonspecific and may be masked by the sequela of chemotherapy, radiation therapy, and/or surgery, and may not be clinically evident until much later in life. Therefore, they are likely to be overlooked if late effects are not actively searched for through regular follow-up. In previous issues of GGH there were 4 reviews of papers dealing with the long-term complications of CCS addressing height,<sup>2</sup> premature menopause,<sup>3</sup> growth hormone therapy and secondary neoplasms,<sup>4</sup> growth hormone deficiency, quality of life and neuropsychological function.<sup>5</sup> A clinic based model for survivors of childhood cancer has been proposed by Hinkle et al.<sup>6</sup>

Yoshikazu Nishi, MD

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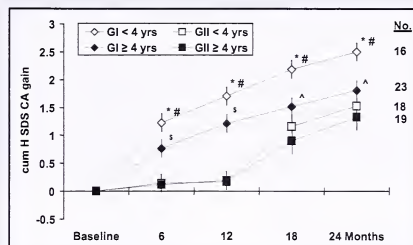
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# Growth Hormone Treatment in Very Young Children Born Small for Gestational Age

Argente and colleagues analyzed the outcome of growth hormone (GH) treatment in a large group of very young children born small for gestational age (SGA). They evaluated 76 children, recruited from 14 public hospitals in Spain, aged 2 to 5 years (37 males and 39 females; 45% less than 4 years of age) born SGA without catch-up growth during their first 2 years of life. The results after 24 months of GH treatment (0.06 mg/kg/day for 2 years, group I) were compared with those of a control group without treatment for 12 months, followed by 12 months of GH therapy (group II). The mean height SDS gain for chronological age in group I children was 2.10, compared to 1.43 in the children of group II. Height SDS for bone age was significantly different between groups only when group II did not receive GH treatment. Growth velocity SDS increased from -2.2 at baseline to 4.7 at 12 months in group I, while no significant changes from baseline values were noted in untreated group II subjects. Children in both groups under 4 years of age had the greatest gain in growth velocity, not only in SDS but also in their absolute increase in centimeters; weight SDS followed the same pattern (Figure). The BMI SDS did not change significantly during the study period and there was no significant acceleration of bone age. Fasting blood glucose, insulin, and HbA<sub>1c</sub> levels remained within the normal range and with no difference among groups throughout the study. Both insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 increased significantly after 6 months of GH therapy and remained at a similar level thereafter, but did not exceed +2 SDS for chronological age during the study period.

Argente J, Gracia R, Ibañez L, et al, on behalf of the Spanish SGA Working Group. Improvement in growth after two years of growth hormone therapy in very young children born small for gestational age and without spontaneous catch up growth: Results of a multicenter, controlled, randomized, open clinical trial. *J Clin Endocrinol Metab.* 2007;92:3095-101.

**Editor's Comment:** Nearly 3% of infants are born SGA—that is with a weight and/or length at least 2 SD below the mean for gestational age. Most of these children undergo catch-up growth, allowing them to reach normal height by 2 years of age. However, close to 10% of SGA children fail to achieve an appropriate catch-up growth and remain short throughout childhood with a height below -2 SD. As demonstrated by a



**Evolution of the SDS of height for chronological age** (cumulative H SDS CA gain) in children less than or greater than 4 years of age in each group.  $p < 0.05$  group I (GI) < 4 years vs GI > 4 years.  $\Delta p < 0.05$  GI > 4 years vs. Group II (GH) > 4 years.  $\# p < 0.05$  GI < 4 years vs. GH < and > 4 years.  $\$ p < 0.05$  GI > 4 years vs. GH < and > 4 years. Group I received GH from the beginning of the study. Group II received GH starting at the 12 month time-point. (Mean and 95% confidence interval).

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number of recent studies,<sup>1,2</sup> when treated with GH these patients can normalize their height during childhood, are able to maintain a normal growth velocity while prepubertal and during puberty, and can attain a normal adult height. Treatment with GH seems to be useful even in non-GH deficient SGA children and in those in whom no detectable cause for the lack of catch-up growth can be detected. However, most studies performed so far have been completed in older SGA children, so that the safety and efficacy of GH treatment in young children born SGA is unknown. In this study by Argente et al it was

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demonstrated that very young SGA children with no spontaneous catch-up growth during the first 2 years of life are able to significantly increase their growth velocity and their height SDS following 2 years of GH therapy. However, continuously high plasma IGF-I and IGFBP-3 levels during therapy were evident. If these persist for years there may be potentially harmful effects.<sup>3</sup> The increase in growth velocity was greatest in SGA patients. Whether early GH treatment will result in a significantly greater adult height in these patients remains to be determined by

long-term follow-up, but these observations seem to suggest the benefits and safety of early GH therapy in short children born SGA.

Roberto Lanes, MD

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## Metabolic Syndrome in Brothers of PCOS Women

Polycystic ovary syndrome (PCOS), defined by hyperandrogenism, chronic anovulation, and/or polycystic ovary disease is one of the most common endocrinopathies in young women and evidence supports a central role of insulin resistance in the pathophysiology. Although previous studies have found high incidences of familial clustering of PCOS, suggesting that it might be a genetic disease, the lack of a male phenotype has made it difficult to assess the genetic component of this disorder. To date no study has looked at the gold standard of euglycemic-hyperinsulinemic clamp methodology in brothers of PCOS women. Baillargeon and Carpentier studied 17 brothers of women with PCOS and 28 male controls. The male controls selected were of comparable age and BMI as the brothers, and had no first-degree relatives with PCOS. Participants in the study were between 18 to 40 years of age with BMIs between 19-40 kg/m<sup>2</sup>. The study protocol included anthropometric measurements of waist circumference and fasting blood samples for steroid levels. A standard OGTT was performed 2 days prior to a standard euglycemic-hyperinsulinemic clamp. In a subgroup of participants rates of oxygen consumption were measured during a 40-minute baseline period and during the last 40 minutes of the clamp to determine total body carbohydrate oxidation using indirect calorimetry. Assays obtained included total testosterone, androstenedione, DHEAS, 17  $\alpha$ -hydroxyprogesterone, sex-hormone binding globulin (SHBG), free testosterone, estrogen, progesterone, FSH, LH, thyrotropin, insulin, TSH, and prolactin. In addition C-reactive protein, total cholesterol, triacylglycerol, and HDL cholesterol were measured and LDL cholesterol was calculated. Fibrinogen, plasminogen activator inhibitor (PAI)-1 and factor VIII levels were also measured. For each variable the difference between the groups was adjusted for age and BMI using multiple linear regression analysis.

Age, BMI, waist circumference, and total fat percentage were comparable between the 2 groups as were systolic and diastolic blood pressure. Free

testosterone levels, androstenedione, and DHEAS were similar in both groups. Fasting triacylglycerol levels were significantly increased in the brothers, but HDL and LDL cholesterol were comparable. PAI-1 and factor VIII were significantly increased in the brothers as was fasting glucose, 2-hour glucose levels and area under the glucose curve. Those with metabolic syndrome, as defined by the US National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III, were 18% in the brothers and 7% in the controls. This difference was not statistically significant. Insulin sensitivity was significantly decreased by 38% in the brothers and insulin stimulated total body carbohydrate oxidation was decreased by 65% in the brothers. After adjustment for age and BMI, the factor VIII levels, 2-hour glucose, as well as AUC<sub>glucose</sub> and AUC<sub>insulin</sub> during the OGTT were still significantly different between the 2 groups, but the differences in triacylglycerol, PAI-1, and fasting glucose were no longer significant. Those individuals with BMI >26.5 kg/m<sup>2</sup> had significantly increased PAI-1 and AUC<sub>insulin</sub> and significantly decreased androstenedione and sex hormone binding globulin.

The authors concluded that the brothers of women with PCOS have a significant decrease in insulin sensitivity associated with decreased glucose tolerance and hypercoagulability as evidenced by increased PAI-1 and factor VIII levels. Additionally, insulin stimulated glucose disposal was decreased by 65%. Thus brothers of women with PCOS displayed insulin resistance, glucose intolerance, and many of the characteristics of insulin resistance syndrome. A unique finding of the study was that glucose intolerance in the brothers of women with PCOS is irrespective of their degree of obesity. The authors noted that the limitations of their studies included a somewhat limited recruitment of all brothers since it was not possible to recruit all members of the affected and non-affected families. However, they concluded that their data suggest that brothers of PCOS women may have inherited insulin resistance and metabolic syndrome typical of PCOS and that these young men deserve careful clinical evaluation and long-term follow-up.



Baillargeon JP, Carpentier AC. Brothers of women with polycystic ovary syndrome are characterized by impaired glucose tolerance, reduced insulin sensitivity and related metabolic defects. *Diabetologia*. 2007;50:2424-32.

**Editor's Comment:** Over the past several years pediatric endocrinologists have been evaluating an increasing number of adolescents with PCOS. It is not uncommon for these teenagers to be accompanied by mothers and sisters who also have obvious signs suggestive of PCOS. It is uncommon, at least in this editor's experience, for brothers of these teenagers to accompany them to the clinic visit. Thus, there is a potentially large group of teenagers and young adults

with significant metabolic abnormalities who are not being evaluated and counseled. With the growing epidemic of obesity one is hesitant to suggest that pediatric endocrinologists actively recruit additional overweight adolescents to their clinics. However, the information presented above by Baillargeon and Carpentier suggests that to exclude the discussion of brothers' health status during the evaluation of girls with PCOS may be a significant omission. It would be of interest to obtain additional clinical information on brothers of adolescents with PCOS. This would appear to be an area for further clinical research.

William A. Clarke, MD

## Consensus Guidelines for Adult Growth Hormone Deficiency 2007

Ten years after the Growth Hormone (GH) Research Society drafted its "Consensus Guidelines for the Diagnosis and Treatment of Adults with Growth Hormone Deficiency (GHD)", a second international workshop was convened (Sydney, Australia) with 30 delegates to create an updated set of guidelines in 2007. Diagnosis of adult GHD was expanded in patient scope since the first document. Testing should be reserved for patients with evidence of hypothalamic-pituitary disease and with intention to treat. This includes patients with signs and symptoms of hypothalamic-pituitary disease (endocrine, structural, or genetic causes), patients who had received cranial irradiation or tumor treatment, and a new group, patients who had sustained traumatic brain injury or subarachnoid hemorrhage. Of note, the degree of pituitary dysfunction does not correlate with the severity of brain injury, and GH testing should be deferred for at least 12 months after injury due to the rate of endogenous GH axis recovery.

Another new patient group discussed is the GHD patient during the transition period, that newly recognized life stage between cessation of statural growth (ie, epiphyseal closure) and acquisition of complete somatic maturation (ie, full development of lean body mass and bone mineralization). Apart from patients with known genetic causes of GHD/hypopituitarism and patients with multiple pituitary hormone deficiencies (who should continue GH treatment without the need for further testing), patients with childhood onset GHD should undergo reevaluation of their GH function after at least one month off GH treatment for assessment of potential treatment during the transition period. A second reevaluation may be considered at the end of the transition period (about age 25) for those with isolated idiopathic GHD or discordant testing (low insulin-like growth factor [IGF]-I but normal stimulated GH peak) at the start of transition. Adult GH treatment is not indicated for patients with non-GHD pediatric indications, such as those born small for gestational age or Turner syndrome.

Adult GH treatment aims to "...correct the metabolic,

functional, and psychological abnormalities associated with adult GHD." Dosing should be based on age and gender, not body weight, and escalated to response in a gradual and individualized fashion. Recommended monitoring of response includes:

1. Anthropometry (including weight, height, BMI and waist circumference): at least yearly
2. Quantified body composition and bone mineral density (DEXA): at baseline and every 2 years thereafter
3. Serum marker for GH dose titration (serum IGF-I): at least yearly and no sooner than 6 weeks after a dose change
4. Cardiovascular risk factors (blood pressure, fat mass, cholesterol panel): yearly, with similar goals as the general population
5. Fasting serum glucose: yearly
6. Quality of life (careful history, not disease-specific quality of life questionnaires)

Although GH treatment is indicated for adult patients with proven GHD, GH supplementation is not recommended for the physiologic age-related decline in GH/IGF secretion. Lower doses are called for in the elderly, to reduce the incidence of side effects and maintain age-dependent normal levels of IGF-I.

Ho KKY on behalf of the 2007 GH Deficiency Consensus Workshop Participants. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *Eur J Endocrinol*. 2007;157:695-700.

**First Editor's Comment:** The reader is encouraged to go through the entire original document, as the guidelines were too extensive to summarize here; this abstract highlights the newer recommendations. Also covered are the dosing recommendations, interactions with other hormone deficiencies, and treatment safety issues.

More than the advances, what struck me were the



*persistent unknowns of the field. Ten years after the first set of guidelines, we remain prey to suboptimal diagnostic testing. The lack of standardization of the GH and IGF-I assays was lamented in the consensus statement, as was the need for better age- and gender-related normative data. There was an entire section devoted to the various GH stimulation tests, their respective indications and limitations, and the multiple cut-off levels which also need better substantiating normative data. It is not surprising that the authors concluded, "...partial GHD is not adequately defined." Unless we can accurately distinguish normal from abnormal hormone levels, how can clinical care and research in the growth field advance effectively?*

Adda Grimberg, MD

**Second Editor's Comment:** *The reader is encouraged to review the article in its entirety. However it may be worth noting a few more pertinent points in addition to those elaborated above. The consensus of experts stated that one stimulation test was sufficient for the diagnosis of adult GHD. They endorsed the use of*

*an insulin or a glucagon tolerance test, and did not recommend clonidine, L-DOPA or arginine. GH releasing hormone (GHRH) + arginine or GHRH + GH-releasing peptide (GHRP) have also been validated, though GHD of hypothalamic origin may be missed, particularly in patients treated with cranial irradiation, then insulin or glucagon tolerance test may be necessary. The peak GH level for diagnosis was <3 mcg/L after insulin, higher levels may be acceptable following GHRH in individuals with a BMI of <25 kg/m<sup>2</sup>. Measurements of circulating IGF-I levels constitute a good screen, though a normal level may not rule out GHD. Sex steroid, glucocorticosteroid and thyroid replacement should be optimized before testing or initiating GH treatment. The efficacy of treatment should be monitored and objective parameters determined, ie, body composition. Where available, DEXA should be utilized to quantitate body composition changes. IGF-I levels are indicated for titration of the GH dosages. Disease-specific quality of life questionnaires that assess the problems need to be validated.*

Fima Lifshitz, MD

## Genetics of Stature

Variation in adult height is a classic polygenic trait, ie, it is determined by many genes each having a small effect. The identity of these genes has been elusive despite delineating many genes that have a major impact on height based on detection of mutations that cause severe growth deficiency. Although linkage studies have pointed to several genomic regions that influence height, there have not been any examples of gene variants that are reproducibly associated with height variation in the general population. However, from analysis of genome-wide association data, Weedon et al now showed that common variants in the *HMG2* oncogene are associated with height.

The investigators began by analyzing data from 4,921 individuals including 1,896 UK individuals with type 2 diabetes from the Wellcome Trust Case Control Consortium and 3,025 Swedish or Finnish participants from the Diabetes Genetics Initiative. More specifically, they performed a meta-analysis of sex- and age-adjusted height z-scores for 364,301 autosomal single nucleotide polymorphisms (SNPs) common across data sets. These SNPs provide 64% coverage of the Utah-based Haplotype Map.

Two SNPs most associated with height were mapped in and 12 kb downstream of the 3' UTR (3' untranslated region) of the high mobility group-A2 (*HMG2*) gene. *HMG2* is a strong biological candidate for influencing height because its homozygous deletion produces the dwarf Pygmy mutant in mice. In replication studies of adults sampled from across the height distribution, each copy of the C allele of the SNP was associated with an increase of 0.07 in the adult height z-score, which is equivalent to ~0.4 cm in height.

To determine the age at which the association appears, longitudinal data from the Avon Longitudinal Study of Parents and Children were analyzed. There was no evidence of association at birth, but strong association with height was observed at age 7 years, suggesting that the effect was on longitudinal skeletal growth. Since the Pygmy mice also displayed greatly reduced fat mass, the investigators sought evidence that the association affects BMI, but none was observed.

The authors discussed the fact that HMG proteins are DNA-binding proteins and often serve an architectural function with regard to chromatin structure and modeling, but they did not suggest possible mechanisms through which the polymorphism might alter bone growth.

Weedon MN, Lettre G, Featthy RM, et al. A common variant of *HMG2* is associated with adult and childhood height in the general population. *Nat Genet.* 2007;39:1245-50.

**First Editor's Comment:** *It is ironic that although normal height is probably one of the most studied polygenic traits in humans, the first gene to show a strong effect in the general population is only now coming to the fore. It will be interesting to see how this story unfolds and what other genes are identified with new genomics analysis technology. The genetics of height variation assessed by linkage studies were reviewed in GGH.<sup>1</sup> These identified proteins, whose genes map to chromosomes 2q21 and 6q21 with locus interacting on an epistatic model, account for approximately 20% of height variation. These gene loci contain RUNX2 transcription factors with known functions on linear skeletal growth.*

William A. Horton, MD

**Second Editor's Comment:** *HMG2 encodes "High Mobility Group AT-Hook 2" and is sited on chromosome 12q14.3. It is expressed in undifferentiated mesenchyme. HMG proteins alter chromatin configuration and thereby gene expression. They do so by the binding of their "AT hook domains" to AT-rich DNA; this alters conformation of the double helix and permits transcription complexes to either promote or inhibit transcription of targeted genes. Microdeletions or mutations of HMG2A have been associated with benign neoplasia (lipoma, salivary adenoma, uterine leiomyoma). Truncation of HMG2A secondary to a pericentric inversion of chromosome 12 with breakpoints at 12p11.22-12q14.3 has been associated*

*with a syndrome of somatic overgrowth, advanced bone and dental ages, multiple lipomas and a cerebellar tumor.<sup>2</sup> Truncations of mouse ortholog Hmg2 (Hmg1c) result in somatic overgrowth, lipomas, and increase in body fat.<sup>3</sup> Homozygous deletion of mouse Hmg2 results in decrease in growth.<sup>4</sup>*

Allen W. Root, MD

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## Height and Health-related Quality of Life

Findings regarding associations between height and psychosocial variables are inconsistent. To address perceived methodological and design weaknesses in previous studies, Christensen and colleagues sought to clarify the nature of this relationship by analyzing data collected through a national health survey. Their primary aim was to assess the relationship between stature and health-related quality of life (HRQoL) in an adult general population sample in the UK. Secondly, they sought to evaluate potential moderating effects of social status, age, gender, and chronic conditions on the relationship between height and HRQoL.

This report is based on secondary analyses of the 2003 Health Survey for England (HSE03), conducted between January 2003 and March 2004, by the UK Department of Health. The HSE03 comprises a random general population sample for those living in private households in England (73% participation rate). Observations for 14,416 adults (>18 years of age) were included in the analyses. Height and weight were measured by a nurse; HRQoL was measured using the EQ-5D questionnaire (EuroQoL). The EQ-5D self-report consists of 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 3 possible levels reflecting no health problems, moderate health problems, and extreme health problems. Using a specific British EQ-5D scoring algorithm which converts total scores to quality adjusted life years, the 5 dimensions were summarized into a single score. An individual who has no problems in any domain scores 1.0 and death equals 0.0.

Mean EQ-5D scores were lower in the shorter compared with taller subjects, as well as being lower than the overall population mean. Based on statistical criteria, the total sample was split into 3 standardized height (HSDS) subgroups: (1) HSDS  $\leq -2.0$ ,  $n=606$ ; (2)  $-2.0 < \text{HSDS} \leq 0$ ,  $n=6580$ ; and (3) HSDS  $> 0$ ,  $n=4760$ . In regression analyses adjusting for potential demographic confounds (age, gender, chronic illness, social class, and body weight), subgroup 1 had significantly lower

EQ-5D scores compared with subgroups 2 and 3, and subgroup 2 received lower scores than subgroup 3. Based on regression coefficients, an increase of 1 HSDS would be associated with a statistically significant increase in the EQ-5D score of 0.061 for subjects  $\leq -2.0$  HSDS, 0.010 for those between  $-2.0$  and  $0$  HSDS, and 0.002 for those  $> 0$  HSDS. The increase in EQ-5D score with increasing height in the  $> 0$  HSDS, although statistically significant, was not considered of clinical significance. The main contributors to the reduced HRQoL in the shortest subgroup were problems with mobility, usual activities, and pain/discomfort. The authors concluded that increasing final height in children with short stature may be beneficial and could enhance HRQoL outcomes barring troublesome side effects and excessive cost of treatments.

Christensen TL, Djurhuus CB, Clayton P, Christiansen JS. An evaluation of the relationship between adult height and health-related quality of life in the general UK population. Clin Endocrinol (Oxf). 2007;67:407-12.

**First Editor's Comment:** *HRQoL should (1) represent a multidimensional construct, including several core dimensions (eg, physical functioning and symptoms, psychological and emotional state, and social functioning), (2) be patient, rather than physician, centered, and (3) reflect subjective evaluations of daily functioning and psychological well-being.<sup>1</sup> The use of patient reported outcomes, such as HRQoL measures, are encouraged and may soon be mandated by the FDA for the evaluation and approval of new drugs and medical interventions.<sup>2</sup> Rigorous standards for the development and psychometric evaluation of HRQoL measures have been promoted by the World Health Organization. It is therefore a positive development to see research published examining the relationship between measured height and subjective reports of QoL. In the FDA's review of growth hormone (GH) treatment for the indication of idiopathic short stature (ISS), HRQoL was not utilized as an endpoint in the approval process.<sup>3,4</sup>*

*Christensen and colleagues acknowledged that*

inferring a causal relationship between height and HRQoL is not possible because of the single point, cross-sectional design of this survey. This limitation notwithstanding, they stated that their study "conclusively show(s) a significant correlation between adult height and HRQoL, which may indicate that improving final height in children with growth disorders who are receiving GH treatment should result in positive HRQoL outcomes, even if studies to date do not always show a benefit in childhood or adolescence." However, the use of a single method (the EQ-5D) makes such a statement (even as speculation) premature. Further, no controlled study, to date, has demonstrated a psychological benefit of increased growth velocity/height through the use of GH treatment.

A curious aspect of this study's findings concerns the pattern of scores for individual EQ-5D dimensions. Based on the authors' review of earlier studies suggesting that short stature exerts a psychosocial stress associated with poorer intellectual, psychosocial, and psychiatric function, it is surprising that the main contributors to reduced HRQoL in the shortest subgroup were problems with mobility, usual activities, and pain/discomfort. It is not obvious why shorter participants would more likely experience problems with walking or be confined to bed (as defined by the instrument), or experience more pain or discomfort unless, however, the short stature was accompanied by other features which compromised function—in which case, it is likely that the features accompanying the short stature, rather than the short stature itself, account for the compromised function.

Finally, the investigators pointed out the associations between height and HRQoL in adulthood are nonlinear; ie, it was only among the shortest survey participants (ie,  $<-2.0$  HSDS) that meaningful improvements in HRQoL with increased height was predicted. Provided we accept correlational findings as evidence of causation, one implication of this is that GH-induced increases in adult height beyond  $-2.0$  HSDS would not yield personal benefit. This finding therefore provides empirical support for the ethical argument of terminating GH treatment at the point at which the individual achieves an adult height within the lower portions of the normative range.<sup>5</sup>

In this context, it is noteworthy that this article is not accompanied by a disclosure statement indicating conflict; the first 2 authors' affiliation is listed as Global Development, Novo Nordisk A/S.

David E. Sandberg, PhD

**Second Editor's Comment:** Projects which have tried to assess the effect of height on QoL, either in childhood or adult life, have been bedeviled by underpowered studies, the fallibility of questionnaires as a technique of QoL assessment, and the apparent extraordinary ability of children to adapt to their physical and environmental circumstances. The analysis of the data of Christensen et al showed a significant correlation between adult short stature and HRQoL. Height had a 6-fold greater correlation with HRQoL in the short adult population (ie, height  $<-2$  SD) compared to the taller population subgroup. Very short subjects (height  $<-3$  SD) were particularly affected and had very low QoL. It is likely that this study will be quoted in order to justify treatment of short children with GH. It should be appreciated that an improvement of adult height from  $-2.0$  to  $-1.0$  SD during GH therapy did not change the HRQoL to a large extent. Nevertheless when GH therapy offers the opportunity to make a large difference in adult height, for example in GH deficiency, adult HRQoL is likely to improve significantly.

Martin O. Savage, MD

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## Effects of Gluten-free Diet in Atypical Celiac Disease

Celiac disease frequently presents growth impairments as evidenced by an inflammatory enteropathy from T-cell hypersensitivity to certain cereal antigens; catch-up growth may be induced by initiation of a gluten-free diet. Street et al sought to study children longitudinally over their first year on the diet. Children with atypical celiac disease (patients with typical gastrointestinal symptoms as well as in an atypical fashion) were followed; outcome measures included changes and correlations in growth parameters, insulin-like growth factor (IGF) axis members, and the proinflammatory

cytokines implicated in celiac disease pathophysiology, interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$ .

Twenty children (9 male), aged 4.2 to 14.2 (mean 9.6) years at diagnosis of atypical celiac disease, were followed; 17 completed the one-year evaluations and 3 were lost to follow-up. Of note, all had atypical celiac disease and presented with recurrent abdominal pain, anemia, nausea, occasional vomiting, and fatigue, or were screened due to family history. None had diarrhea or malnutrition, 11 children were prepubertal at diagnosis, and during the year's follow-up, 2 boys progressed from



**Summarized results (mean ± SEM).**

| Parameter             | Celiac disease |            |                | P value <0.05:<br>C= vs ctls<br>D= pre/post diet<br>N= neither |
|-----------------------|----------------|------------|----------------|--|
|                       | Controls       | Baseline   | 1 year on diet |  |
| Height z-score        | 0.09 ± 0.3     | 0.51 ± 0.3 | 0.88 ± 0.4     | D  |
| Target height z-score | -0.4 ± 0.5     | -0.4 ± 0.3 | -              | -  |
| BMI z-score           | 0.5 ± 0.3      | -1.6 ± 0.1 | 0.89 ± 0.3     | C, D   |
| IGF-I (ng/ml)         | 392 ± 47       | 208 ± 32   | 305 ± 35       | C, D   |
| IGF-II (ng/ml)        | 1098 ± 255     | 952 ± 52   | 1008 ± 119     | N  |
| IGFBP-1 (ng/ml)       | 61 ± 7         | 54 ± 8     | 45 ± 9         | N  |
| IGFBP-2 (ng/ml)       | 306 ± 35       | 493 ± 41   | 388 ± 74       | C, D   |
| IGFBP-3 (ng/ml)       | 4216 ± 286     | 4087 ± 300 | 4108 ± 281     | N  |
| IL-6 (pg/ml)          | 1 ± 0.1        | 2 ± 0.6    | 2 ± 0.7        | C  |
| TNF-α (ng/ml)         | 4 ± 1          | 2 ± 1      | 2 ± 0.4        | C  |

Tanner 2 to 3. Mean bone age at diagnosis was 9.4 years, SEM = 0.9 years. Eighteen healthy children (5 male), aged 5.6 to 14.6 (mean 11.1) years, matched for pubertal stage, were evaluated as controls at baseline (Table). Their bone age data were not available.

The authors further examined various IGF/IGF binding protein (IGFBP) molar ratios, simple linear regression analyses and step-wise linear regression analyses to find additional correlates with baseline and treatment values. They concluded, "the data from this study confirm changes in the IGF and cytokine systems at diagnosis of celiac disease which tend to normalize on the gluten-free diet."

Street ME, Volta C, Ziveri MA, et al. Changes and relationships of IGFs and IGFBPs and cytokines in celiac disease at diagnosis and on gluten-free diet. Clin Endocrinol. 2008;68:22-8.

**Editor's Comment:** There are several limitations to this study in considering the authors' conclusions.

The discussion section contains many conjectures about the mechanistic links between cytokines, IGF axis, growth, and disease of patients with celiac disease, stretching even to the increased risk of malignancy in patients with long-standing untreated celiac disease. All the data in this paper were associative. Associations are never sufficient to prove causation, as directionality and confounders remain unknown. There were no supporting mechanistic

studies. Likewise, the paper measured serum concentrations. Local (ie, intestinal) concentrations of the cytokines and IGF axis members are more pertinent to disease activity, and changes may not be reflected in the serum levels. Finally, the study's ability to generalize is limited. The subjects all had atypical celiac disease, so the results do not necessarily support conclusions about celiac disease in toto. However, it is this very limitation that makes the findings of this paper intriguing. None of the patients studied had diarrhea, signs of malnutrition or history of celiac crisis. Although the BMI at baseline was significantly lowered, it was still within the normal range. Likewise, the gluten-free diet improved the height z-score, which was already normal, and even better than target height, at baseline. The finding of significant alterations in serum cytokines, IGF-I and IGFBP-2 within this population speaks to the sensitivity of the IGF system to this disease process.

Adda Grimberg, MD

## Levothyroxine Therapy on Ventricular Function in Neonates with Congenital Hypothyroidism

Decreased thyroid hormone levels are associated with poor left ventricular contractility and relaxation in hypothyroid adults. These abnormalities can be reversed by levothyroxine substitution therapy. Few studies have been done in neonates with congenital hypothyroidism and to date the results have been conflicting. Only standard echocardiography has been used to assess left ventricular function. Tissue Doppler echocardiography (TDE) is a new method that permits evaluation of regional and global left and right systolic and diastolic ventricular function and color codes the velocity of myocardial movement allowing for more accurate quantification.

Fifty neonates (17 to 28 days of age) who were full term and diagnosed with congenital hypothyroidism (TSH >5.6 mIU/L) with a depressed serum free thyroxine

(FT<sub>4</sub> <10 pmol/L) or total thyroxine (TT<sub>4</sub> >54 nmol/L) were studied. A control group of 35 healthy neonates with normal thyroid function levels matched for age, sex, body surface area, and BMI were studied. None of the subjects had congenital heart disease as assessed by clinical and routine echocardiographic studies. Each neonate was studied with both conventional M-mode pulsed wave Doppler and with TDE. The infants were sedated with oral chloral hydrate for the studies. M-mode echocardiography measured left atrial aortic diameter, left atrial/aortic ratio, left ventricular fractional shortening, and left ventricular ejection fraction. In addition diastolic mitral and tricuspid inflow velocity was measured. The TDE permitted measurement of peak early diastolic mitral annular velocity, peak late diastolic mitral annular



velocity, and peak systolic mitral annular velocity, as well as similar measurements for tricuspid velocity.

Using conventional Doppler echocardiography, markers of left ventricular systolic global function were significantly lower in the infants with congenital hypothyroidism. In addition, early and late mitral and tricuspid valve diastolic function were significantly lower in the infants with congenital hypothyroidism. After a month of levothyroxine (L-T<sub>4</sub>) therapy several of the left ventricular parameters improved, but left atrial and aortic diameter did not change. Significantly reduced mitral systolic and early diastolic velocity was found by TDE in the group with congenital hypothyroidism. These significantly increased after therapy, while the peak annular mitral and tricuspid velocity remained unchanged.

Mao et al pointed out that their study was the first comprehensive report of systolic and diastolic function of both ventricles in neonates with congenital hypothyroidism and their data showed impaired left ventricular systolic function which normalized with L-T<sub>4</sub> therapy. Their data also showed that infants with congenital hypothyroidism do not have abnormal left atrial structure. The use of the TDE confirms subclinical impairment of both left and right ventricular contractile function in neonates with congenital hypothyroidism, as well as diastolic dysfunction

of both ventricles. The authors concluded that their data underscore the importance of early detection and treatment of infants with hypothyroidism.

Mao S, Wang Y, Jiang G, Zhao Z. Effects of levothyroxine therapy on left and right ventricular function in neonates with congenital hypothyroidism: a tissue Doppler echocardiography study. *Eur J Pediatr*. 2007;166:1261-5.

**Editor's Comment:** *This is a very interesting and comprehensive study which shows convincing evidence that there is significant cardiac dysfunction in neonates with congenital hypothyroidism. The presence of a control group adds to the significance of the findings. It is interesting that this study, conducted in China, was performed on infants aged 17 to 28 days, prior to the initiation of L-thyroxine therapy. Details of screening for congenital hypothyroidism in China were not presented. It is disturbing that treatment of a hypothyroid infant would be delayed as long as 28 days. One would hope that with improvement in screening techniques such a delay could be reduced. Clearly the authors have presented significant information demonstrating the need for early identification and treatment of this disorder.*

William L. Clarke, MD

## Uterine Development in Turner Syndrome

Bakalov and associates performed a cross-sectional study evaluating uterine development in 86 women with Turner syndrome (TS), aged 18 to 45 years, who were participating in a comprehensive NIH study. All subjects had a karyotype by G-banding consistent with TS in at least 70% of 50 white blood cells. The women were evaluated by either transabdominal (n=68) and/or by transvaginal (n=20) ultrasonography. Longitudinal and anterior posterior fundal diameters were calculated as well as the maximal transverse uterine diameter. Normative data were used to characterize uterine maturity. Historical and treatment data including pubertal development, age of initiation of hormone replacement therapy, type of estrogen used, years of estrogen use, and history of growth hormone therapy were recorded. In the case of spontaneous menarche, the time interval from menarche to the development of amenorrhea was noted.

The mean age of the study population was  $31.8 \pm 7.3$  years. Most subjects (93%) had a karyotype consistent with TS, while 6 (7%) had mosaicism. None had a Y chromosome (intact or abnormal), 15% had spontaneous menarche at age  $12.2 \pm 1.7$  years, but had developed amenorrhea by their late teens. All other subjects (73/86) had started estrogen at an average age of  $15.7 \pm 4.1$  years. Thirty percent (26/86) had also been treated with growth hormone. Almost one quarter (24.4%; 21/86) had a fully developed uterus both in size and shape, while

many (44%; 36/86) had a smaller size uterus (transitional) and 31.4% (27/86) had an immature (cylindrical shaped) uterus. Regression analysis demonstrated that uterine size was influenced significantly by age, years of estrogen use, current use of hormone replacement therapy, history of spontaneous menarche and the type of estrogen medication. There was no correlation between age of first exposure to estrogens and the size of the uterus. The degree of uterine maturity was positively associated with years of estrogen use, history of spontaneous menarche, and negatively associated with the lack of current hormone replacement therapy.

The authors reviewed recent studies from Germany<sup>1</sup> which showed that only mosaic females develop normal uterine size and that karyotype was the only significant predictor of normal uterine development. Findings in the current study were significantly different and 57% of the subjects with a mature uterus had a 45,X karyotype. This may be explained by an average longer duration of estrogen exposure. The authors stated that these findings are encouraging for those women with TS who wish to carry a successful pregnancy. A recent review of women with TS in the US participating in oocyte donation programs found that 69% became pregnant and these pregnancies resulted in the birth of a live infant.<sup>2</sup>

Bakalov VK, Shawker T, Cenicerios I, Bondy CA. Uterine development in Turner syndrome. *J Pediatr*. 2007;151:528-31.

**Editor's Comment:** These authors presented some truly encouraging information for endocrinologists to share with their patients with TS. Indeed hormone replacement therapy is associated with normal uterine development while the age of starting hormone replacement therapy is not a critical factor. Thus those women with TS who wish to participate in oocyte donation programs should be encouraged to do so or may be encouraged to do so with reasonably good assurance that their uterus should

be capable of sustaining a normal pregnancy. As the authors noted, their study could have unexpected biases due to its cross-sectional nature.

William L. Clarke, MD

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## GH Treatment Effects on Body Composition in SGA

The use of growth hormone (GH) therapy in small for gestational age (SGA) children with short stature, now approved and licensed both in the US and Europe, requires critical appraisal. Body composition in childhood may be affected by alteration of fetal growth. SGA infants who show catch-up growth tend to become obese and may be at risk for metabolic syndrome in adult life. However, SGA children who remain short are thin and have a low BMI and possibly compromised bone mineral density. The group of 25 SGA subjects (birth weight and current height <2 SD) reported in this study were prepubertal and randomized to receive either GH therapy (n=16) or act as untreated controls for 3 years and then start GH therapy (n=9). Heights in both groups were <2 SD and the daily GH dose was 1 mg/m<sup>2</sup> body surface area.

Clinical characteristics were comparable in the 2 groups. In the untreated subjects lean body mass (LBM) decreased during the 3 years (P<0.01) contrasting with the GH-treated group which showed catch-up increase of LBM. When the untreated subjects started GH, their LBM SDS also increased significantly. Therefore GH therapy, in the dose described, induced catch-up of LBM. However percentage body fat decreased in the GH-treated subjects. Bone mineral density SDS

measured by DEXA increased significantly in the GH-treated group compared to the untreated subjects.

Willemsen RH, Arends NJ, Bakker-van Waarde WM, et al. Long-term effects of growth hormone (GH) treatment on body composition and bone mineral density in short children born small-for-gestational-age: six-year follow-up of a randomized controlled GH trial. Clin Endocrinol (Oxf). 2007;67:485-92.

**Editor's Comment:** These findings are of interest, but their clinical relevance remains uncertain. The anabolic effects of GH on muscle bulk and bone mineralization are demonstrated, as is its lipolytic effect. However the benefit to the child of these changes is difficult to assess. Is the improvement in BMD really going to prevent development of osteoporosis and increased fracture risk in adult life? The answers are unknown. Is the reduced LBM in the untreated short SGA child actually a disadvantage to the child? Again we are not certain. However, in this report the carefully studied longitudinal changes in body composition which occur during GH therapy are useful in documenting the anabolic and lipolytic effects of GH in short SGA children.

Martin O. Savage, MD

## Widespread Monoallelic Expression of Human Autosomal Genes

With certain exceptions, it is generally assumed that maternally and paternally-derived copies (alleles) of each gene are expressed at comparable levels in humans. The first exception is inactivation of most of the genes residing on the X-chromosome in females—so called X-inactivation. Half of the cells in an embryo on average randomly inactivate the paternal X chromosome and half inactivate the maternal X chromosome around the time of implantation. The second exception involves imprinting of autosomal genes, such as IGF-2, on a parent-of-origin basis. A third exception is a small group of autosomal genes that are subject to random monoallelic expression; these include genes encoding odorant receptors, T cell receptors, interleukins, and natural killer cell receptors. There is new evidence that monoallelic expression of autosomal genes may be

much more extensive than previously believed.

Gimelbrant et al exploited the growing number of single nucleotide polymorphisms (SNPs) and advances in gene chip (array) technology to survey allele-specific transcription of about 4,000 genes in lymphoblastoid cell lines from 3 individuals. They took advantage of the observation that once a cell decides to express one of 2 alleles, the clonal descendants of this cell continue to express the selected allele. Since lymphoblastoid cells are polyclonal, they were able to derive clonal B cell lines using single-cell cloning.

To perform the genome-wide screen for monoallelic transcription, the investigators developed protocols to distinguish polymorphic allele expression based on detection of SNPs in nuclear RNA, which is enriched in intronic RNA, where most SNPs associated

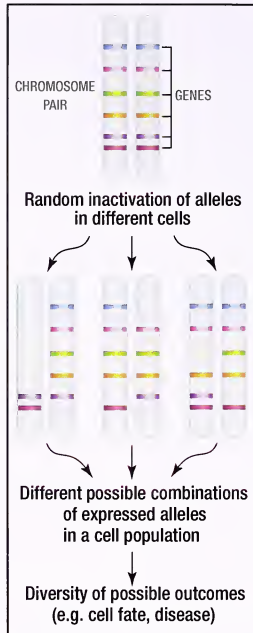
with genes reside. Conversion of this RNA to double-stranded cDNA and analysis on a SNP array generated "transcriptosome-derived genotypes" that allowed monoallelic expression to be identified. Filters were used to minimize cDNA genotyping artifacts. About 10% of SNPs were reliably called from this analysis, which was expected since most of the other SNPs are likely present in regions of the genome that are not expressed by B cell lines that were studied.

As proof-of-concept, the investigators first showed that random inactivation of X-chromosome genes could be detected in the clonal cell lines and then demonstrated as an example of their approach that monoallelic expression of the amyloid precursor protein gene could be detected. They next turned to genome-wide screening.

On the array used for analysis, there were SNPs present for ~11,000 genes. They were able to detect allele-specific transcription for ~4,000 genes in 2 or more cell clones. Of the ~4,000 genes examined, 2.2% were detected as monoallelically expressed with multiple informative SNPs per gene per clone. An additional 7.3% of assessed genes were identified as monoallelically expressed based on a single informative SNP per gene per clone. The genes included both B cell-specific genes and ubiquitously expressed genes. The investigators suggested a conservative estimate that over 1,000 genes are subject to random monoallelic expression in humans.

Several interesting observations were made. For example, the choice of expressed allele was made independently for each gene within a given clonal cell line. This is in contrast to the chromosomal-wide coordination characteristic of X-inactivation. Another finding was that a disproportionately large fraction of genes coding for cell surface proteins—transmembrane receptors and surface proteins was detected.

The authors concluded by suggesting that at least 1,000 human genes display random monoallelic transcription



**Generating diversity.** Alleles are randomly inactivated on a pair of chromosomes in a human somatic cell. The various patterns of inactivation in progeny cells are then stabilized (epigenetically). This can generate diverse cellular and physiological outcomes. Reprinted with permission Ohlsson R. Science. 2007;318:1077-8. Copyright © 2007 AAAS. All rights reserved.

that could contribute to genetic diversity within tissues of an individual as well as between individuals. A commentary by Ohlsson<sup>1</sup> notes that although monoallelic expression has been known in humans, this study by Gimelbrant expands the concept further especially by documenting it in a much larger number of genes than previously appreciated. He briefly discusses possible mechanisms that could account for the phenomenon as well as its potential role in modulating disease.

Gimelbrant A, Hutchinson JN, Thomson BR, Chess A. Widespread monoallelic expression of human autosomes. Science. 2007;318:1136-40.

**Editor's Comment:** This is one of several publications in recent years that challenges what we were taught about mendelian genetics. Of note, several genes relevant to human growth disorders were identified as displaying monoallelic expression including the growth hormone receptor gene (GHR) and genes that harbor mutations responsible for Ellis van Creveld syndrome (EVC) and the trichorhinophalangeal syndrome 1 (TRPS1). It seems quite plausible that monoallelic expression of these genes could contribute to the clinical variability of these conditions.

Lymphoblastoid cells have very different functions compared to chondrocytes, osteoblasts and other cells that contribute to skeletal growth; and their patterns of gene expression may differ dramatically. Screening the latter cells for monoallelic transcription would be technically much more difficult than for lymphoblastoid cells, but it would likely reveal monoallelic expression of additional growth related genes.

William A. Horton, MD

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## Stroke, Cardiac Disease and Diabetes Mellitus in Hypopituitarism

The impact of long-term growth hormone deficiency (GHD) and of long-term growth hormone (GH) treatment on cerebrovascular and cardiovascular diseases and diabetes mellitus is unknown. Holmer et al evaluated

the incidence of nonfatal stroke and cardiac events and the prevalence of type 2 diabetes mellitus (T2DM) in a cohort of GHD patients and healthy controls. The authors also studied the effects of cardioprotective drugs and 6



years of GH-replacement treatment in this population. The incidence of nonfatal stroke and cardiac events was estimated retrospectively from questionnaires in 750 GHD patients (53% males and 47% females) and in 2314 matched population controls. GHD patients were recruited from the departments of endocrinology at all Swedish University hospitals and one county hospital. All patients were diagnosed as having severe GHD by dynamic testing (peak GH <3 mcg/L). The lifelong incidence of nonfatal stroke was tripled in GHD women and doubled in GHD men. A decline was noted in both genders following the detection of the first pituitary hormone deficiency and GHD, a period of time during which most patients received GH therapy. The lifelong incidence of nonfatal cardiac events declined in GHD men; GHD women had a higher prevalence of T2DM. Women were twice as likely to be taking lipid-lowering drugs as the population controls, while GHD men had a 28% higher prevalence for the use of antihypertensive medication. The authors concluded that the decreased risk of nonfatal stroke in both genders and of nonfatal cardiac events in GHD men may be due to the larger prescription of cardioprotective drugs and to 6 years of GH-replacement. The increased prevalence of T2DM in GHD women can be partly attributed to a higher body mass and to decreased physical activity.

Holmer H, Svensson J, Rylander L, et al. Nonfatal stroke, cardiac disease and diabetes mellitus in hypopituitary patients on hormone replacement including growth hormone. *J Clin Endocrinol Metab*. 2007;92:3560-7.

**Editor's Comment:** *An increased incidence of cerebrovascular and cardiovascular mortality in patients with hypopituitarism on conventional hormone treatment, but without GH therapy, has been reported in recent epidemiological studies.<sup>1,2</sup> GHD is believed to be responsible for the early atherogenesis in hypopituitarism,*

*as cardiovascular risk factors have been improved with GH treatment in this group of patients. Glucose intolerance, T2DM, and hypertension are increased in GHD.<sup>3</sup> Diastolic blood pressure tends to decrease with GH treatment, while insulin sensitivity is impaired following initial GH replacement, but may improve later as fat mass is reduced. This study showed that hypopituitary patients had a higher lifelong incidence of nonfatal stroke (triple in GHD women and double in GHD men), although cerebrovascular events decreased in men and women during the periods following the diagnosis and the treatment of the pituitary hormone deficiencies and of GHD. This decline was probably due to the long-term use of GH and the replacement of thyroxine and glucocorticoids. Additionally, patients may also benefit from the increased administration of lipid-lowering and antihypertensive medications. The increased prevalence of T2DM in GHD women could not be attributed to overtreatment with GH as the IGF-I level was at mid range. Additionally, acromegaly and Cushing's disease were excluded in these patients, thus the increased prevalence of T2DM was partly attributed to their higher BMI and their lower physical activity. Long-term surveillance for cardiovascular disease and T2DM seems necessary in hypopituitary patients; the institution of appropriate treatment with hormone replacement and cardioprotective drugs plays a positive role in decreasing the risk of nonfatal stroke and the risk of nonfatal cardiac events in men; an increased prevalence of T2DM seems to be present in GHD women.*

Roberto Lanes, MD

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## Growth and Metabolism in In Vitro Fertilization Children

In vitro fertilization (IVF) singleton children have an increased risk of malformations and low birth weight. They also face an increased risk of disorders with overgrowth partly due to abnormal methylation patterns of imprinted genes. Nutritional manipulation early in fetal life has also been shown to reduce methylation and over expression of non imprinted genes. Miles et al conducted a study regarding the long-term outcome of IVF children, an area in which there is still a lack of information. The authors investigated growth and changes in the metabolic and hormonal profile of this population. Healthy prepubertal children aged 4 to 10 years, born at term, after singleton pregnancy, were recruited into IVF and control groups. All subjects had been breastfed. There were 69 IVF children (5.9 years) and 71 control children (6.9 years). Anthropometric measurements and BMI were recorded, focusing on fat and glucose metabolism, and insulin-like

growth factor (IGF)-I levels. Both groups were matched for parental anthropometry, socio-economic factors and dietary conditions. IVF children were taller than controls (and girls were even more so) when height was corrected for parental height. This increase in stature was proportionate. It occurred despite a lower birth weight. The corrected BMI was lower in the IVF group and there was no difference in percent fat assessed by DEXA. There was a trend toward higher IGF-I levels in the IVF group with patients above 7 years of age having the highest levels. The IGF-I/IGF binding protein (IGFBP)-3 ratio was also increased. IGF-II was elevated as well in the IVF group without any age related effect.

For all children there was an association between tall stature and high IGF levels. A favorable metabolic profile was found in the IVF group with higher HDL, lower triglycerides and a low total to HDL/cholesterol



ratio. There was no difference in body composition. The authors speculated that IVF results in epigenetic changes altering genes involved in growth and metabolism that could be similar to the changes shown in specific syndromes like Beckwith-Wiedemann.

Miles HL, Hofman PL, Peek J. In vitro fertilization improves childhood growth and metabolism. *J Clin Endocrinol Metab.* 2007;92:3441-5.

**Editor's Comment:** Since this technique was introduced, IVF has accounted for a growing number of births.<sup>1</sup> Only recently have follow-up studies focused on the postnatal outcome of these children. A few informative and elegant studies have already drawn our attention to epigenetic changes that induced major malformations. The most investigated area is the overgrowth disorder of Beckwith-Wiedemann with a variable clinical expression from the full syndrome to isolated overgrowth. It has been shown that there are imprinting disorders observed in humans and animals born after the use of assisted reproductive technology. The genomic imprinting defects relate to an epigenetic marking of certain genes, resulting in monoallelic expression in a parent-of-origin-dependent manner. Imprinting control elements are characterized by differentially methylated regions in which the imprinted allele is methylated and the other parental allele is unmethylated. Imprinting is established during the development of the germ cells and must be maintained at a critical stage of pre-implantation development when the rest of the genome is subjected to a wave of

demethylation. These imprinted genes have a major role in fetal growth and development. All imprinting disorders observed after assisted reproductive technology involve the maternal side inducing a maternal to paternal switch, with activation of non-coding RNA on the maternal side. The cause of association with IVF is unknown. One can only suggest that nutritional and environmental factors, or periconceptual or preimplantation conditions could result in these alterations. Furthermore, we do not know whether the clinical changes already observed are reversible. Hence their significance remains unclear.

Miles et al described an increased incidence of tall stature in prepubertal children which was accompanied by increased levels of IGF-I and IGF-II. It was suggested that this "overgrowth" might be the consequence of programmed endocrine changes related to the IVF process. Remarkably, in this group of children the body composition and the lipid profile were normal. However, in another recent study the offspring of those conceived by IVF presented significantly higher peripheral adipose tissue.<sup>2</sup> It is difficult to compare the metabolic status of the 2 populations because of age differences and methods used in both studies. Appropriate follow-up should be established for all IVF children.

Raphaël Rappaport, MD

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## Hypopituitarism Following Traumatic Brain Injury and Subarachnoid Hemorrhage

The hypothalamus and pituitary are vulnerable to injury and dysfunction following traumatic brain trauma (TBI) and subarachnoid hemorrhage (SAH). These constitute worldwide public health problems and leading causes of death and disability in young adults. Survivors of both TBI and SAH are at a great risk of significant neuroendocrine dysfunction, adverse physical and/or psychological problems, depression, and sleep disturbances that result in disturbed quality of life (QOL).

Schneider et al searched the MEDLINE database for articles published between 2000 and 2007 pertaining to TBI and SAH. They identified 19 studies including 1137 patients (1015 TBI patients and SAH 122 patients). Only 2 of these studies (with 74 patients) reported on pediatric populations. The authors investigated 13 studies (with 809 TBI patients and 102 SAH patients) that were performed at least 5 months following the injury (chronic phase). They excluded studies in the early phase after injury to avoid the confounding effect of acute critical illness on neuroendocrine function and the pediatric populations for reasons of homogeneity. The pooled prevalence of anterior hypopituitarism in the chronic phase after TBI and SAH was 27.5% (95% CI, 22.8%-28.9%) and

47% (95% CI, 37.4%-56.8%), respectively. The pooled prevalence of hypopituitarism was greater in patients with severe TBI, as compared with those with mild or moderate TBI (as defined by the Glasgow Coma Scale). On the contrary, clinical severity of SAH did not help discriminate between patients at high and low risk of developing hypopituitarism. Early neuroendocrine abnormalities were transient in some patients while hypopituitarism evolved over time in others.

The authors considered that hypopituitarism appears to be a common occurrence following TBI and SAH and might contribute to morbidity and poor recovery after brain injury although most cases remained unrecognized and untreated. All patients hospitalized for TBI or SAH should be evaluated for endocrine alterations long-term.

Schneider HJ, Kreitschmann-Andermahr I, Ghigo E, Stalla GK, Agha A. Hypothalamic-pituitary dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage. A systemic review. *JAMA.* 2007;296:1429-38.

**Editor's Comment:** Another article regarding TBI published in the recent literature was reviewed in GGH last year.<sup>1</sup> The current paper by Schneider et al provides

specific information regarding patients with SAH. Considering the large number of individuals who have TBI and SAH each year, post-traumatic hypopituitarism is an important public health issue. TBI and SAH pose substantial risks to hypothalampituitary dysfunction. Hypopituitarism after TBI and SAH might contribute to a delayed or hampered recovery during rehabilitation. However, in both adults and children, a large number of patients with hypopituitarism after TBI or SAH remain undiagnosed and untreated.

Possible causes of hypopituitarism include hemorrhage, infarction, ischemia, necrosis, fibrosis, swelling, stalk transection, or direct trauma to the hypothalamus, stalk, and/or pituitary region. The severity of TBI seems to be an important risk factor for developing hypopituitarism, however, post-traumatic hypopituitarism can also manifest after even mild TBI. Whereas hypothalampituitary dysfunction occurred without regard to the severity of SAH.

The signs and symptoms associated with hypopituitarism are often nonspecific and mimic the sequelae of TBI and SAH such as depression, neuropsychological deficits, or personality changes. They are likely to be overlooked if endocrine dysfunction is not actively assessed. Moreover, hormonal deficits may contribute to the

chronic disability and the physical, cognitive, health, and social sequelae in patients with TBI and SAH. Therefore, accurate endocrine evaluation and long-term follow-up of TBI and SAH patients are necessary in order to detect the occurrence of hypopituitarism, regardless of clinical evidence for hypothalampituitary dysfunction. In order to improve outcome and quality of life of TBI and SAH patients, adequate hormone replacement therapy may be necessary in those who develop hypopituitarism. It is necessary for physicians as well as patients and family members to know that hypothalampituitary dysfunction following TBI and SAH may occur long after the initial trauma. A close collaboration among neurosurgeons, neurologists, rehabilitation specialists, internists, pediatricians, and endocrinologists is essential to achieve a coordinated approach to the care of patients with TBI and SAH. The consensus guidelines for assessment and for clinical practice of such patients have been published.<sup>2,3</sup>

Yoshikazu Nishi, MD

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2. Ghigo E, Masel B, Aimaretti G, et al. Brain Inj. 2005;19:711-24
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## FTO Gene Association with BMI and Obesity

Frayling et al and Dina et al have both linked a common variant in a set of single nucleotide polymorphisms (SNPs) in the first intron of FTO (fat mass and obesity associated gene; OMIM 610966, chromosome 16q12.2) with early onset of severe obesity in children and adults of European ancestry. FTO has 9 exons; its product and function are as yet unknown. In the report of Frayling et al, a genome-wide association study of 490,032 SNPs and their relationship to type 2 diabetes mellitus (T2DM) was conducted and 10 SNPs in intron 1 of FTO (designated A allele) was found to be closely related to this disorder. Further analysis revealed an even stronger association between BMI and the FTO intron 1 SNPs variant. In adults of all ages and both genders, each A allele was associated with an increase in BMI of 0.10 z-score units (~0.4 kg/m<sup>2</sup>). Adult carriers of one A allele had an odds ratio of 1.31 for being overweight (BMI >25 kg/m<sup>2</sup>) and of 1.18 for being obese (>30 kg/m<sup>2</sup>); subjects homozygous for the A allele had 1.38 risk of being overweight and a 1.67 risk of obesity. Similar studies in children and adolescents between 7 to 14 years of age revealed that those with one A allele had an odds ratio of 1.27 for being overweight and of 1.35 for being obese. Waist circumference, skin-fold thickness, and DEXA measurement of fat mass were increased in children with the A allele. Frayling and co-workers found no functional variants in the exonic

sequences of FTO relative to the SNPs variation in intron 1. Thus, the manner in which this variant of FTO affects weight accumulation is as yet unknown. Dina et al also associated the A allele with severe obesity in adults (BMI >40 kg/m<sup>2</sup>) as well as with early-onset obesity in children, but found no mutations in the coding regions of FTO. Both groups concluded that a variant in SNPs in intron 1 of FTO is associated with an increased risk of obesity in children and adults, but the mechanism of the effect remains unexplained at present.

Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316:889-94.

Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet. 2007;39:724-6.

**Editor's Comment:** Experimentally in mice, deletion of the chromosome segment in which FTO is located is embryonically lethal in the homozygotic animal and is marked by fused toes and thymic hyperplasia in the heterozygotic mouse that is of normal weight. Therefore, the composition, structure, and functional properties of the product(s) of FTO variants may need to be identified by methods other than those that attenuate (or enhance?) expression of FTO in experimental animals. If these goals can be successfully accomplished and the

*functional relationships between variants of FTO and the regulation of energy metabolism and conservation elucidated, then it may be possible to design agents that can be directed to sites of FTO action that will ultimately lead to improved methods of weight control.*

*Interestingly, the presence or absence of the A allele was not associated with birth weight.*

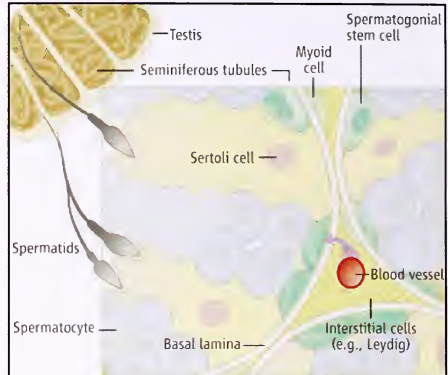
Allen W. Root, MD

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## A Niche for Undifferentiated Spermatogonia

In human males, spermatogenesis proceeds over several decades. Scattered throughout the spermatogenic tubules of mammalian testes are spermatogenic stem cells (cells that are able to self-renew and to differentiate into cells with more specialized functions) that appear to be localized to specific regions within the tubule (Figure). In mice, undifferentiated spermatogenic stem cells constitute less than 1% of testicular cells and periodically differentiate into primitive type A single (As) spermatogonia that then give rise to daughter cells—A paired (Apr) and A aligned (Aal)—chains of 4 to 32 cells—that in turn evolve into more mature spermatogenic cells.<sup>1</sup> The tubular regions that harbor the most primitive and undifferentiated spermatogenic stem cells are termed “niches” and are deemed important because of the environment provided therein that enables the undifferentiated A cells to survive and from which daughter cells migrate and populate the spermatogenic tubules permitting the decades-long process of spermatogenesis. Yoshida and co-workers have identified the sites of As localization by labeling undifferentiated A cells with green fluorescent protein (GFP) expressed in response to a regulatory sequence of a gene (Ngn3) expressed in spermatogenic cells. Utilizing time-lapse imaging to follow the course of GFP cellular expression in intact mouse testes, they localized the earliest mouse spermatogenic stem cells (As) to specific regions in spermatogenic tubules; these cells reside in a basal tubular compartment adjacent to the interstitium and across from blood vessels that are surrounded by interstitial cells (including Leydig cells); these sites are characterized by turns in the spermatogenic tubule and by branching of their associated blood vessels. As As cells transitioned to Apr and Aal cells, they migrated from the site of origin and spread throughout the basal tubular compartment giving rise to more differentiated spermatogonia, spermatocytes, spermatids, and sperm. The investigators confirmed these observations by transplantation of testicular fragments from donor testes that had been cleansed of vessels and interstitium to sites beneath the tunica albuginea of recipient testes in vivo. Three months later, the grafts had revascularized, the interstitium had been reconstituted, and spermatogenesis was normal; As cells were again localized to turns in the tubules across from branch points of the blood vessels that were themselves encased in interstitial cells. The authors suggested that the niche for As cells by proximity of the tubular basal compartment to the branch point of blood vessels and to abundant interstitial cells provides



**At home, in small narrow places.** Spermatogenic stem cells localize to interstitial regions between seminiferous tubules in the mouse testis. This implies that interstitial cells and branching blood vessels secrete factors (arrow) that influence stem cell fate.

Credit: Adapted by P. Huey/Science, Reprinted with permission DiNardo S, Braun R. Science.2007;317:1696-7. Copyright © AAAS 2007. All rights reserved.

a microenvironment in which “signals” from these cells recruit, nourish, and stimulate differentiation of spermatogenic stem cells. The biochemical nature of these signals is unknown but likely include testosterone, a factor known to be important for the earliest stages of spermatogonial differentiation, as well as products of the Sertoli cells. That niches can be reconstituted (as demonstrated by the testicular graft experiments) indicates that new niches can be developed, a process that would support long-term spermatogenesis.

Yoshida S, Sukeno M, Nabeshima Y-I. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. Science. 2007;317:1722-6.

**Editor's Comment:** Identification of the sites within the spermatogenic tubule that harbor undifferentiated spermatogenic stem cells may prove beneficial in isolating such cells. Inasmuch as these are cells with the diploid number of chromosomes (ie, prior to the first meiotic division), spermatogenic stem cells may ultimately provide a source of pluripotent stem cells.<sup>1</sup>

Allen W. Root, MD

#### Reference

1. DiNardo S, Braun RE. Science. 2007;317:1696-7.



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## GROWTH HORMONE ADMINISTRATION: IS IT SAFE AND EFFECTIVE FOR BODYBUILDING AND IMPROVED ATHLETIC PERFORMANCE?

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### INTRODUCTION

Athletes and the media have demonstrated great interest in the subject of administration of growth hormone (GH), popularly referred to as *doping*. Thus, a review of the evidence for safety and efficacy in athletes, especially, adolescents, is warranted. Many other drugs are administered off-label, particularly a majority given to children and adolescents. However, recombinant human GH (rhGH) is unusual because off-label prescribing and

administration is illegal if given for indications not approved by the US Secretary of Health and Human Services (HHS). In this article—following short sections on the physiology of GH and the clinical role of rhGH—the data pertinent to athletic performance and problems with detection of doping are presented. This article ends with the specific legal issues and the activity surrounding further legal action with reference to rhGH and athletes.

The consideration of rhGH as an ergogenic aid and its potential to enhance athletic performance and/or body composition goes back many decades.<sup>1</sup> Due to the banning of rhGH use for officially sanctioned sport, there has been a large effort to detect its

### From The Editor's Desk

Dear Colleague:

We have applied for and expect to be granted the opportunity of granting CME credits for reading GGH. Thus, while you enjoy the lead article and each one of the reviews you may also meet your requirements for licensure in the comfort of your home or office. In the near future the CME application and questionnaire will be posted on line. Once you complete it you will attain the necessary credits. Hopefully this feature will add to the value you place to the journal.

The current issue of GGH includes a very timely review of the safety and effectiveness of growth hormone for body building and improved athletic performance. The article by Dr. Alan Rogol brings us up to date and clarifies the issues that were widely discussed during this past summer's Olympic Games in China. However the article should also serve the pediatric endocrinologist to guide their patients and their families who seek this treatment to enhance their children's abilities. It should also serve as a resource to warn them of the illicit use of this product for such purposes, as well as to caution them to avoid falling prey to the multiple ineffective, expensive, and unregulated products available for purchase through the Internet. I also want to bring to your attention the reviews on the genetics of stature and the genetics of dwarfism. These excellent reviews include a synthesis of the state of the art of the most current papers and concepts in the field. Also noteworthy is the review dealing with the limited workforce of pediatric endocrinologists.

The economic situation of GGH continues to worsen with the downturn of the economy, yet we do not qualify for a bailout. Therefore I would appreciate your support in the form of a generous contribution so we may continue fulfilling your educational needs. I am sure you are being swamped with donation requests, please put GGH on top of your list and make your tax-deductible contribution to Pediatric Sunshine Academics, Inc. at [www.PedSacademics.org](http://www.PedSacademics.org) or mail to 1040 Alston Road, Santa Barbara, CA 93108.

Happy Holidays and Best Wishes for 2009  
Fima Lifshitz, MD  
Editor-in-Chief



presence in athletes.<sup>2,3</sup> More than ten years and millions of dollars have been spent on devising and implementing tests to detect doping. Despite anecdotal reports of the widespread use of doping no athlete has been sanctioned for the use of rhGH, even with the multiple seizures of rhGH from athletes and teams.

Why should pediatric endocrinologists be concerned about this seemingly esoteric subject? Pediatric endocrinologists have been counseling children, adolescents, and their parents about the height-increasing properties of rhGH for decades. The use of rhGH is legitimate in children who are truly small, such as those with GH deficiency, and other disorders (Table 1). As athletics and sports play an ever increasing and important role in the lives of children and adolescents, parents seek a competitive edge for their children. Families spend thousands of dollars on coaching and equipment in hopes of the possibilities of college scholarships and/or professional contracts. Therefore, pediatricians are now being asked to prescribe rhGH because of parental "beliefs" that it will improve athletic performance in children and adolescents. Although very expensive, parents may consider rhGH as seemingly little different from very expensive coaches, equipment, and training camps.

**Table 1. FDA-approved indications for rhGH therapy in children**

|   |
|---|
| Growth hormone deficiency   |
| Chronic kidney disease  |
| Turner syndrome   |
| Small-for-gestational age infants who fail to catch-up to the normal growth percentiles |
| Prader-Willi syndrome   |
| Idiopathic short stature  |
| SHOX gene haploinsufficiency  |
| Noonan syndrome   |

### PHYSIOLOGICAL ROLE OF GH

The physiological role of GH is to increase linear growth in children, to promote anabolic (tissue building) metabolism, and to alter body composition as part of this anabolic role. Growth hormone actions include the hepatic and local synthesis and release of its main mediator-protein, insulin-like growth factor (IGF)-I. The growth-promoting effects of GH include longitudinal bone growth by actions at the epiphysis and the differentiation of the prechondrocytes. GH shares some of these roles with IGF-I, meaning that the direct effect of GH and/or local production of IGF-I are both necessary for optimal growth.<sup>4</sup>

Stimuli to GH release include deep sleep, exercise, stress—including heat stress—hypoglycemia, and some amino acids. Some pharmacological agents are also stimuli to

the release of GH, for example, beta-2 adrenergic agonists, clonidine, L-DOPA, and estrogens and androgens (through an estrogen dependent mechanism). Inhibitory influences include obesity or ingesting a carbohydrate-rich diet. The direct effects of GH lead to increased glucose availability, increased free fatty acid levels and an increase in amino acid uptake by muscle. Longer term effects are mediated via IGF-I and include endocrine and paracrine effects in muscle and bone.<sup>4</sup>

Alterations in GH-deficient subjects include: the reduction of lean body mass, an increase in body fat, and a reduction in bone mineral density. From this the major metabolic effects of GH can be deduced. Administering rhGH reverses many of these alterations. However, it is not quite so simple, because GH has different effects depending upon the time following natural secretion (GH) or exogenous administration (rhGH). It is insulin-like in the first few minutes, but after several hours GH becomes diabetogenic and is anti-insulin at the liver and at peripheral sites, glucose utilization is decreased, lipolysis is increased, and the tissues are refractory to the acute insulin-like effects for several hours. The direct actions of GH include amino acid transport in muscle permitting protein synthesis and an increase in nitrogen balance, increased fat mobilization through lipolysis (increased triglyceride hydrolysis to free fatty acids and glycerol and reduction in fatty acid re-esterification) and an augmentation of lipid oxidation (Figure 1). These effects may be detected not only by decreases in body fat and in adipocyte size, but also by a decrease in lipid content per adipocyte.<sup>4</sup>

### CLINICAL ROLE OF rhGH

Short children are prescribed rhGH to promote linear growth<sup>5</sup> (Table 1) and that is the most visible result of rhGH treatment in infants, children, and adolescents. Additionally, rhGH prevents hypoglycemia in some infants with congenital hypopituitarism. In adults, rhGH is administered<sup>6</sup> to promote physiologic and psychological well-being (Table 2).

**Table 2. FDA-approved indications for rhGH therapy in adults**

|                                |
|--------------------------------|
| Growth hormone deficiency      |
| Muscle wasting due to HIV/AIDS |
| Short bowel syndrome           |

The outcome of rhGH replacement therapy in a GH-deficient child or adolescent may be an increase in fat-free mass, both body cell mass (muscle) and total body water (especially the extra-cellular compartment), and a decrease in body fat with a redistribution from central to peripheral.<sup>7</sup> Controlled experiments in hypopituitary adults have shown that the baseline decrease in functional capacity of approximately 20% reverts to

normal when measured as maximal oxygen uptake ( $\text{VO}_{2\text{max}}$ ), aerobic capacity, maximal power output, or ventilation threshold with rhGH treatment.<sup>8,9</sup> The increase in  $\text{VO}_2$  was proportionate to the increase in lean body mass (the respiring tissue). A decrease in fatigue was also reported; this is likely due to the decrease in the ventilatory threshold or lactate threshold as a percentage of maximal oxygen uptake. This was perceived as being able to work within a comfortable range.

How might this happen? One should note that the requirements for the increase in work require metabolic fuels, oxidation (intermediary metabolism), and useable energy. The immediate burst comes from the oxidation of glucose and the more prolonged capacity from the oxidation of free-fatty acids. By increasing ventilation and oxygen transport by hemoglobin GH directly leads to an enhanced delivery of substrate and oxygen to respiring muscle. Increased cardiac output (stroke volume and left ventricular ejection fraction) permits the distribution of the oxygen to the capillary network and

to the extraction of the oxygen by muscle fibers, either to be used directly or stored in myoglobin. Other effects that enhance the delivery of oxygen include diminished systemic vascular resistance. In all of these physiologic responses GH and exercise are likely additive and, perhaps, synergistic. Indirect effects of GH (likely mediated by IGF-I) are related to the alteration in lean body mass and in more efficient thermoregulation.

## GH AND ATHLETIC PERFORMANCE

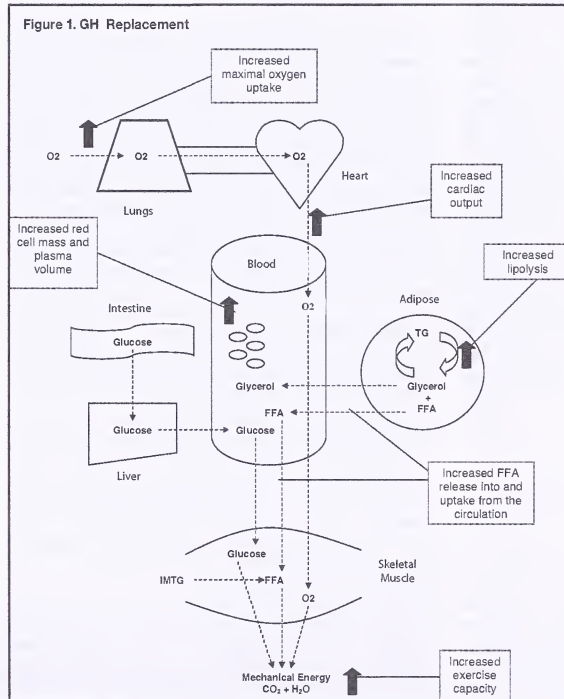
In the 1940s the first GH was extracted from human cadaver pituitary glands.<sup>10</sup> The GH that was derived was in such limited quantities that there was none available for the purposes of testing it for athletic performance.<sup>11</sup> Only human and monkey pituitary GH has efficacy in man.<sup>12,13</sup> In 1985 synthesized rhGH received FDA approval. Thus a virtually unlimited supply became available and clinical studies were undertaken in children and adolescents with subnormal growth and in adults with GH deficiency, aging, as well as for performance or aesthetic purposes. The evidence is neither clear nor robust that rhGH produces

salutary ergogenic and performance benefits among athletes.<sup>14</sup>

## Definition of Doping

The International Olympic Committee (IOC) defines doping as the "use of an expedient (substance or method) which is potentially harmful to athletes' health and/or capable of enhancing their performance, or the presence in the athletes' body of a prohibited substance or evidence of the use thereof or evidence of the use of a prohibited method". There is no mention of intent or of how the substance entered the body. If the substance is in the athlete's body then the athlete is responsible. That is the basis for sanctions for testing positive for a prohibited substance. Sir Arthur Porritt, first chairman of the IOC Medical Commission, noted, "To define doping is, if not impossible, at best extremely difficult, and yet everyone who takes part in competitive sport or who administers it knows exactly what it means. The definition lies not in words but in integrity of character."

In fact, there are huge pressures to excel. Athletes are driven to perform their best and along with the pressure to win there is often an attitude that doping is necessary to achieve success. Expectations about success include potentially lucrative financial rewards with winning, such



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as collegiate scholarships and salary as a professional athlete. The rationale for taking ergogenic effectors such as rhGH is that by becoming bigger and stronger the athlete will perform better. It should be noted that performance is much more than just strength or endurance; for the athlete must produce, control, and efficiently use the energy in a fashion that maximizes athletic performance.

There is a system in place for therapeutic use exemptions to doping for those athletes who require the *substance* for health; for example, insulin is permissible for those with diabetes mellitus. This is a formal process overseen by the US Anti-Doping Agency (USADA) as the local agent for the world anti-doping effort as directed by the World Anti-Doping Agency (WADA).

### Abuse of rhGH

The illegal indications for rhGH are listed in Table 3. Growth hormone is listed under class S2 of hormones and related substances in terms of the 2006 WADA and IOC prohibited list of doping agents. Other peptides in this category include erythropoietin (EPO), corticotrophin (ACTH), IGF-I, and insulin. It is likely that rhGH is being abused at an increasingly prevalent rate. However, it should be noted that much of what is purported to be rhGH—especially products promoted on the Internet—is not. Of course, any drug taken orally cannot be rhGH. Many of the products advertised online and in magazines are GH releasers, mainly amino acids and rarely, analogues of GH releasing hormone (GHRH).<sup>15</sup> It is also worth noting that these are considered “dietary supplements” and not subject to FDA oversight. The notion that amino acids release GH is on solid scientific ground given that tests for GH sufficiency may include arginine, or the closely related amino acid, ornithine. What isn’t stated is that very concentrated solutions of these amino acids are administered intravenously before GH is released. Also not prominent is the physiologic concept of the absolute and then relative refractory period following GH release, irrespective of the cause.

**Table 3. Off-label/illegal use of rhGH**

|                                  |
|----------------------------------|
| Anti-aging                       |
| Athletic performance enhancement |
| Body building                    |

A casual Internet search (in June, 2008) using the key words “hGH AND sport performance” yielded approximately 158,000 web links, mainly to sites that had multiple supplements to sell. Many of the listed products require administration for many months. A few examples included:

- Chromium, l-ornithine, l-arginine, l-lysine, l-glutamine, l-glycine, “pituitary” powder, colostrums, placental

extract and choline. 60-day supply \$49.95

- hGH energizer containing: vitamin B-6, tribulus, l-arginine, l-leucine, l-glutamine, l-lysine, gamma-aminobutyric acid (GABA), l-isoleucine, l-valine, colostrums, l-ornithine and l-glycine. It is touted as an “all natural hGH supplement.” 90-day supply \$29.95
- A nasal spray. It contains: alpha GPL, GABA, multiple amino acids, many as noted above, l-DOPA, bean extract, momiyo extract and alpha-ketoglutarate—I suspect that many other substances are included! 90 day supply \$59.95

Finally, something that might be rhGH for injection, but one must complete a form for a free (medical) consultation and thus presumably for a physician to write a prescription. It is important to note that if a prescription is written for anti-aging, body building, or athletic performance, a felony has been committed by the prescriber, the recipient, and (presumably) the dispenser (Table 3). Cost is not noted, but likely ranges in the \$5,000 to \$50,000-range depending on the size of the recipient and the dose per kg.

There are many reports that have noted an increasing prevalence of rhGH abuse. These primarily come from anecdotal “information” on the benefits of GH posted on the Internet, as well as a dated, but very favorable write-up in *The Underground Steroid Handbook*.<sup>16</sup> The press has reported an increasing number of seizures from elite athletes including cyclists and swimmers. What is it that athletes expect to obtain from taking rhGH? The athletes want improved performance, but such studies are difficult to do, either as alleged “clinical trials” or observational studies in athletes, for they rarely take agents singularly, but often a “cocktail” of multiple dietary supplements and one or more doping agents.

Although rhGH has not been shown to unequivocally increase muscle strength or to improve performance,<sup>14</sup> it is considered one of the drugs of choice, because it is extremely difficult to prove that one is receiving it. The structure of rhGH is identical to the main isoform of naturally secreted GH. The pulsatile secretion of native GH means that its levels fluctuate widely, from undetectable to clearly within the doping range. Both GH and rhGH have a short half-life in the circulation. Exercise is potent stimulus to GH release and release may be modified by variations in nutrition and legitimate nutritional supplements.

### Studies of rhGH in Athletes

Liu and colleagues<sup>14</sup> have systematically reviewed the effects of rhGH on athletic performance. Using stringent criteria for a meta-analysis, they scanned 7599 titles from the largest databases, reviewed 252 abstracts in detail, and retrieved 56 articles for full-text evaluation. Following their review, 44 articles representing only 27



unique studies met the strict inclusion criteria. A total of 303 participants received rhGH for an average of 20 days but a significant number received rhGH only once. The subjects were mainly young men (average age 27 years) and were recreational and not elite athletes. The average dose was 36 µg/kg/day which is approximately 5- to 10-fold the therapeutic dose for adults with GH deficiency. Lean body mass increased in the rhGH-treated groups compared to those not treated (2.1 kg [95% CI, 1.3 to 2.9 kg]) with a small, not statistically significant, decrease in fat mass (-0.9 kg [CI, -1.8 to -0.0 kg]). Body weight did not change significantly. Only 2 studies appropriately evaluated change in strength;<sup>17,18</sup> these were the longest trials of 42 and 84 days duration. On 1-repetition maximum voluntary strength (1-RM) testing, those who received rhGH showed no change in biceps strength (-0.2 kg [CI, -1.5 to 1.1 kg]) or quadriceps strength (-0.1 kg [CI -1.8 to 1.5 kg]). In the second study none of the 7 other muscle groups evaluated showed a positive change in strength.

Minor effects of rhGH have been noted on basal metabolism with a slight decrease in respiratory exchange rate reflecting the preferential burning of fat rather than carbohydrate, at rest. Additionally, very little effect on exercise capacity has been reported. The results may be summarized by noting that lactate levels trended higher, plasma free fatty acid concentrations and glycerol concentrations were significantly increased—reflecting the lipolytic metabolic effect of rhGH—but the respiratory exchange ratio did not change. These studies showed very little ergogenic effects of rhGH in recreational athletes. The studies were of short duration and most likely did not represent how elite athletes administer rhGH, either with reference to dose, duration of doping, or addition of other supplements—both legal and illegal. Based on countless reports in the media, it is clear that many athletes abuse steroids in addition to the *noted* amounts of rhGH. None of the studies would have been able to detect differences of 0.5 to 1.0 % in “performance”. These small differences are those that are relevant to the time (track) events, distance or height (field) events that separate the champion from any other finishing position. Similar issues relate to a host of sports other than track and field, but may be even more difficult to quantitate.

Recently, rhGH (19 µg/kg/day) administered for one week was noted to increase strength, peak power output, and IGF-I levels in a group of abstinent dependent users of anabolic androgenic steroids.<sup>19</sup> Great care was taken to be certain that no anabolic steroids were detected in appropriately obtained urine samples. Body weight increased—this was likely water retention—as did peak power output. Although this is a very special group of athletes and is a single study, it was quite carefully performed.

Adverse events were common in the larger group of studies in the Liu et al meta analysis.<sup>14</sup> These mirrored those of adult subjects who administered rhGH in what were at that time, child and adolescent doses. Adverse events included soft tissue edema, joint pain, carpal tunnel syndrome, and excessive sweating. Most were related to fluid retention and considered to be secondary to the rhGH effects on salt and water balance by the kidney.

In a clinical trial designed to determine the pharmacodynamics of rhGH abuse, Nelson and co-workers<sup>20</sup> administered rhGH or placebo and testosterone (in men only) or placebo, or both in a double-blind study to young recreational athletes for 8 weeks. The final doses of rhGH were approximately 4-fold (women) and 6-fold (men) the normally prescribed dose for GH deficient young adults. Although there were no “efficacy” data with reference to body composition or athletic performance, the data are important with reference to adverse events. Although no subject discontinued the study due to adverse events related to rhGH, minor adverse events were reported in all groups, including the placebo groups. Swelling was reported in a greater number of rhGH subjects than placebo subjects (men: 67% versus 2.5%,  $P=0.02$ ; women: 65% versus 31%,  $P=0.06$ ). Subjects receiving rhGH reported more joint pain and *pins and needles* sensations; however, statistical significance was reached only in the men ( $P=0.02$  and  $P=0.03$ ). These data show the relatively small “therapeutic” index for rhGH and likely have implications for those athletes purportedly administering much higher doses.

In a clever sub-analysis of the placebo group, only reported in abstract form,<sup>21</sup> this group of investigators queried the placebo group about whether they were receiving active drug. The male athletes who believed that they were administered rhGH, even though they received the placebo, had both *perceived* improvement in performance measures and improvement in one of several *measured* indicators of physical performance. Although the study design<sup>20</sup> was not powered for this endpoint, it certainly does complicate the outcomes of trials with rhGH for performance endpoints.

Virtually all studies reviewed by Liu and colleagues<sup>14</sup> had significant limitations. The major ones included:

- Very few studies evaluated strength and exercise capacity
- Small effects would not have been found
- Short duration of the studies, many for only one dose
- Doses of rhGH and other supplements are very likely different in the real world.

Liu and colleagues concluded, “*Claims regarding the performance-enhancing properties of growth hormone are premature and are not supported by our review of the literature. The limited published data evaluating the*



*effects of growth hormone on athletic performance suggest that although growth hormone increases lean body mass in the short term, it does not appear to improve strength and may worsen exercise capacity. In addition, growth hormone in the healthy young is frequently associated with adverse events."*<sup>14</sup>

#### CURRENT DETECTION OF rhGH DOPING

The ability to detect rhGH has been quite a difficult task for analytical chemists, because the amino acid sequence of rhGH is identical to that of the main GH isoform secreted by the pituitary; unlike other peptide hormones it has no N-linked glycosylation sites; its secretion is pulsatile with a short half-life (16 to 20 minutes); there are circulating GH-binding proteins; potential cross reactivity with other peptide hormones (eg, prolactin); and it is stimulated by exercise and stress. Blood sampling is required for all detection methods, because less than 0.1% may be found in the urine. Its renal secretion is poorly understood and greatly variable within and between subjects.<sup>22</sup>

The analytical approaches rely on immunoassays as opposed to the more established doping tests for anabolic steroids, which depend on GC/MS technology (Figure 2). There are 2 general approaches to detection of doping with rhGH. The first, (direct) approach measures the GH isoform composition by the differential immunoassay method.<sup>23</sup> For this approach one constructs pairs of antibodies whose primary focus is all of the isoforms of GH and a second set which is virtually restricted to the 22kD isoform—the one that is 100% of the rhGH. The first assay is called *permissive* (pituitary) and the second *specific* (recombinant). The rationale is that the more one takes of the rhGH (22kD), the less pituitary GH (especially, 20kD)

will be secreted; implying that the ratio of the assay of the *specific* to the *permissive* will rise. As an example, the ratio rises from 0.6 to 1.5 in subjects administered rhGH, but this assay would only be valid within a few days of the last injection of rhGH. The validation of this technique requires knowledge (ie, testing) of the effects of exercise on the recombinant/pituitary ratio, an independent confirmatory test, knowledge of the *window of opportunity*, and data from athletes—both recreational and elite. This method is unable to detect doping<sup>22</sup> with pituitary derived GH or the abuse of the GH secretagogues, IGF-I itself or in combination with its major circulating binding protein, IGFBP-3 (IGF-I/IGFBP-3).

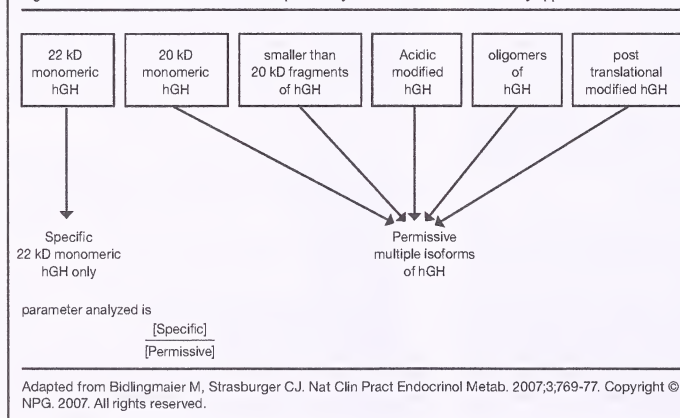
The second is the indirect approach in which specific analytes dependent on GH (or IGF-I) are measured. Variables from the IGF system and collagen/bone have been chosen because they change markedly during rhGH administration and it appears that combinations of variables using discriminant functions are the most promising. Detection of rhGH supplementation is possible at least until 2 weeks following the last administration, although there is progressively decreasing sensitivity after the first week. Normative data in athletes have been established.<sup>24</sup> The physiological changes in GH-dependent markers in adolescent athletes are far more dramatic than in older athletes, thus making it quite difficult to detect doping in this age range without constructing a complex algorithm that would depend more on maturational age than it would on the chronological age—another complication for doping control.<sup>25,26</sup> Data using this approach have noted only minor effects due to trauma or micro-injury or ethnic background.<sup>3,27</sup> As with any assay, rigorous standardization is required

and interference by concomitant drug abuse, especially anabolic steroids, is a likely complication. For the moment the most informative combination of analytes is IGF-I and procollagen III peptide levels and individual discriminant functions for men and women.

#### FUTURE RESEARCH IN DOPING

The doping-detection field in the future will require the determination of combinations of rhGH-dependent

Figure 2. Rationale for detection of hGH in plasma by the differential immunoassay approach



analyses that remain detectable for a longer period of time than the ones currently available, and perhaps other methods for the direct determination of the IGFs and GH-secretagogues. It would seem that abuse of rhGH (or other peptide hormones) manufactured by the major global pharmaceutical companies could be markedly diminished by adding, for example, an inert fluorescent marker that would be excreted in the urine. Detection of that unnatural marker might then be considered a doping offence. Most likely this would markedly diminish, but not stop, doping offences with these hormones. One can only speculate what is stopping the pharmaceutical manufactures from doing so.

The era of gene doping, for example adding GH or IGF-I genes to specific muscles, is upon us. Experiments have been done in animals.<sup>28</sup> No detection methods presently available could detect this type of doping.

### LEGAL ISSUES

As is true for most drugs, physicians may prescribe off-label, meaning that trials for that particular condition have not been performed but that it is logical to use an already approved drug for a specific patient. However, rhGH is quite different; it is *illegal* to prescribe rhGH off-label for age-related conditions (anti-aging) or for performance enhancement (Table 3). Unlike most FDA-approved medications, rhGH can only be prescribed for indications specifically authorized by the Secretary of HHS (for indications, see Table 1). Because it is not administered orally and it was formerly classified as a drug, rhGH is not considered a dietary supplement and is not subject to the Dietary Supplement and Health Education Act (DSHEA).

The precise language of the Federal Drug and Cosmetic Act<sup>29</sup> (FDCA) under section 303 is:

1. *Except as provided in paragraph (2), whoever knowingly distributes, or possesses with intent to distribute, human growth hormone for any use in humans other than the treatment of a disease or other recognized medical condition, where such use has been authorized by the Secretary of Health and Human Services under section 505 and pursuant to the order of a physician, is guilty of an offense punishable by not more than 5 years in prison, such fines authorized by title 18, or both.*
2. *Whoever commits any offense set forth in paragraph (1) and such offense involves an individual under 18 years of age is punishable by not more than 10 years imprisonment, such fines as are authorized by title 18 or both.*
3. *Any conviction for a violation of paragraphs (1) and (2) of this subsection shall be considered a felony violation of the Controlled Substances Act for the purposes of forfeiture under section 413 of such Act.*
4. *As used in this subsection the term "human growth*

*hormone" means somatrem, somatropin, or an analogue of either of them.*

5. *The Drug Enforcement Administration (DEA) is authorized to investigate offenses punishable by this subsection.*

### SUMMARY AND CONCLUSIONS

There are a number of legitimate uses of rhGH in infants, children, adolescents, and adults. It is different from most drugs in that its off-label use is illegal for those unapproved indications related to athletic performance, body building and anti-aging. Although difficult to show any ergogenic advantage in clinical trials, none of the trials have been large enough or have narrow enough end points to have a valid outcome given the changes in performance that are relevant to world-class athletes. Some progress is being made in the ability to detect doping with rhGH, but to date no national or international athlete has been sanctioned for abusing rhGH. This does not mean that rhGH is not being used by athletes, just that the testing is not yet robust enough to capture those abusing rhGH. Further research is clearly needed to improve the detection techniques, and also to determine if rhGH as administered to athletes is actually ergogenic or enhances one's image in body building.

As difficult as it is to note either changes in performance or body composition in adults, it is much more difficult to detect these alterations in adolescent athletes, whose natural pubertal progression involves a marked ramping-up of the GH/IGF-I system, as well as the analytes that are being considered for the detection of doping.

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#### Editor's Comment:

The lead article in this issue of GGH entitled "Growth Hormone Administration: Is it safe and effective for bodybuilding and improved athletic performance?" by Dr. Alan Rogol reviews the current state of the misguided use of human growth hormone. This topic has been of high interest as prominent professional athletes have been the subject of investigation and hearings by the US Congress. These high profile governmental activities may only be reflecting the tip of the iceberg of a prevalent practice in our society that may be permeating our youth. It is not possible to track the number of individuals receiving illegally distributed growth hormone, but it may account for a \$2 billion per year business in the US.<sup>1</sup> This is primarily a cash only business as the vast majority of users pay out of pocket for the drug. The New York State Bureau of Narcotic Enforcement uncovered highly profitable, illegal distribution of growth hormone; an investigated compounding pharmacy purchased 25 grams of imported growth hormone for \$75,000 and converted each gram into 3000 IUs of growth hormone, then sold the drug for \$6 to \$18 per IU—yielding \$450,000 to \$1,350,000.<sup>2</sup> In 2007, in this case alone, the company entered into a deferred prosecution agreement with the Massachusetts US Attorney's Office and was fined \$10.5 million over the illegal distribution of growth hormone for non medical uses. Additionally,

there are many other sales through the Internet of multiple products purportedly marketed as growth hormone. These practices preclude the detection and monitoring of adverse events and the potential health consequences of the illegal use of growth hormone.

Pediatric endocrinologists are well acquainted with the wish of children and their parents to administer growth hormone for growth augmentation purposes and are often consulted for its use as an agent for enhancement of their athletic capability and bodybuilding. Beware that the administration of growth hormone for the later purpose is illegal and its efficacy and safety for bodybuilding and athletic performance has not been demonstrated, as discussed in the lead article by Rogol. However, as long as our culture seeks perceived physical enhancements with products like growth hormone, we will have to be aware of the extensive distribution and promotion to our youth and actively participate in curtailing its use. This is quite a challenge, as this and other medications are easily found and sold online. According to *The New York Times*<sup>3</sup> there are over 365 Internet sites that advertise and/or sell controlled medications by mail and offering to supply the drugs without a proper prescription. The US Drug Enforcement Administration found that 85% of all Internet prescription sales involved controlled drugs, compared with 11% of those filled through traditional pharmacies, suggesting that online



*sales are destined for misuse. Mr. Califano, a former secretary of Health and Human Services, said: "Abuse of prescription drugs has exploded among college students, and we think that one way they get these drugs is over the Internet."*

Fima Lifshitz, MD  
Editor-in-Chief

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Genetics of Stature

Adult height is primarily (approximately 80% to 90%) determined by hereditary factors. Socioeconomic status, nutrition, and disease influence only a relatively small proportion of attained stature. It has long been suspected that there are a multitude of genes that impact upon this polygenic trait, with each gene exerting an additive but only very limited effect. From genome-wide association studies employing single nucleotide polymorphism (SNP) analyses in approximately 80,000 individuals of European ancestry (UK, Scandinavia, Holland, Iceland), these 3 investigative groups have identified more than 30 chromosomal sites and the potential genes that appear to be partially involved in the regulation of adult stature in humans (Table). Gudbjartsson et al divided the candidate genes into 3 functional groups—those associated with skeletal development (eg, *BMP2*, *BMP6*), those that encode zinc-dependent metalloproteinases (*ADAMTS10*) and glycoproteins (eg, *FBN1*) that affect cartilage composition, and those that are involved with the processes of chromosome segregation and mitosis (eg, *CDK6*, *HMG2*). The gene most frequently associated with stature in all 3 studies was *ZBTB38*. This zinc-finger protein binds methylated DNA—specifically the methylated allele of the differentially methylated region of H19/IGF2.<sup>1</sup> This is the site at which epigenetic errors of imprinting result in either the Beckwith-Wiedemann syndrome (OMIM 130650) of somatic overgrowth or the growth retardation syndrome of Russell-Silver (OMIM 180860).<sup>2</sup> *ZBTB38* represses transcription of methylated regions. Thus, it is interesting to speculate that *ZBTB38* might affect adult stature through regulation of the production of insulin-like growth factor (IGF)-II, perhaps during in utero development when IGF-II is known to be one of the determinants of fetal growth. Independent of its effect on methylated DNA, *ZBTB38* also regulates transcription of *TH*, the gene encoding tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis. Other commonly identified gene candidates were *HMG2* encoding a chromatin architectural factor and *CDK6* encoding a cyclin dependent kinase regulator of the cell cycle.

While each of these candidate genes has only a small effect upon adult height (estimated 0.4 cm), collectively they can exert significant influence and account for only approximately 4% of adult stature. The more "tall" alleles one has, the taller the individual (Figure). In the study of Weedon et al, there was a 5 cm difference in adult stature between subjects with 17 or fewer "tall" alleles compared to those with 27 or more.

Gudbjartsson DF, Walters GB, Thorleifsson G, et al. Many sequence variants affecting diversity of adult human height. *Nat Genet.* 2008;40:609-15.

Lettre G, Jackson AU, Gieger C, et al. Identification of 10 loci associated with height highlights new biological pathways in human growth. *Nat Genet.* 2008;40:489-90.

Weedon MN, Lango H, Lindgren CM, et al. Genome-wide association analysis identifies 20 loci that influence adult height. *Nat Genet.* 2008;40:573-83.

**First Editor's Comment:** *These reports are of great interest as they dramatically illustrate just how many genes must be involved in the determination of adult stature. They also illustrate the quantitative problem that the clinician will face in identifying the "cause" of genetic short stature in a specific patient. However, it was difficult to critically examine the data because some of it was derived by meta-analysis of previously published reports. Thus, it was unclear whether or not there may have been some overlap between analytical data utilized in the 3 reports. The reports are also difficult to interpret because the investigators employed different probes for similar or related SNP sites. For example, ZBTB38 was identified as SNP rs724016 in the report of Lettre et al, as SNP rs6440003 in the report of Weedon et al, and as SNP rs6763931 in the report of Gudbjartsson et al. [A brief expository review of genome-wide association studies and SNPs has been written by Christensen and Murray.<sup>3</sup>]*

Allen W. Root, MD

**Second Editor's Comment:** *Fisher proposed in 1918 that many genetic factors, each having an individually*



**Chromosome loci and candidate genes highly associated with adult stature**

| Chromosome | Gene     | Mutated Human Disease (OMIM) | Function  |
|------------|----------|------------------------------|---|
| 2          | EFEMP1   | 601548                       | Fibrin-like matrix protein. Retinal dystrophy (126600)  |
| 3          | ZBTB38   | -                            | Binds to and represses methylated DNA   |
| 4          | LCORL    | 611799                       | Transcription Activator   |
| 4          | HHIP     | -                            | Regulates hedgehog signaling  |
| 6          | LIN28B   | 606178                       | Promotes cell growth  |
| 6          | BMP6     | 611044                       | Bone morphogenetic protein  |
| 6          | GPR126   | 112266                       | Orphan G protein receptor   |
| 7          | CDK6     | 603368                       | Cyclin dependent kinase-regulator of cell cycle   |
| 7          | GNAI2    | 604394                       | Guanine nucleotide binding protein-with mitogenic properties  |
| 9          | PTCH1    | 601309                       | Receptor for Sonic, Indian & Holo-prosencephaly (610828) Desert hedgehogs. Basal cell nevus syndrome (109400) |
| 12         | HMG2     | 600698                       | Chromatin architectural factor. Tall stature, lipomas   |
| 12         | SOCS2    | 605117                       | Suppresses cytokine signaling – via Janus kinase and signal transducer and activation of transcription (STAT) |
| 14         | TRIP11   | 604505                       | Interacts with TRP/T3   |
| 15         | ADAMTSL3 | 609199                       | Component of extracellular matrix   |
| 15         | AGC1*    | 155760                       | Aggrecan – chondroitin sulfate. Spondyloepiphyseal dysplasia – Kimberly (608361) proteoglycan core protein    |
| 15         | FBN1     | 134797                       | Fibrillin-connective tissue matrix. Marfan syndrome (154700)  |
| 18         | DYM      | 607461                       | Transmembrane protein Osteochondrodysplasia (607326, 223800)  |
| 19         | DOT1L    | 607375                       | Histone-3 methyltransferase   |
| 19         | ADAMTS10 | 60899                        | Metalloproteinase. Weill-Marchesani syndrome (277600)   |
| 20         | GDF5     | 601146                       | Cartilage morphogenetic protein, Chondrodysplasia (201250, 200700, 113100), TGF $\beta$ subfamily             |
| 20         | BMP2     | 112261                       | Bone morphogenetic protein, stimulates bone formation   |

\*Designated ACAN in reports

(Data culled from the reports of Lettre et al, Weedon et al, and Gudbjartsson et al.)

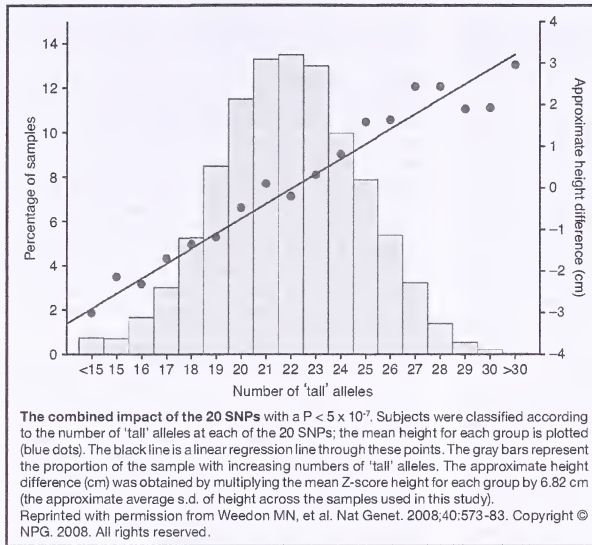
small effect, explain the heritability of height.<sup>4</sup> Much attention has been devoted since that time to identifying these factors. For instance, numerous genes have been identified that harbor mutations responsible for the osteochondrodysplasias and other syndromes associated with severe short stature, but in general these genes do not seem to influence the normal continuous variation in stature. Although linkage studies have elucidated chromosomal regions that affect height variation, they have not identified specific gene loci that influence height in the general population. It has not been until the recent application of genome-wide association (GWA) studies that significant headway has been made. This approach takes advantage of high-throughput analysis of single nucleotide polymorphisms (SNPs) identified through the so called HapMap project, a growing

number of patient groups for whom DNA is available for analysis and advances in computational methods that enable such analysis and permit datasets to be combined. Indeed, one of the first GWA investigations of height was reviewed in GGH.<sup>5</sup> This reviewed study has now been expanded substantially and joined by 3 other large GWA studies as reported in the May 2008 Nature Genetics. The new investigations have utilized more rigorous multi-stage experimental designs to analyze hundreds of thousands of SNP markers in ~63,000 individuals measured for adult height.

The report by Weedon et al identified 20 genetic variants which, in the aggregate, account for ~3% of height variation in adults of European ancestry. The identified SNP markers do not influence height per se, but they implicate genes within which or nearby to which they reside. One can envision how most of the candidate genes implicated in this manner could influence growth as they encompass growth factors and their receptors, proteins that interact with or alter the extracellular milieu of growth

factors and proteins that modulate intracellular signaling or are linked to cell cycle regulation or cancer. Most notable here are Indian hedgehog (IHH), Hedgehog interacting protein (HHIP) and Patched 1 (PTCH1), which belong to the Hedgehog pathway, growth and differentiation factor 5 (GDF5), suppressor of cytokine signaling 2 (SOCS2) and cyclin-dependent kinase-6 (CDK6). The previous association with a marker near the high mobility group-A2 (HMG2) gene locus was confirmed.

The report by Lettre et al identified 10 loci associated with height variation also in adults of European ancestry, 4 of which were the same as in the Weedon report including HHIP. These authors emphasized that 3 of the candidate genes—HMG2, the histone methyltransferase DOT1L and the methyl-DNA-binding transcriptional repressor gene ZBT38—are involved in chromatin remodeling. They



note that the 3' untranslated region of *HMGA2* contains the largest number of *let-7* microRNA binding sites and that 3 of the other implicated genes, *CDK6*, *DOT1L* and *LIN28B*, a gene upregulated in hepatocellular carcinoma, are considered targets of *let-7*. MicroRNAs, such as *let-7*, are small, nontranslated RNAs that down regulate expression of target genes.

The report by Gudbjartsson et al detected 27 genomic regions in which SNP variants were associated with adult height. Their data came from individuals with Icelandic, Dutch, European- and African-American ancestries and results accounted for 3.7% variation in adult height. Several of the implicated genes were the same as in the other 2 reports, but a few additional genes were identified including *BMP2*, *BMP6* and the *TGF- $\beta$*  and *BMP* inhibitor, *Noggin* (*NOG*).

In contrast to the GGH abstract<sup>6</sup> describing a single SNP association with adult height published in May 2008, these new reports identify 54 gene loci that influence variation in height in adults primarily of European descent. As noted in the accompanying editorial by Visscher,<sup>6</sup> it is reassuring that SNPs previously observed to associate with height were confirmed, SNPs in 3 genes were found associated with height in all 3 studies, and 7 genes were implicated in 2 of the 3 investigations. It is not surprising that variation in genes involving growth factors or modulation of growth factor signaling pathways influence height. More intriguing and novel is the implication of genes involved in chromatin remodeling and in microRNA regulation of gene expression. The papers illustrated the power of GWA studies and also the necessity of very large sample sizes creating consortia of research groups and even consortia of

consortia as stated by Visscher.<sup>6</sup>

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## Gender of Growth Hormone Recipients in the US and Globally

The investigators examined gender-based patterns of recombinant human growth hormone (rhGH) use in the US and how it compares to that of other countries, in the context of findings of previously reported gender disparities and the fact that rhGH has entered its third decade of clinical use. Data from all patients enrolled in the International Growth Study (KIGS) registry were included in the analysis. Patients were categorized into 4 geopolitical regions: US; Europe/Australia/New Zealand; Asia; and Rest of the World

(ROW; Argentina, Brazil, Colombia, Egypt, El Salvador, Guatemala, México, South Africa, and Venezuela). The US portion of the database was further divided into 10 geographic regions, according to US Postal Service zip code. To minimize the diagnostic inconsistencies across investigators, geographic regions and time, over 100 KIGS diagnoses were collapsed into 8 categories: (1) congenital GH deficiency; (2) organic acquired GH deficiency; (3) renal insufficiency; (4) Turner syndrome; (5) Prader Willi syndrome (PWS); (6) small for

gestational age (SGA), intrauterine growth retardation (IUGR); (7) familial short stature/constitutional growth delay/idiopathic short stature (FSS/CGD/ISS); and (8) idiopathic, neurosecretory, and transient GH deficiencies (IGHD).

Analyses depicted a consistent male predominance among US pediatric rhGH recipients, at almost 2:1. The gender ratio did not change significantly across the 3 time periods defined by the sequence of FDA-approved indications: 1992 and before comprised the classic GH-deficiency era; 1993-2000 the non-GH deficiency pathophysiology era; and 2001 onwards, the height-based era. All indications except PWS (and Turner syndrome because it is female-limited) significantly exceeded 50% males. The male predominance for all non-organic indications combined (72%) exceeded that for the organic indications, with or without Turner syndrome (38% and 59% male, respectively;  $P < 0.0001$  for both). Males outnumbered females at all ages, but with increased disparity during the second decade. With regard to male predominance across US regions, the areas with maximal and minimal percentages differed for each indication and the predominance did not correlate with either the number of children in each geographic area or the ratio of pediatric endocrinologists to children in each area. Comparing the US with global patterns demonstrated the US to have the second greatest male predominance, exceeded by Asia (mostly Japan), but greater than Europe/Australia/New Zealand. Recipients of rhGH in the ROW region were only 47% male.

The authors concluded that male predominance among US pediatric rhGH recipients, described at the introduction of rhGH, persisted into this third decade of use. The factor that most consistently affected the gender distribution was the diagnostic indication, with the greatest disparity appearing in indications without clear organic etiologies, ie, ISS. The absence of male predominance in the ROW region raises the question of cultural influences on rhGH use. The authors also noted that of the 10 greatest rhGH users, the US is the only country with a commercial third-party payer health system as well as being the only country in which ISS became a government-approved indication for pediatric rhGH therapy. The investigators concluded that medical care providers need to be aware of the reported practice bias, and carefully consider girls with growth failure to ensure timely diagnosis and treatment of underlying health problems.

Grimberg A, Stewart E, Wajnrajch MP. Gender of pediatric recombinant human growth hormone recipients in the United States and globally. *J Clin Endocrinol Metab.* 2008;93:2050-6.

**Editor's Comment:** *The persistent trend in the disproportionate number of males treated with rhGH should raise a number of concerns as well as provoke questions regarding the likely cosmetic (rather than medical necessity) rationale for rhGH treatment. Grimberg and colleagues previously reported that girls were referred for short stature half as often as boys and were more likely to have an identifiable underlying condition.<sup>1</sup> It could be concluded that in the shift from monitoring growth, as a general indicator of physical health to measuring height and treating short stature, that girls, in general, are placed at higher risk of having serious medical conditions diagnosed later than boys.*

*Given the incremental cost-effectiveness of rhGH therapy for ISS is approximately \$52,000 per inch,<sup>2</sup> it is well worth pondering why the US is the only country in which ISS became a government-approved indication for pediatric rhGH therapy. As Grimberg and colleagues noted, the data from this study suggest that social and cultural differences, in conjunction with perceived acceptability of rhGH expenditures, foster greater gender disparities in pediatric rhGH use in Japan and the US compared with other world regions.*

*One of the largest gender disparities was found to be within the category of rhGH initiation starting at 15-20 years of age. Multiple studies have demonstrated that age of rhGH initiation is one of the best predictors of growth response, with the younger the age at initiation, the better the response.<sup>3,4</sup> Initiating rhGH treatment in boys significantly more than girls between the ages of 15-20 years, with the knowledge that replicated clinical findings predict minimal growth response outcomes for this age range, lends support to the interpretation of over-treatment of boys.*

David E. Sandberg, PhD

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## Height Velocity Targets for First Year Growth Hormone Responses in Short Children

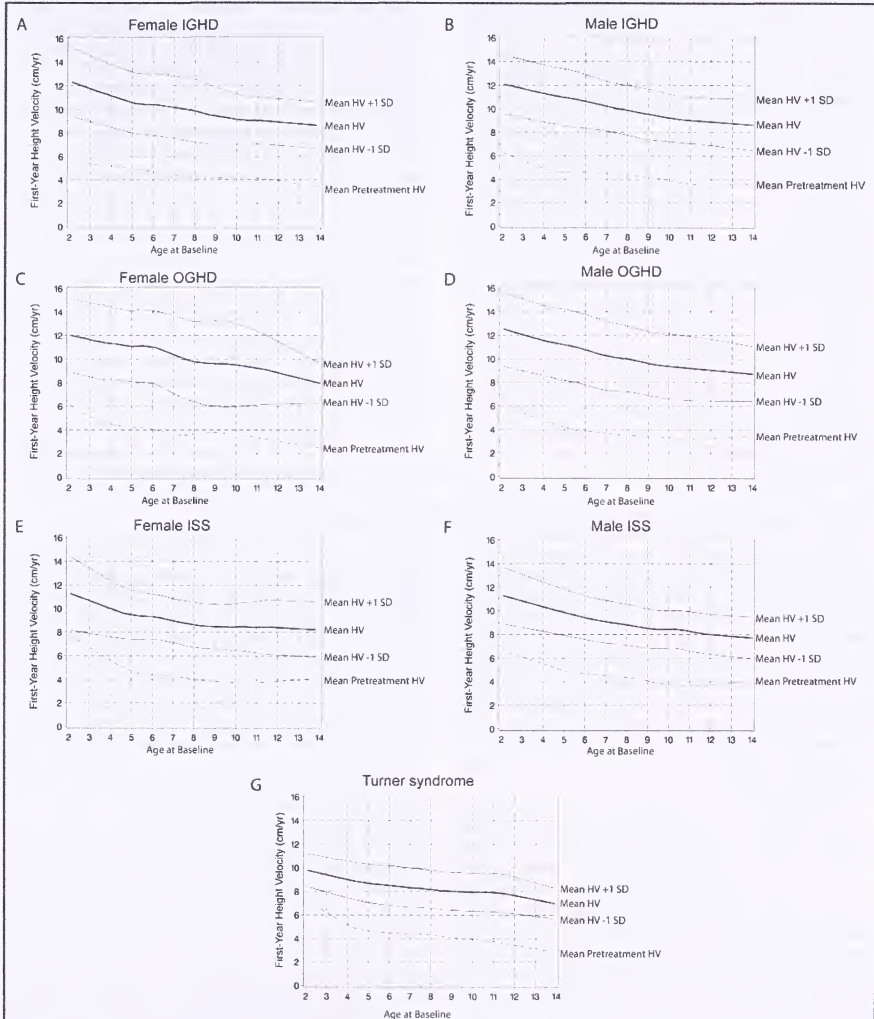
Recombinant human growth hormone (rhGH) has been used for treating a number of conditions. Several attempts have been made to define and predict GH response with the development of mathematical models, mostly in GH-deficient (GHD) patients. Such

models do not account for the variability observed in GH responsiveness (or sensitivity) in different types of short stature. Therefore, the authors presented evidence-based data criteria for defining the GH responsiveness. Their aim was to provide clinicians



with age-specific targets, considering the first year of treatment with standard daily doses of rhGH in prepubertal short children. Using data from the National Cooperative Growth Study (NCGS), GH response

curves were constructed for the first year of treatment. All children were new to treatment and prepubertal. Data were collected from 4297 boys and 3061 girls with idiopathic/organic GHD, idiopathic short stature,



First-year growth responses to daily GH expressed as HV at age of treatment onset (x-axis) in naive, prepubertal females and males with IGHD (A, B), OGHD (C, D), and ISS (E, F), and females with TS (G). Data given for mean and mean  $\pm 1$  SD.

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and Turner syndrome. All data were cross-sectional, mean  $\pm 1$  SD for first year height velocity (HV) on rhGH were plotted (in cm) against subject age at onset of rhGH treatment, as well as the mean pre-treatment HV data. Height velocity plots of each category as a factor of age at baseline were developed. Mean  $-2$  SD HV plots approximated the pre-treatment HV. The results were presented in a series of plots; these were primarily graphical. Interestingly, each graph contained the curve for the treatment growth velocity of different stages and types of patients (Figure).

There was considerable variability in the response to therapy with rhGH in children receiving a standard weight-based dosing schedule. The wide range of clinical responsiveness to therapy may also denote a challenge to the traditional fixed weight-based dosing generally employed. It emphasized the importance of age at initiation of treatment. Another point of interest was the similarity of the growth response pattern across etiologies of short stature. These growth response curves should also be viewed as conservative and to include some limitations, ie, an unknown amount of non-compliance in all groups, GHD may be part of multiple pituitary deficiencies, etc. Nevertheless, it is suggested that these data offer the clinician a tool to assess progress of an individual patient within an evidence-based frame.

Bakker B, Frane J, Anhalt H, Lippe B, Rosenfeld RG. Height velocity targets from the National Cooperative Growth Study for first-year growth hormone responses in short children. *J Clin Endocrinol Metab.* 2008;93:352-7.

**Editor's Comment:** These data, derived from a large population study, are welcome and provide a context of the well known large variability in growth responses to rhGH therapy. The growth response graphs provided an additional but useful tool in contrast to numerous previous studies which proposed more theoretical and mathematical predictive approaches: prediction of the first year response to rhGH and prediction of adult height based on the first year growth response. These mathematical models were also derived from post-marketing long-term follow-up data, but they did not provide us with practical tools in clinical practice. The new growth response curves showing the first year of therapy may help evaluate the initial catch-up growth and the adjustment of rhGH doses after the first year of therapy. This could be performed in relation to age. In any case, they focus our attention on the first year of treatment and provide information which may turn out to be useful for the patient and the family, particularly in the group of idiopathic short stature patients and in those that may have compliance problems.

Raphaël Rappaport, MD

## Adult Height of Treated Congenital Adrenal Hyperplasia Patients

Noting reports indicating that patients with the 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) often fail to reach their target height, Hoepffner and colleagues reviewed medical charts of 56 patients with 21-hydroxylase deficiency to examine the effects on final height of strictly controlling hydrocortisone and fludrocortisone. Sixty-two patients were followed continuously at a university children's hospital by the same physician; 6 were excluded from analyses due to receiving additional medications that may effect CAH therapy and/or growth ( $n=2$ ) or due to bilateral adrenalectomy ( $n=4$ ).

Participants were divided into 5 subgroups. Patients in the first 3 groups were diagnosed within their first year of life: (1) adult patients born before 1975 ( $n=13$ , all salt-wasting, 5 males); (2) adult patients born in or after 1975 ( $n=26$ , 21 salt-wasting, 8 males); (3) 7 to 15 years of age and had not yet attained final height ( $n=9$ , all salt-wasting, 4 males); (4.1) pre-pubertal bone age, late diagnosed, therapy initiated at 3.5 to 6 years of age ( $n=5$ , 3 salt-wasting, 3 males); and (4.2) pubertal bone age, late diagnosed, therapy initiated at 5.5 to 9 years of age ( $n=3$ , 1 salt-wasting, 2 males). All patients received therapy monitoring exclusively by the outpatient unit and had regular 3-month measurements of height, weight, blood pressure, and bone age (BA).

Hydrocortisone was administered 3x/day, every 8-10 hours. Management was designed so the course of BA followed the course of chronological age (CA), ie, patients received an increased hydrocortisone dose if BA was higher than expected over a 6-month observation period, and vice versa. Fludrocortisone was administered 2-3x/day with the hydrocortisone. Dosage was monitored by blood pressure; to avoid fludrocortisone overdosage, blood pressure values were not allowed to exceed the upper normal range. Change from DOCA to fludrocortisone was gradually introduced to patients in groups 1, 4.1, and 4.2 since the late 1970s when the current therapy regime was initiated. Authors provided specific hydrocortisone and fludrocortisone dosages by age group within group. Beginning in 1992, morning 17-hydroxyprogesterone (17-OHP) in saliva was measured by immunoassay every 3 months and, sometimes more frequently after reaching 5 to 6 years of age. Occasional plasma rennin concentration measurements were added to the monitoring regime. Target height standard deviation scores (htSDS) were based on measured parental height. All values for data analysis were collected via retrospective chart review. Statistical analyses focused mainly on groups 1 and 2. The authors recommended that readers consider results pertaining to groups 4.1

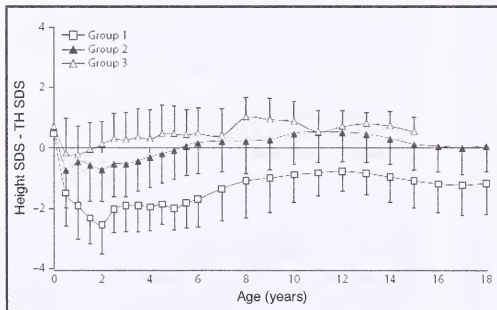
and 4.2 as clinical observations due to the small sample size and to view group 3 results as a demonstration of current corticoid dosage since patients had not reached their final height.

Results showed patients in group 1 had a mean corrected height (ie, measured htSDS minus target htSDS) of approximately -2SDS during their first years of life; this increased to approximately -1 htSDS by age 8 where it remained through adolescence to adulthood (ie, 18-years old) (Figure). In the 1st, 2nd, 4th, and 5th years of life, group 1 growth rates were significantly less than in group 2; in each year (0 to 18 years), the mean group 1 htSDS was significantly lower than that for group 2. With regard to bone age, group 1 experienced significant suppression between ages 1 to 6 years compared with the BA SDS in group 2, followed by a recovery to 1.8 SDS at age 8. Regarding BMI, patients in group 2 showed a continuous and statistically significant increase from ages 2 to 8 years to approximately 1 SD, with no increase after this age. Patients in group 2 reached their target height (0.1 corrected final htSDS). The authors noted that the corrected final mean htSDS of -1.2 of group 1 (those born before 1975) was similar to values reported in the literature and was due to likely excessive corticoid dosages particularly during the first 2 years of life. Use of lower corticoid doses during the following years of life resulted in extremely fast bone maturation up to approximately 1.8 SD, which exceeded growth velocity. In contrast, combined early treatment involving substitution therapy with hydrocortisone and fludrocortisone (ie, group 2) was associated with patients reaching their target height. A similar pattern emerged in groups 3, 4.1, and 4.2. Hoepffner and colleagues credit success with attaining target height to strict medication adherence and monitoring, specifically, by keeping BA the same as CA through combined corticoid administration every 8 hours. For patients with classic CAH who are treated early and following the recommended regimen, they see no need

for other forms of therapy (eg, growth hormone (GH), gonadotropin-releasing hormone analogs [GnRHa], antiandrogens, or aromatase blockers).

Hoepffner W, Kaufhold A, Willgerodt H, Keller E. Patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency can achieve their target height: The Leipzig experience. *Horm Res* 2008;70:42-50.

**First Editor's Comment:** At a time when different medications are introduced in an effort to assist children with CAH attain their target heights,<sup>1,2</sup> Hoepffner and colleagues findings are quite exciting. Children with CAH can attain their target height with strict monitoring and careful hydrocortisone and fludrocortisone administration. This finding is particularly important as we discover more about the risks of various alternative strategies to increase height. While a full review of all medication side effects is not possible here, I offer the following for thought: GnRHa treatment, in conjunction with GH, is one tool used to arrest pubertal progression in persons with CAH and, thereby, prolong the time over which linear growth can occur. The use of this medication is, however, not without drawbacks. In a study of visuospatial working memory pre- and post-GnRHa treatment in young women, results suggested that hormone withdrawal following GnRHa administration alters the neural circuitry underlying performance of the visual working memory.<sup>3</sup> Specifically, although behavioral responses appeared unimpaired, event-related fMRI under GnRHa exposure was found to be associated with attenuated left precuneus and posterior cingulate cortex activation at encoding and cerebellar activation at recognition. These effects were observed at an 8-week assessment. It could be argued that these changes may return to pre-GnRHa-treatment levels following discontinuation; however, alterations to typical brain function during adolescence (the developmental stage at which GnRHa would be administered to youth with CAH) may have organizational effects that extend beyond the point GnRHa is withdrawn. Evidence for this possibility comes from experimental research by Schulz and Sisk<sup>4</sup> which demonstrated that pubertal gonadal hormones act in lasting ways on the juvenile brain. For example, the authors showed that male Syrian hamsters deprived of gonadal steroid hormones during their pubertal phase of development failed to demonstrate typical masculine reproductive behavior even when those hormones were later replaced. They found that adolescent exposure to testicular hormones causes male behaviors that communicate moment-to-moment dominance status between animals. Similarly, adolescent exposure to ovarian hormones defeminizes female reproductive behavior in the Syrian hamster. Comparable "organizational" effects of steroid hormone



Pattern of corrected height SDS (height SDS - target height SDS)  $\pm$  SD of patients of groups 1-3. Reprinted with permission from Hoepffner W, et al. *Horm Res*. 2008;70:42-50. Copyright © Krager 2008. All rights reserved.

exposure during adolescence are seen in female rats. Species differences notwithstanding, there is as yet no basis to guarantee families of the long-term safety associated with experimental protocols such as GnRHa, antiandrogens, or aromatase blockers to optimize height. It is therefore reassuring to learn that patients with CAH can achieve their target height through vigilant surveillance of hormone replacement alone.

David E. Sandberg, PhD

**Second Editor's Comment:** The paper by Hoepffner et al clearly illustrates that CAH patients who receive an appropriate treatment usually attain their target height, whereas those who do not receive the best medications or do not follow a strict adherence and monitoring of the treatment regimen may not reach their genetically determined height. This observation is very important and should raise our awareness of the difficulties that patients face with demanding long-term treatment protocols for chronic conditions like CAH. When such a patient's growth is faltering additional therapies ie, GnRHa may compound the problem, not withstanding cost, and may lead to other potential concerns. Dr. Sandberg's commentary focuses on new experimental data that suggested that manipulation of the timing of puberty can affect neuroendocrine function and behavior, at least in animals. These potential effects

need be investigated in patients with precocious puberty who are regularly treated with GnRHa. In patients with CAH, as well as in others with chronic conditions, the first challenge is to deal with the adherence and compliance of the patients. This issue was reviewed in GGH<sup>5</sup> and despite the importance of medication in treatment, compliance ranged from 11% to 93%.<sup>6</sup> Lack of response or inappropriate response to medication may be indicators of poor adherence.<sup>7</sup>

Fima Lifshitz, MD

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## Combined GH and Aromatase Inhibitor Therapy in GHD Adolescents

In this study, Mauras et al investigated whether the potent aromatase inhibitor, anastrozole, could delay bone age acceleration and increase adult predicted height in boys with growth hormone deficiency (GHD). They studied 52 GHD adolescents on recombinant human GH therapy who were randomized to co-treatment with anastrozole or placebo daily for 36 months. Fifty subjects completed 12 months, 41 completed 24 months, and 28 completed 36 months of treatment. Bone age advancement was significantly slower in the anastrozole vs the placebo group both at 2 and 3 years of therapy ( $1.8 \pm 0.1$  vs  $2.7 \pm 0.1$  yr and  $2.5 \pm 0.2$  vs  $4.1 \pm 0.1$  yr, respectively [ $p < 0.0007$ ]). This resulted in an increase in predicted adult height of  $+4.5 \pm 1.2$  cm at 24 months and of  $+6.7 \pm 1.4$  at 36 months of therapy in the anastrozole group when compared with a 1 cm gain at both time points in the placebo group. While serum testosterone concentrations increased more in the anastrozole group after 12 months of therapy, this difference was not significant at 24 months. Estradiol and estrone concentrations increased gradually in the placebo group during the 3 years of treatment, while they remained stable in the anastrozole treated group. Insulin-like growth factor (IGF)-I levels were similar at baseline and increased in a similar fashion in both groups throughout the study. The pace of pubertal progression was similar between groups as measured both by testosterone

concentrations and testicular volumes. Fasting lipids, glucose concentrations, complete blood count, urinalysis and liver profiles were normal in both groups at baseline, with no significant differences over time. There was no difference in lumbar spine bone mineral density between groups. The authors concluded that anastrozole increased the adult height potential of adolescent boys on GH therapy while maintaining a normal pubertal progression.

Mauras N, Gonzalez de Pijem L, Hsiang HY, et al. Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. *J Clin Endocrinol Metab.* 2008;93:823-31.

**Editor's Comment:** Treatment with GH has been shown to improve the final height of GHD children. However, once puberty has begun the time available to increase linear growth in GHD patients is limited. Gonadotropin releasing hormone (GnRH) analog therapy in addition to GH treatment has proven to improve the near final height of GHD patients in puberty.<sup>1</sup> However, this form of therapy is relatively expensive, requires long-term parenteral administration and careful follow-up, and recent studies have demonstrated substantial changes in body composition and in intermediary metabolism, with an increase in adiposity and a decrease in protein synthesis, lipid oxidation, energy expenditure and muscle



strength following analog use.<sup>2</sup> Additionally, prior studies by Maura et al<sup>3</sup> have demonstrated increased loss of urinary calcium and in the rate of calcium resorption, with a significant decrease in measures of bone formation in treated patients.

In this study, Maura et al demonstrated how combined anastrozole (a potent aromatase inhibitor which blocks the conversion of androstenedione to estrone and testosterone to estradiol) and GH administration to adolescent GHD boys increased their adult height potential. Growth velocity remained similar to that of GH and placebo treated children, but with a slower increase in bone maturation, resulting in a net increase in predicted adult height. Anastrozole was well tolerated and free of side effects with no negative effects on fasting lipids or glucose, liver function tests or changes in fat-free mass or percent fat mass. While the increase in lumbar bone mineral density was less in the anastrozole treated group at 24 months, this difference was not noted at 36 months and osteocalcin concentrations, a measure of bone formation, were similar during the whole treatment period in both anastrozole and placebo treated patients. The pace of pubertal progression as determined by changes in testosterone concentrations and in testicular volumes was also similar in both groups. Therefore, treatment with an aromatase inhibitor and GH

may offer an alternative in promoting growth in GHD boys who have entered into puberty. However, this conclusion is based on limited data, as patients have not been followed to final height, studies have been performed only in males and in a limited number of patients, and only one randomized double-blind, placebo-controlled study has been performed in this group of patients. More long-term data regarding bone health, potential effects on spermatogenesis and sperm motility, lipid and carbohydrate metabolism will be necessary in assessing the pros and cons of aromatase inhibitor therapy in GHD children and in short boys in general.

Roberto Lanes, MD

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## Factors Predicting Ante- and Postnatal Growth

In an attempt to better understand factors which contribute to both antenatal and postnatal growth, a series of conditional analyses were performed on data collected prospectively from 1218 mother and infant pairs. The study subjects had to be Caucasian, have a prenatal visit before 20 weeks, and had to demonstrate a structurally normal single fetus which was carried to term. Maternal steroid use or thrombotic disorders disqualified the pairs. At the first prenatal visit maternal height was measured as well as paternal height when available. Maternal weight was measured and the history was taken with regard to tobacco use. Socioeconomic status was determined from information regarding education, marital status, occupation, partner's occupation, and social class. Assignment was made using the classification system of the UK Office of Population, Census, and Surveys. Placental weight was recorded after the membranes were trimmed. Birth weight was measured using self-calibrating scales, length by infantometer, and head circumference with a metal tape. In addition, skin fold measurements were made at the triceps, subscapular, and quadriceps areas. These measurements were repeated at 6 months of infant age. Feeding practices were noted at birth and reassessed at 3 months. These were classified as totally breast fed, mixed, or totally bottle fed.

The cohort of women in this study was not different anthropometrically from the UK population and social

class distribution was also representative. Non-smokers comprised 71% of the cohort. Placental weight was shown to be related to birth weight, birth length, and head circumference. Factors determining placental weight, when birth weight was excluded, were gestational age at delivery, maternal height, weight at first prenatal visit, and paternal height. One factor—increasing parity—had a negative effect. These factors explained 7% of the variance in placental weight. When the analysis was redone including birth weight, length of gestation, and smoking during pregnancy, these also influenced placental weight in a positive manner. Female gender was associated with reduced placental weight. These factors explained 40% of the variance in placental weight.

Placental weight, parity, maternal weight at first prenatal visit, and gender of the infant did not influence weight, length, or head circumference at 6 months of age. Weight at 6 months was influenced by maternal and to a lesser extent paternal height. Smoking was associated with a relatively heavier infant at 6 months and lower socioeconomic status had an additional effect as did breast-feeding. Duration of pregnancy was also an important factor. Length SDS at 6 months was influenced by maternal and paternal height. Smoking had no effect on length at 6 months. Head circumference at 6 months was influenced by maternal weight, height, duration of pregnancy, and maternal smoking whereas breast-feeding at 6 months was associated with a reduction in



head circumference SDS.

These data demonstrate an important impact of maternal and paternal stature on the size of the infant at 6 months. Parity, placental weight, and birth weight, although important to the size of the infant at birth, have little effect on growth during the first 6 months. The effect of parity is mediated by determination of size of the infant at birth and this is mostly mediated by placental weight. The findings demonstrated that small and large babies have small and large placentas respectively. The authors pointed out that the factors that might be modified to determine placental weight and therefore size at birth are rather limited, the most important being smoking. Smoking during pregnancy is associated with a lower birth weight, shorter length and reduced head circumference. But there is compensation in growth during the first 6 months of postnatal life. They also pointed out that of all 3 anthropometric measures at 6 months, maternal and paternal stature impacted the most with maternal height having more effect on weight and head circumference. They concluded that the data highlight the importance of factors such as smoking and parity that can be manipulated by public health education

and others such as gestational length that can be hopefully manipulated by careful prenatal care and attendance at prenatal clinics.

Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC, Cole TJ. Factors predicting ante- and postnatal growth. *Pediatr Res.* 2008;63:99-102.

**Editor's Comment:** *This interesting manuscript attempts to characterize factors that influence growth during infancy—and particularly at an age which is effected primarily by nutrition and for the most part is growth hormone independent. It is important to note that all of these data were collected from uncomplicated pregnancies. However, this large cohort is socioeconomically and anthropometrically representative of the UK population. Thus, the information is of extreme importance in obtaining a better understanding of how infants grow and which factors may be important. It is also important for pediatric endocrinologists to better understand the factors that may be contributing to growth failure in infants referred to their practices.*

William L. Clarke, MD

## Height Sparing in Anorexia Nervosa?

Reports of height in girls with anorexia nervosa (AN) have conflicted between stunting and sparing. While under-nutrition and low insulin-like growth factor (IGF)-I levels would be expected to stunt statural growth, high levels of growth hormone (GH), with its direct effects on the growth plate, and hypogonadism, resulting in delayed skeletal maturation, would be expected to preserve height.

Towards a better understanding, Prabhakaran et al compared 110 girls with AN (mean duration of illness  $11.6 \pm 13.2$  months) to 98 age-matched controls (aged 12-18 years); 63 girls with AN and 79 controls were followed prospectively for one year. Girls were premenarcheal at baseline; 25 girls had AN and 10 were controls. At baseline, girls with AN had significantly lower BMI (mean  $18.5 \pm 2.1$  vs  $22.0 \pm 3.2$  kg/m<sup>2</sup>, respectively), lower IGF-I levels (15.8% of girls with AN had IGF-I concentrations below the reference range and 52.6% had levels within the lowest quartile for pubertal stage, compared to 18.4% of the controls), and higher nadir GH levels on overnight sampling (mean  $2.14 \pm 1.17$  vs  $1.04 \pm 1.01$  ng/mL, respectively). Bone ages were similar, though the difference between bone age and chronologic age was lower by a few months in the AN group.

Midparental target heights (based on parental reports) and baseline heights were slightly higher for the AN group than controls (the latter  $164.3 \pm 6.9$  vs  $162.5 \pm 6.5$  cm, respectively). Height parameters did not differ significantly between the groups at 12-month follow up. Associations between nadir GH levels and z-scores for both height and predicted adult height (by Bayley-Pinneau method) were stronger in immature subjects and in controls. For girls with

AN, these height parameters were associated with IGF-I levels instead, and inversely with duration of illness. The one-year increase in height z-score for immature girls with AN was predicted by baseline delay in bone age relative to chronologic age.

The authors concluded that hypogonadism (delayed skeletal maturation), not higher GH levels, preserves final height in girls with AN. The duration and severity of illness (and hence, IGF-I levels) also played an important role in height outcome.

Prabhakaran R, Misra M, Miller KK, et al. Determinants of height in adolescent girls with anorexia nervosa. *Pediatrics.* 2008;121:e1517-23.

**Editor's Comment:** *Endocrinologists are frequently consulted for the hypogonadism associated with AN. Patients' families present with the chief complaint of amenorrhea and raise concerns over bone mineralization.<sup>1</sup> While these are valid concerns, this paper supports the notion that treatment of the underlying disease process (ie, nutritional repletion and psychological correction of the distorted body image) is preferable to hormone replacement therapy, especially for girls who are still growing. The reader must keep in mind that the height outcomes found in this study are dependent on the duration and severity of illness and may not generalize to other groups.*

Adda Grimberg, MD

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## Growth Plate Changes of Catch-up Growth Following Caloric Restriction: Morphologic and Gene Expression Changes, Especially HIF1 $\alpha$

To study the mechanism of catch-up growth, Even-Zohar et al examined growth plate morphology and gene expression in prepubertal male Sprague-Dawley rats subjected to 10 days of 40% caloric restriction. This degree of caloric reduction long-term had been shown to increase rat longevity, and was calculated based on the ad lib feeding of similar age and weight rats in a previous experiment (access to water remained unlimited). Rats were randomized into 3 groups: ad libitum (AL; unlimited food access throughout the experiment), food restricted (RES; 60% of the same chow throughout the experiment), and catch-up (CU; the 60% intake for 10 days followed by ad libitum feeding for the next 7 days).

Growth parameters confirmed the experimental design, though the catch-up growth was partial. Weight gain was 6.5 gm/day in the AL group, and only 1.2 gm/day in the RES group. The CU period augmented weight gain to 15.1 gm on the first day of ad lib feeding, followed by an average daily weight gain rate of 8% their total body weight compared to 4.5% in the AL group. Nonetheless, the CU group failed to completely regain their weight deficit by the end of the week. Similarly, humeral length was significantly reduced in the RES group throughout, and significantly improved at day 7 of CU but still shorter than the AL group.

The humeral epiphyseal growth plates (EGP) reflected the gross growth parameters. The EGP length (from the reserve zone to the ossification front of the metaphyseal bone) was constant in the AL group, shorter in the RES group, and showed progressive catch-up in the CU group. The average number of chondrocytes per column (proliferative zone through the last hypertrophic cell) was reduced in RES, and improved in the CU group from day 2 to 7 of ad lib feeding. The ratios of proliferative to hypertrophic zones were unaffected by the nutritional interventions.

Towards a mechanistic understanding, the EGPs were microdissected and total RNA was pooled from at least 15 sections from each zone of each animal to be studied by Affymetrix microarray. Between the RES and AL groups 4144 probes differed significantly. Interestingly, the gene expression profile of the CU group differed from RES by the first day of liberated feeding, yet remained

different from AL for the remainder of the week. The investigators focused on genes with a so-called "up-down-up" (UDU) expression profile (highly expressed in AL, reduced in RES and increased again in CU). At least 2-fold changes were shown in 714 genes, going down from AL to RES and up from RES to CU; of these, 550 were differentially expressed among all 3 groups on the first day of refeeding. In silico analyses of functional groups and promoter of cluster revealed the UDU genes to be enriched for synthetic (macromolecule metabolism, RNA processing and translation, protein transport, secretion and degradation) rather than proliferative functions.

Of the 7 transcription factors whose downstream targets were enriched in the UDU gene list, hypoxia inducible factor (HIF)1 $\alpha$  was selected by the investigators for further study. Messages of HIF1 $\alpha$  and 3 of its target genes (one representing each function: glycolysis, proliferation/survival and chondrogenic/structural activity) were quantified by RT-PCR to validate the microarray findings. The UDU expression changes were specific to the growth plates, as hepatic expression of HIF1 $\alpha$  did not differ among the 3 groups on the first day of liberated feeding. Immunohistochemistry of the proximal humeral EGPs showed HIF1 $\alpha$  protein most intensely in the AL and CU proliferative zone cells, mainly in the nucleus (consistent with its function as a transcription factor); RES cells stained weakly for HIF1 $\alpha$ . Microdissection and RT-PCR of tibial EGPs showed proliferative zone cells to exceed hypertrophic zone cells in HIF1 $\alpha$  message levels as well; the 3-fold expression difference between the zones was constant across experimental conditions.

In summary, the authors found that nutritional

### Growth, Genetics & Hormones

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### Growth, Genetics & Hormones

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restriction/refeeding has a significant effect on expression of HIF1 $\alpha$  and its targets in the prepubertal rat EGP, and proposed that HIF1 $\alpha$  plays an important role in regulating chondrogenesis. They further speculated on the mediators of HIF1 $\alpha$  regulation by nutritional restriction/refeeding, including oxygen tension (presumably decreased in CU growth plates due to the increased EGP dimension and/or increased oxygen consumption, both of which can be expected from the rapid growth) and circulating hormone levels (such as insulin-like growth factor [IGF]-I, which is known to induce HIF1 $\alpha$  and is itself regulated by nutritional status). One can easily see how this paper has opened the door for many follow-up studies, not just of HIF1 $\alpha$  but of the other identified genes as well.

Even-Zohar N, Jacob J, Amariglio N, et al. Nutrition-induced catch-up growth increases hypoxia inducible factor 1  $\alpha$  RNA levels in the growth plate. *Bone*. 2008;42:505-15.

**Editor's Comment:** *This elegantly designed study marks an exciting new era of growth research. Historically, endocrinologists studied growth by focusing on changes in circulating hormone levels. Creation of the LID mouse (liver-specific IGF-I deficient via the cre-lox technique) shocked the traditional paradigm. The LID mice grew normally despite a 75% reduction in*

*circulating levels of IGF-I, highlighting the surprising importance of autocrine/paracrine IGF-I for growth.<sup>1</sup> Thus, "the action" is now understood to be local, in the growth plates, rather than the circulation. Growth plate studies have increasingly permeated the growth literature, some of which have been reported in previous issues of GGH.<sup>2,3</sup> By applying technological advances, like the microarray and gene database analyses, to the newer growth-plate focus, this paper's study design has the power to not only spotlight previously expected "players" but identify new ones as well that are important for mediating growth and its various perturbations. Such experimental approaches are likely to herald accelerated advances in the growth field, though confirmatory evidence through alternative models are still required to validate the biologically significant findings and to rule out the potential for inter-species differences.*

Adda Grimberg, MD

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## High Growth Rate of Girls with Precocious Puberty Exposed to Estrogenic Mycotoxins

Zeranol ( $\alpha$ -ZAL;  $\alpha$ -zearalanol) is an anabolic estrogen that has been used for increasing muscle mass in cattle and poultry. It is produced by *Fusarium*, and is thus a mycotoxin. Earlier studies have shown estrogenic mycotoxins in 5 of 36 girls with early thelarche in southeastern Hungary and a high incidence of central precocious puberty (CPP) in a northwest region of Tuscany (22 to 29 times higher than that in neighboring areas), suggesting an environmental estrogen exposure as causative. Zearalenone (ZEA) and its metabolites (ie,  $\alpha$ -ZAL,  $\beta$ -zearalanol [ $\beta$ -ZAL],  $\alpha$ -zearalanol [ $\alpha$ -ZOL], and  $\beta$ -zearalanol [ $\beta$ -ZOL]) are apparently able to adopt a chemical configuration that resembles 17 $\beta$ -estradiol ( $E_2$ ) and binds to estrogen receptors in target cells thus exerting estrogenic (agonist) action.

The aim of this study by Massart and colleagues was to test the hypothesis that environmental estrogenic exposure through mycotoxins could be associated with CPP in girls from the Viareggio countryside of northwest Tuscany, Italy. Thirty-two girls with CPP—defined as history of increased growth velocity, Tanner 2 breast development, and bone age advanced more than one year, LH and FSH responses to gonadotropin releasing hormone (GnRH) stimulation in the pubertal range,  $E_2$  levels  $>25$  pg/mL, and chronologic age  $\leq 8$  years—were studied. Group A comprised 17 girls came from the Viareggio countryside and group B were 15 girls from

Pisa. In addition 31 age- and sex-matched control subjects from Viareggio (n=15, group C) and Pisa (n=16, group D) were studied as controls. Following diagnosis, all 32 girls with CPP were treated with triptorelin (TR) depot IM every 28 days for more than 12 months. Auxologic data and pubertal development were recorded initially and at 3- and 6-month intervals and bone age was done yearly and read by Greulich and Pyle method. Italian standards were used to determine height SDS, weight SDS, and height velocity SDS. Mycotoxin (ZEA,  $\alpha$ -ZOL,  $\beta$ -ZOL,  $\alpha$ -ZAL, and  $\beta$ -ZAL) levels were determined using high performance liquid chromatography from sera during the GnRH stimulation test at diagnosis and at 12 months of treatment.

All 63 girls were born at term and appropriate for gestational age. At the start of the study there were no significant differences between the CPP groups and the control groups. The only mycotoxins detected were ZEA and  $\alpha$ -ZOL. At diagnosis 6 of the 17 girls (35%) at Viareggio had higher serum ZEA and  $\alpha$ -ZOL levels than the other 3 groups; ZEA and its metabolites were not detected in the 15 CPP girls from Pisa or in the control subjects. All 32 girls with CPP (groups A and B) had undetectable mycotoxin levels after 12 months of GnRH agonist treatment. In order to study the differences between the girls who were mycotoxin positive versus those in whom mycotoxins were undetectable, 2 additional groups were formed. The 6 girls



who were mycotoxin positive (group E) were compared with the 26 girls who were mycotoxin negative (group F). At diagnosis there were no differences in chronologic age, target SDS in all 3 groups, and bone age and bone age/chronologic age ratio were not different in the CPP groups before and 12 months after treatment. However, group E and group F had different growth trends during treatment. Group E height SDS for chronologic age significantly increased from baseline during treatment while height SDS for chronologic age declined slightly in group F. Similarly, weight SDS for chronologic age increased from baseline in group E, but not in group F. The BMI did not differ in groups with CPP at diagnosis or after 12 months of therapy. In addition, after 12 months of therapy, height SDS for bone age was higher in Group E than in Group F even though no difference was detected at the time of diagnosis. Height velocity SDS for chronologic age was also higher in group E than in group F and the control subjects, while height velocity SDS for chronologic age in group F was constant during treatment. At diagnosis serum ZEA levels correlated with height, SDS for chronologic age, weight SDS for chronologic age, and height SDS for bone age. There was no correlation detected for  $\alpha$ -ZOL; at 12 months no correlations were detected. No differences were found in the groups with CPP at diagnosis and during treatment for LH, FSH, or for  $E_2$ .

The authors reviewed information known about ZEA as a non-steroidal mycotoxin produced by *Fusarium* species on several grains. Of note, beside the estrogenic activity, ZEA also has anabolic properties and ZEA food contamination could be either direct or indirect by carryover of mycotoxin in animal tissues such as milk and eggs after intake of contaminated feeds. In the US  $\alpha$ -ZAL has been used widely as a growth promoter to fatten cattle. This application was banned in the EU in 1985. The ZEA metabolites mimic estrogens and act as estrogen receptor agonists; they have limited or no binding to carrier proteins. Thus they have easier access to estrogen target tissues and a potency that may be as much as 50 times greater than their actual concentrations suggest. The finding that the girls who were mycotoxin positive had a higher growth rate during TR treatment than those who were mycotoxin negative may be related

to the anabolic effect of accumulated ZEA that persists despite effective GnRH agonist treatment. The authors referenced a publication that showed a prepubertal dose of estrogen replacement during TR treatment in girls with CPP is effective for at least 2 years in maintaining a height velocity of about +1 SDS without accelerating bone maturation.<sup>1</sup> Finally, the authors noted that although ZEA is stored in adipose depots, a single dose has a half-life of only about 22 hours in human blood. Thus incidental exposure may be time limited, but could induce a central maturation of the hypothalamic pituitary gonadal axis.

Massart F, Meucci V, Saggese G, Soldani G. High growth rate of girls with precocious puberty exposed to estrogenic mycotoxins. *J Pediatr*. 2008;152:690-5.

**Editor's Comment:** *There is a growing body of evidence concerning endocrine disruptors. Much of the information has been gathered from animal studies but there have been a few human studies that demonstrate a clear-cut association between such environmental agents and early puberty in children. Massart et al, in this carefully controlled study, demonstrated a significant association between an "endocrine disruptor" and CPP. The disruptor, ZEA and its metabolites, is a naturally occurring mycotoxin. Thus, the relationship between at least one estrogen disruptor and the occurrence of CPP in pediatric patients cannot be dismissed. This study should give encouragement to those who are attempting to identify other potential environmental contaminants that may be associated with endocrine disruption in the pediatric population and also should underscore the importance of taking a careful dietary and exposure history from each of our patients who present with similar clinical findings. It is disturbing that a derivative of this mycotoxin has been widely used as a growth promoter to fatten cattle in the US. Only with careful, well-documented information can these important associations be identified and exposures be limited or reduced.*

William L. Clarke, MD

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## Long-term Follow-up of Idiopathic CPP Treated with GnRHs

Pasquino et al evaluated the impact of gonadotropin-releasing hormone analog (GnRHa) treatment on the adult height (AH), BMI, bone mineral density (BMD), and reproductive function of 87 girls with idiopathic central precocious puberty (ICPP). Patients were treated with depot triptorelin at a dose of 100 to 120 mcg/kg every 21–25 days for a period of  $4.2 \pm 1.6$  years (range 3–7.9) and were then observed for  $9.9 \pm 2.0$  years (range of 4–10.6) after discontinuing treatment; 32 untreated girls with ICPP served as controls. The AH of treated girls was  $159.8 \pm$

5.3 cm, significantly higher than predicted adult height (PAH) with a gain in centimeters between PAH and AH of  $5.1 \pm 4.5$ . Although, on the whole, BMI increased, BMI SDS for chronological age was not different at the beginning or at the discontinuation of treatment, or years afterwards; patients who were overweight or obese at the beginning of treatment remained so by the end of therapy. Gonadotropin and estradiol levels decreased significantly with GnRHa therapy and rose above pre-treatment levels one year after discontinuation of therapy.



Ovarian volumes, were reduced during treatment and increased thereafter, while uterine length was unchanged during therapy and increased one year after discontinuing therapy. Menarche appeared at the age of  $13.6 \pm 1.1$  years after withdrawal of GnRHa at  $0.9 \pm 0.4$  years (range of 0.3–2.0); 82 patients had a pattern of regular menses, while 5 had oligomenorrhea due to intensive physical activity (which resolved when this activity was decreased), and 6 girls became pregnant and delivered normal offspring. The BMD calculated both by area and volume were decreased at discontinuation of therapy when compared to controls, but after complete resumption of gonadal activity were not significantly different from controls. The authors concluded that GnRHa treatment of girls with ICCP is safe for the reproductive system, BMD, and BMI and is helpful in reaching an AH close to target height.

Pasquino AM, Pucarelli I, Accardo F, Demiraj V, Segni M, Di Nardo R. Long-term observation of 87 girls with idiopathic central precocious puberty treated with gonadotropin-releasing hormone analogs: Impact on adult height, body mass index, bone mineral content and reproductive function. *J Clin Endocrinol Metab.* 2008;93:190-5.

**Editor's Comment:** The long-term follow-up by Pasquino and colleagues of a large cohort of girls with ICCP treated with GnRHa, suggests that this form of therapy is safe—leading to a normal resumption of gonadal function one year after discontinuation of therapy. This was manifested by an increase of gonadotropins and estradiol to normal levels for age, an increase in ovarian and uterine dimensions, appearance of menarche at a mean of  $0.9 \pm 0.4$  years after discontinuation of treatment with the maintenance of a normal menstrual pattern thereafter, and with normal pregnancies and deliveries of healthy offspring in 6 girls. This, as well as previous data<sup>1</sup> should assure physicians and parents of the safety of this medication in regard to gonadal function and the future reproductive health of girls with ICCP treated for prolonged periods with GnRHa. Recent data suggest that children with ICCP may have an increased BMI and that GnRHa treatment might

contribute to the worsening of this parameter.<sup>2</sup> Although, as a whole, BMI increased during therapy, it remained in the same centile or SDS throughout treatment and patients who were already obese or overweight at the beginning, remained so at the end of treatment. There is still controversy regarding the beneficial effect of GnRHa treatment on the AH of treated girls with ICCP.<sup>1,2</sup> In this study AH of treated girls was significantly increased when compared to PAH before the beginning of therapy and, as a whole, patients reached or overcame their TH. When the AH height of untreated control subjects was compared to that of treated individuals it was found to be about 5 cm shorter—more than 4 cm below their TH and with no significant gain over their PAH. However, while GnRHa therapy seems helpful in reaching an AH close to TH, it is clear that there is a marked variability in individual response. Another worrisome issue is the effect of suppression of ovarian activity on BMD, both during therapy and long-term.<sup>3</sup> Even though this study demonstrated a decrease in bone accretion during GnRHa therapy, bone mineral density calculated both by area and volume seemed to normalize after the complete resumption of ovarian activity and peak bone mass was reached. Although GnRHa therapy has been widely used in the treatment of girls with ICCP for the last 20 years, many doubts in regard to its long-term benefits and safety remain. This long-term follow-up of a large cohort of treated girls may ease concerns.

Roberto Lanes, MD

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## Genetics of Dwarfism

The centrosome is a cytoplasmic organelle that prepares the mitotic spindle for chromosome segregation and also regulates progression of the cell cycle through mitosis.<sup>1</sup> Pericentrin 2 (PCNT2) is a centrosomal protein that is essential for the integrity of the mitotic spindle as it links the microtubules of the mitotic spindle apparatus to the centrosomal core. PCNT2 is also involved in the process of normal cell division at the G2-M checkpoint. Thus, loss of PCNT2 likely results in cell death because of defects in both chromosome segregation and mitosis. Rauch et al and Griffith et al have described clinical syndromes associated with biallelic loss-of-function mutations in the gene encoding PCNT2—also termed kendrin (PCNT2 - chromosome 21q22.3-qter - OMIM 605925).

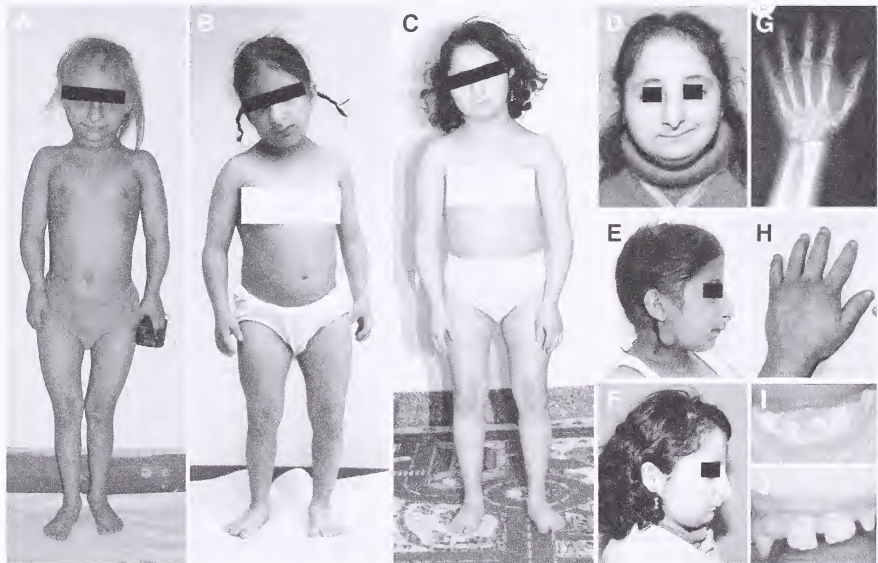
Microcephalic osteodysplastic primordial dwarfism (MOPD) is characterized by intrauterine and postnatal growth retardation, short limbs (brachymelia), and microcephaly (Figure 1). The humeri and femora are broad, shortened, and bowed. Clinically, MOPD has been subclassified into types I (OMIM 210710), II (OMIM 210720), and III (OMIM 210730). Types I and III are considered to be variations of the same disorder and are associated with dysplasia of the skull, vertebrae, and limbs and malformations of the brain and early death; no gene mutation has as yet been identified in these subjects. In patients with MOPD II, an autosomal recessive disorder, facial features are similar to those of patients with Seckel syndrome (*vide infra*); birth weight is <1.5 kg at term;

average adult height is 100 cm; adult head circumference is 40 cm, mentation is reasonably normal. Adults with MOPD II have a shortened life-span because they are at increased risk for development of type 2 diabetes mellitus, obesity, and cerebrovascular accidents. MOPD II is not considered a syndrome of premature aging as these patients are not at risk for development of neoplasia nor do their chromosomal telomeres display an accelerated rate of shortening. Utilizing the families of patients with MOPD II born to consanguineous parents and genome wide linkage analysis, Rauch et al localized this disorder to chromosome 21q22.3—the site of *PCNT2*. After analysis of the 47 exons of *PCNT2* in 25 unrelated patients with MOPD II, these investigators identified 29 distinct null mutations (12 stop and 17 frameshift) scattered through the gene. Interestingly, in patients with MOPD II, *PCNT2* is transcribed (mRNA levels are normal or slightly decreased) but not translated (*PCNT2* protein levels are absent or low), as its mRNA is subjected to nonsense-mediated mRNA decay directed by pretranslational mRNA surveillance mechanisms. Heterozygous (*PCNT2*<sup>+/−</sup>) parents synthesize less *PCNT2* protein than normal subjects and are

reported to have significantly short adult stature raising the possibility that variants of *PCNT2* are involved in determination of stature in the normal population.

Seckel syndrome is also a heterogeneous, autosomal recessive disorder that has been subclassified into types 1 through 4 depending on linkage to different chromosomal regions (3q22, 18p11, 14q, 21q22.3). It is characterized by *symmetrical* prenatal and postnatal growth retardation, microcephaly with developmental delay, and “bird-like” facial features (small head, large eyes, beak-like nose, micrognathia). The clinical characteristics of patients with Seckel syndrome type 4 (chromosome 21q22.3-*qter*; OMIM 611860) are similar to those of patients with other subtypes. Griffith et al, utilizing a genome wide association procedure in 2 consanguineous families with Seckel syndrome members, also localized the disorder to chromosome 21q22.3 and identified homozygous inactivating (nonsense, single base pair deletion or insertion) mutations in *PCNT2* in affected patients.

The reason that loss-of-function mutations in *PCNT2* result in 2 clinically similar (microcephaly, facial features, growth retardation) but distinct (proportionate versus non-



**Figure 1. Phenotype of MOPD II patients.** (A) P18 at age 8 years 3 months with a height of 84 cm corresponding to a normal size for a female infant aged 1 year 3 months; (B and E) P1 at age 8 years 8 months with a height of 85 cm; (C and F) P2 at age 12 years 6 months with a height of 95 cm and at age 14 years with a height of 96 cm (D) corresponding to a normal size for a female aged 3 years. Note short lower arms especially in P18, mild truncal obesity and premature puberty in P1, significant facial asymmetry in P2 (D), and absence of a sloping forehead typical of microcephaly syndromes. All three patients demonstrate a long nose with prominent tip and hypoplastic alae and small mandible described as typical for patients with MOPD II. (G and H) X-ray and an image of the dorsum of the left hand of patient P2 showing generalized brachydactyly with diaphyseal constriction (overmodeling) of metacarpals and phalanges, as well as abnormal flat shape of the distal radius and ulna epiphyses. (I and J) Hypoplasia and partial agenesis of teeth from patient P18, enamel hypoplasia in teeth from patient P18.

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symmetrical short stature, reasonably normal mentation versus developmental delay) disorders of MOPD II or Seckel syndrome is uncertain. It has been suggested that in MOPD II, the PCNT2 mutations may adversely affect function of the centrosome, while in Seckel syndrome the mutations may impair mitotic progression.<sup>1</sup>

Rauch A, Thiel CT, Schindler D, et al. Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. *Science*. 2008;319:816-9.

Griffith E, Walker S, Martin C-A, et al. Mutations in pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. *Nat Genet*. 2008;40:232-6.

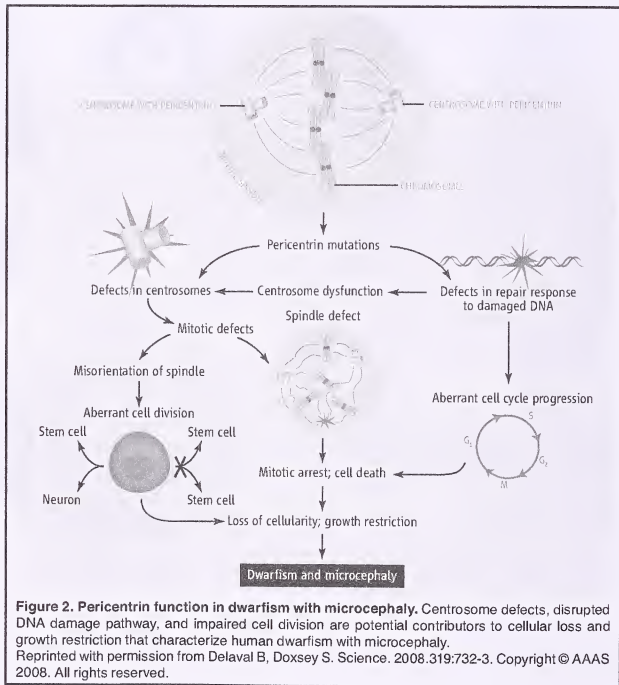
**First Editor's Comment:** Seckel syndrome type 1 has been ascribed to inactivating mutations in the DNA damage detection and repair ataxia-telangiectasia and Rad3-related gene (ATR; chromosome 3q22-24, OMIM 601205). Inactivation of PCNT2 adversely affects function of ATR protein-dependent effects on monitoring of the cell cycle; PCNT2 acts at a point downstream of ATR. Mutations in several genes that encode centrosomal and mitotic spindle-related proteins have been associated with isolated primary microcephaly with normal stature (CDK5RAP2, ASPM, MCPH6) and primary microcephaly with short stature (MCPH1). *Homo floresiensis* is species of hominids whose fossils have been found in Indonesia and who have several features in common with MOPD II including an adult height of 100 cm, small brain but normal intelligence, and skeletal anomalies raising speculation that they may have been humans with MOPD II or defects elsewhere in the DNA damage-repair pathways.

The findings in patients with MOPD II and Seckel syndrome may be compared with those of Hutchinson-Gilford progeria,<sup>2,3</sup> a syndrome of premature aging due to a monoallelic mutation in the gene (LMNA) encoding lamin A. Progeric subjects are characterized by postnatal growth retardation, small head circumference, abnormalities of the skin (altered pigmentation, sclerosis, alopecia), hypodontia, lipodystrophy, restricted joint mobility, cardiovascular abnormalities, and early death. Lamin A (chromosome 1q21.2, OMIM 150330) is an essential component of the protein network found within the nuclear

membrane. Ninety percent of patients have a C-to-T substitution at nucleotide 1824 resulting in substitution at codon 608 of glycine GGC for glycine GGT. This nucleotide change activates a cryptic splice donor site that removes 150 nucleotides from transcribed LMNA mRNA. The translated protein retains farnesyl groups that link mutant and wt lamin A molecules and prevents their release from the inner nuclear membrane, thereby interfering with cell mitosis and gene expression. Experimentally, prevention of farnesyl attachment to mutated lamin A allows the protein to separate from the inner nuclear membrane. A drug that inhibits farnesyl transferase ameliorates a mouse model of progeria. An open-label trial of this agent is now underway in progeric patients.

Allen W. Root, MD

**Second Editor's Comment:** Seckel syndrome refers to a genetically heterogeneous group of autosomal recessive conditions (SCKL1-4) characterized by severe pre- and postnatal growth deficiency and marked microcephaly. While all 4 conditions have been chromosomally mapped, the gene locus is known only for SCKL1; it encodes ATR, which functions to coordinate cellular responses to DNA damage. More specifically, ATR signaling responds to



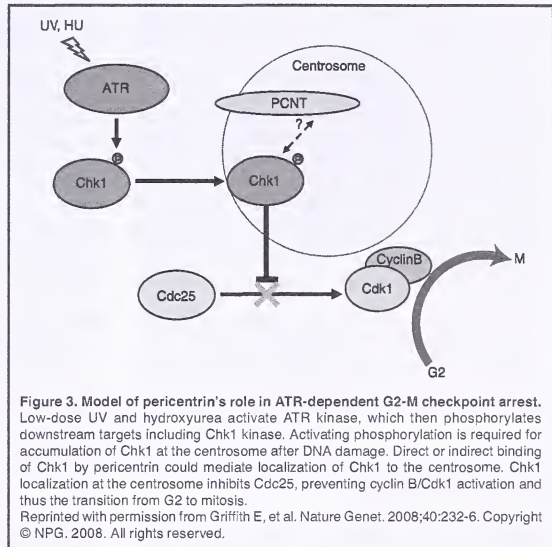
**Figure 2. Pericentrin function in dwarfism with microcephaly.** Centrosome defects, disrupted DNA damage pathway, and impaired cell division are potential contributors to cellular loss and growth restriction that characterize human dwarfism with microcephaly. Reprinted with permission from Delaval B, Doxsey S. *Science*. 2008;319:732-3. Copyright © AAAS 2008. All rights reserved.



single-stranded DNA damage.

Griffith and colleagues carried out an SNP-microarray genome-wide scan to detect regions of homozygosity on 2 consanguineous families with individuals clinically diagnosed with Seckel syndrome and showing evidence of defective ATR signaling. The scan identified a region on chromosome 21q22.3, which corresponds to the SCKL4 locus, that contained the gene encoding the centrosomal protein, PCNT. Pericentrin was considered a candidate because mutations in other centrosomal protein genes were known to cause primary microcephaly (Figure 2). Genomic sequencing revealed a homozygous nonsense mutation in exon 4 in affected members of one family and a homozygous single basepair deletion in the other; both were predicted to result in loss of function. A similar PCNT mutation was detected in a third patient with typical features of Seckel syndrome.

Pericentrin localizes in cells to the pericentriolar material where it is believed to interact with several structural centrosomal proteins involved in the attachment of microtubules during mitotic spindle formation. It also appears to act as a scaffold to recruit signaling molecules, such as protein kinase A (PKA) to centrosomes. The authors carried out a number of experiments to document the absence of centrosomal pericentrin in patient cells. They also induced DNA damage with UV light and showed that ATR-dependent DNA damage response signaling that is normally activated during the cell cycle was defective similar to that observed in cells from patients with SCKL1 (Figure 3). This step is often referred to as G2-M checkpoint arrest, a process that prevents cells from entering into the M (mitotic) phase of the cell cycle with damaged DNA. The authors postulated that pericentrin deficiency interferes with growth through a general impairment in the progression of cells through mitosis. They also noted that identification



**Figure 3. Model of pericentrin's role in ATR-dependent G2-M checkpoint arrest.** Low-dose UV and hydroxyurea activate ATR kinase, which then phosphorylates downstream targets including Chk1 kinase. Activating phosphorylation is required for accumulation of Chk1 at the centrosome after DNA damage. Direct or indirect binding of Chk1 by pericentrin could mediate localization of Chk1 to the centrosome. Chk1 localization at the centrosome inhibits Cdc25, preventing cyclin B/Cdk1 activation and thus the transition from G2 to mitosis. Reprinted with permission from Griffith E, et al. *Nature Genet.* 2008;40:232-6. Copyright © NPG. 2008. All rights reserved.

of pericentrin mutations provides an interesting convergence between microcephaly genes implicated in ATR signaling and those involved in centrosomal function. It makes sense that the profound growth deficiency of Seckel syndrome is due to a disturbance in the machinery that directs cell division, but it would have been impossible to predict the specific defect without recent advances in genomic technology.

William A. Horton, MD

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## Genetics Influences Allelic Expression Patterns

Maternal and paternal alleles of autosomal genes were historically assumed to be expressed at the same levels. However, recent observations suggest that their expression may differ, ie, differential allelic expression (DAE), and that this difference may contribute to variability of clinical phenotypes in dominantly inherited disorders. To determine if there is a genetic component to DAE, Cheung et al examined patterns of allele expression in monozygotic twins.

The authors studied lymphoblastoid B cells from 21

monozygotic twins and 10 unrelated individuals. They took advantage of single nucleotide polymorphisms (SNPs) that map to exons so that they could be detected in mRNA and identified 285 instances in which "A" and "B" alleles could be distinguished in the respective mRNA transcripts. To determine the extent of differential expression of these alleles regardless of twinning, they first examined DAE in one member of each twin pair and the unrelated individuals using deviation from equal expression of the 2 alleles as a measure of DAE. Deviation was considered



nominally statistically significant for half of the allele pairs, and 17% displayed an expression difference of 2-fold or more for one allele over the other.

Next they searched for DAE in the monozygotic twins utilizing 211 SNPs that were found to be heterozygous in 5 or more twin pairs and did an analysis of variance to determine the significance of twin resemblance. The results revealed much greater similarity between twins than predicted by chance. In a few instances in which more than one informative SNPs mapped to the same gene, the results were concordant. Twin resemblance for DAE was detected not only for genes whose alleles deviated substantially from equal expression, but also for genes whose alleles are expressed at relatively similar levels.

The authors drew 2 conclusions from their results. First, at least 50% of genes expressed in lymphoblastoid B cells show some degree of DAE. The difference is greater than 2-fold for some genes. Second, much of the observed DAE seems to be under genetic control.

Cheung VG, Bruzel A, Burdick JT, Morley M, Devlin JL, Spielman RS. Monozygotic twins reveal germline contribution to allelic expression differences. *Am J Hum Genet.* 2008; 82:1357-60.

**Editor's Comment:** *This investigation provides another explanation for why monozygotic twins are so similar. A paper was recently reviewed in GGH<sup>1,2</sup> suggesting that patterns of epigenetic modification diverge in monozygotic twins as they age. Since epigenetic modification influences expression of genes, one wonders if DAE varies with age or correlates at all with such modifications. Similarly, it would be interesting to know the extent to which DAE occurs in cell types other than lymphoblastoid B cells.*

William A. Horton, MD

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## Growth Hormone Therapy Improves Mental and Motor Development in Young Prader-Willi Patients

Prader-Willi syndrome (PWS) is increasingly diagnosed in early infancy because pediatricians and neonatologists are more aware of the clinical picture (muscular hypotonia, feeding difficulties, failure to thrive, and psychomotor delay). The genetic cause of PWS is an alteration in the long arm of paternal chromosome 15 (by deletion, microdeletion, maternal uniparental disomy, mutation of imprinting centre, chromosomal rearrangement). It is well known that methylation analysis is an efficient tool for early and reliable diagnosis of PWS. Children with PWS have an abnormal body composition with a relatively high body fat percentage and a low lean body mass (LBM). Even in PWS infants who are underweight, body fat percentage is high.

Treatment with human growth hormone (hGH) in older children with PWS results not only in an increased growth response but also in an improvement in body composition, with a decline in fat percentage and an increment in LBM, resulting in increased muscle strength and agility. The effects of hGH therapy on psychosocial development in PWS have not been well studied.

Festen and colleagues evaluated psychomotor development in PWS infants and toddlers during hGH treatment compared to controls. Forty-three PWS infants were evaluated at baseline; 29 of them were randomized into a GH group (n=15) receiving 1 mg/m<sup>2</sup>/day of GH or a non-GH-treated control group (n= 14). At baseline, and after 12 months of GH treatment, an analysis with Bayley Scales of Infant Development II (BSID-II) was performed. Data were converted to percentage of expected development for age, and changes during follow-up were calculated.

Infants in the GH group had a median age of 2.3 years (interquartile range [IQR] 1.7–3.0) and the median age of the control group was 1.5 years (IQR 1.2–2.7) ( $p=0.77$ ). Both mental and motor development improved significantly during the first year of study in the GH group vs the control group: median (IQR) change was +9.3% (–5.3 to 13.3) vs –2.9% (–8.1 to 4.9) ( $p<0.05$ ) in mental development and +11.2% (–4.9 to 22.5) vs –18.5% (–27.9 to 1.8) ( $p<0.05$ ) in motor development, respectively. Thus, one year of hGH treatment significantly improved mental and motor development in PWS infants compared to controls. Infants with lower developmental age had the greatest improvement in motor development. There was also a normalization of head circumference and a significant increase in height SDS in the GH group, but not in the control group after one year of hGH treatment. The hGH was well tolerated; compared to randomized controls, hGH did not induce disadvantageous effects on sleep-related breathing disorders, carbohydrate metabolism and thyroid hormone levels.

Festen DAM, Wevers M, Lindgren AC, et al. Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. *Clin Endocrinol.* 2008;68:919-25.

**Editor's Comment:** *The best point of time to initiate hGH therapy for PWS remains unknown. Eiholzer et al<sup>1</sup> do not recommend starting hGH therapy in PWS in the first year of life because of an increased risk of sudden infant death during this period. Festen and colleagues evaluated whether hGH treatment started at an early age could contribute to an improvement in mental and motor development in a group of PWS patients. They*

found a significant improvement of both mental and motor development in the hGH group compared to the control group. Children with lower developmental age had the greatest improvement in motor development, suggesting that hGH treatment might be considered at an early developmental age to optimize the hGH effects on motor development. They also found that hGH did not induce disadvantageous effects on sleep-related breathing disorders.

In their study, insulin-like growth factor (IGF)-I levels increased rapidly during hGH treatment from below the normal range to the high-normal range. IGF-I receptors have been localized in several areas in the human brain, indicating that IGF-I may have a neuroregulatory role in the central nervous system. Theoretically, IGF-I may directly influence the central nervous system or hGH might induce local IGF-I expression in brain tissue,

thereby improving psychomotor development. Another possible explanation for the improvement in mental development during hGH treatment might be that, because of the improved motor development, children are able to sit, stand and walk independently, enabling them to explore and interact with the environment and resulting in a subsequent improvement in mental development. The results of this study suggest that early start with hGH might be beneficial in PWS. However, long-term double-blind studies are needed to evaluate the efficacy and safety of the early treatment with hGH on cognition in childhood and adulthood.

Yoshikazu Nishi, MD

## References

1. Eiholzer U, Allermann D, Schlupf M, Rousson V, Gasser T, Fusch C. Growth hormone and body composition in children younger than 2 years with Prader-Willi syndrome. *J Pediatr* 2004 ;144:753-8.

## Central Adrenal Insufficiency, Pituitary and Neuroradiological Alterations in Prader-Willi

Prader-Willi syndrome (PWS; OMIM 176270) is a genetic disorder caused by an alteration in the long arm of paternal chromosome 15 (by deletion, microdeletion, maternal uniparental disomy, mutation of imprinting centre, chromosomal rearrangement). PWS is characterized by a complex clinical picture (short stature, uncontrollable hyperphagia, obesity, hypogonadism) and growth hormone deficiency that seem to be a central hypothalamic/pituitary dysfunction.

The annual death rate of PWS patients is very high (3%). Many of these deaths are sudden and unexplained. Because most deaths occur during infections and PWS patients suffer from various hypothalamic insufficiencies, de Lind van Wijngaarden and colleagues investigated whether PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions. Twenty-five children genetically confirmed PWS were randomly selected. Twelve patients had paternal deletion (63%), 6 had maternal disomy (32%), and one an imprinting center mutation (5%). Median age of patients with PWS was 9.7 years (range 3.7 to 18.6 years). All were treated with recombinant human growth hormone (rhGH). Overnight single-dose metyrapone tests were performed. Metyrapone (30 mg/kg) was administered at 2330 h. At 0400, 0600, and 0730 h, ACTH, 11-deoxycortisol, cortisol, and glucose levels were measured. Diurnal salivary cortisol profiles were also assessed on a different day at wake-up, 30 minutes after wake-up, at 1400 h, and at 2000 h. Fifteen patients (60%) showed an insufficient ACTH response at the metyrapone test. There was no significant difference in age, gender, genotype, and BMI SD score between patients with CAI and those without. Morning salivary cortisol levels and diurnal profiles were normal in all children, suggesting that CAI becomes apparent only during stressful conditions.

Moreover, Iughetti and colleagues retrospectively analyzed 91 patients with PWS (42 females, 49 males; age range 0.7 to 16.8 years) by cerebral MRI to determine whether there was any diminution in the anterior pituitary gland or other neuroradiological alterations. All subjects were genetically confirmed as PWS (58 microdeletions, 8 deletions, 28 maternal uniparental disomy). Of these 91 patients, MRI analysis showed a reduction in pituitary height (height <1 SD) in 45 patients (49.4%: 23 cases <2 SD; 20 males, 25 females) with 4 cases of empty sella, a complete absence of the posterior pituitary bright spot in 6 patients (6.6%) and other neuroradiological alterations in 10 patients (11%: 8 cases of ventricular enlargement, 2 cases of thin corpus callosum). Altogether, neuroradiological alterations were present in 61 of the 91 (67%) patients. No genotype-phenotype relationship was shown. These results of both de Lind van Wijngaarden and Iughetti indicate that CAI and neuroradiological alterations are more frequent in PWS patients than has been reported to date.

de Lind van Wijngaarden RF, Otten BJ, Festen DAM, et al. High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome. *J Clin Endocrinol Metab*. 2008;93:1649-54.

Iughetti L, Bosio L, Corrias A, et al. Pituitary height and neuroradiological alterations in patients with Prader-Willi syndrome. *Eur J Pediatr*. 2008;167:701-2.

**Editor's Comment:** These are very interesting observational studies, which provide important information for physicians who care for those with PWS. Strikingly, de Lind van Wijngaarden and colleagues reported 60% of PWS patients had CAI; the high percentage of CAI in PWS patients might explain the high rate of sudden death in these patients, particularly during infection-related stress. Because metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a

situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are therefore considered as having CAI during stressful conditions such as infection and surgery. In view of the importance of an adequate function of the hypothalamus-pituitary-adrenal axis for survival, the high prevalence of CAI may be an explanation for the high death rate in PWS patients. In addition to CAI, the condition of acutely ill PWS patients is further compromised by an increase in those with sleep apnea and sudden death during upper respiratory infection. Therefore, de Lind van Wijngaarden and colleagues stated that PWS patients

should be considered to have CAI during stress until proven otherwise with a metyrapone test and they recommended hydrocortisone treatment for PWS patients during stressful conditions including mild upper respiratory infections.

From these results, both neuroradiological alterations and CAI may relate mutually and may be important risk factors for a tendency of sudden, unexpected death in PWS patients. Further studies, including functional and longitudinal neuroradiological investigation, are needed to clarify these problems in PWS patients.

Yoshikazu Nishi, MD

## Genital Function and Sensitivity Following Feminizing Surgery

Like other disorders of sex development (DSD), congenital adrenal hyperplasia (CAH) in 46,XX can be associated with ambiguous genitalia at birth. Clinical management commonly involves surgery performed during infancy and childhood to feminize the appearance of genitals. However, it has been suggested that surgery to the clitoris potentially disrupts neurological pathways and compromises erotic sensation and pleasure. In a cross-sectional investigation, Crouch and colleagues investigated the genital sensitivity of women with CAH and 10 healthy controls (23 to 38 years old). Sensitivity thresholds for the clitoris and upper vagina were measured using a GenitoSensory Analyzer and sexual function by standardized self-report questionnaire including 7 subscales assessing sexual anorgasmia, satisfaction, sensuality, communication, vaginal penetration difficulties, frequency of intercourse and avoidance. Thirty-two of 56 eligible women with CAH (17 to 39 years of age) agreed to participate: 25 with classic CAH, 4 with non-salt losing CAH, and 3 with late-diagnosed CAH. A total of 28 of 32 women participated in sensory testing, including 4 who had not undergone prior genital surgery. The sample is heterogeneous with regard to the type of genital surgery (clitoridectomy versus clitoral reduction and with or without surgery to the lower vagina), age at surgical

procedures, and number of surgical procedures.

Clitoral sensation (temperature) testing indicated relative impairment for those who underwent clitoridectomy. As anticipated, clitoral sensation was not impaired in those with CAH who had not undergone surgery. In comparison with control group participants, women who had undergone clitoral reduction had a higher median threshold for warmth detection and a lower median threshold for cold. Vaginal sensitivity (vibratory) testing could not be assessed in some participants due to introital vaginal stenosis which prevented insertion of the vaginal probe. In addition, some control group participants chose not to undergo vaginal testing. For those who did, no difference was observed in vaginal sensation between the CAH group and control group participants (regardless of prior vaginal surgery).

Assessment of sexual function also proved to be challenging in this study; only 19 of 32 CAH participants adequately completed the questionnaire because of

**GRISS sexual function scores in women with CAH** divided into those with and without surgery compared to normal controls

|                                  | Median CAH (range) |            | Median Normal<br>(range) | P Value<br>(Kruskal-Wallis test) |
|----------------------------------|--------------------|------------|--------------------------|----------------------------------|
|                                  | Surgery            | No Surgery |                          |                                  |
| No. pts                          | 15*                | 4          | 10                       |                                  |
| Global score                     | 5 (1-9)            | 4 (1-5)    | 2 (1-8)                  | 0.029                            |
| Infrequent intercourse           | 8 (1-9)            | 6 (3-8)    | 5 (1-7)                  | 0.030                            |
| Non communication                | 4 (1-9)            | 5 (2-6)    | 5 (3-9)                  | 0.884                            |
| Dissatisfaction                  | 4 (1-9)            | 3 (2-5)    | 3 (1-6)                  | 0.195                            |
| Avoidance                        | 6 (1-9)            | 5 (4-7)    | 2 (1-7)                  | 0.043                            |
| Non sensuality                   | 5 (1-8)            | 3 (2-6)    | 2 (1-9)                  | 0.331                            |
| Vaginal penetration difficulties | 6 (1-9)            | 1 (1-2)    | 1 (1-2)                  | 0.006                            |
| anorgasmia                       | 6 (3-9)            | 4 (3-6)    | 3 (2-9)                  | 0.065                            |

Score range 1 to 9 with 5 or greater indicating difficulty.

\*One respondent excluded since she did not indicate a history of surgery.

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lack of sexual activity upon which responses depended. Those women with CAH who had undergone surgery reported worse scores on the intercourse frequency, vaginal penetration difficulties, and anorgasmia ( $p=0.065$ ) subscales compared with healthy controls and women with CAH who had not undergone surgery. Scores on global sexual dysfunction and avoidance were similar in women with CAH with and without surgery (Table). Significant correlations were detected between self-reported global sexual dysfunction and clitoral sensitivity impairment (Figure). The authors concluded that surgery is associated with a loss of sensitivity, and that impaired clitoral sensitivity is a result of surgical damage to the innervation of the clitoris. The authors further concluded that surgery is associated with sexual difficulties, citing a moderate but significant linear relationship between impaired clitoral sensitivity and the severity of sexual difficulties.

Crouch NS, Liao LM, Woodhouse CRJ, Conway GS, Creighton SM. Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia. *J Urol*. 2008;179:634-8.

**Editor's Comment:** These findings support previous research<sup>1,2</sup> suggesting an association between sexual dissatisfaction and genital surgery. This study goes beyond earlier research, however, by providing evidence for a mechanism mediating this association. Several challenges to interpretation of these data are worthy of note. Detailed operative records were available for only 15 women. This number is too small to enable comparisons of the effects of different surgical techniques. In addition, a significant number of women were unable to undergo vaginal testing due to proximal vaginal stenosis. On one hand, this difficulty provides important information, as the participants had reportedly undergone previous vaginoplasty to overcome penetration difficulties, yet penetration challenges clearly remain. A similar problem was seen in the effort to assess sexual function. Nineteen of 32 participants were unable to complete the questionnaire, citing lack of sexual experience necessary to complete questionnaire items.

It can be argued that clitoridectomy is rarely performed these days, and as such, numbness associated with this operation does not apply to the types of techniques currently performed. However, the authors indicated that only a third of the women in their sample who had undergone a more conservative technique, clitoral reduction, reported normal clitoral sensitivity. Most participants in this study underwent surgery in the early 1980s. The authors indicated that most of the clitoral procedures these participants experienced were based on the dorsal neurovascular bundle preservation approach described in 1981 by Mollard,<sup>3</sup> a procedure the authors noted that has been widely used after 1985 and which has become the basis of current practice.

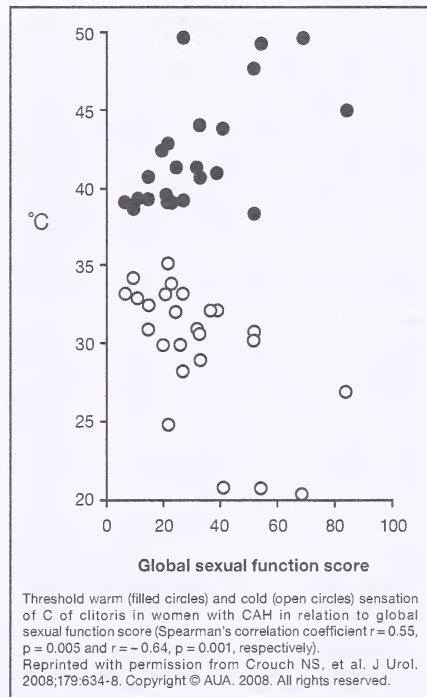
If the findings from this study are replicated in future studies, important issues need be carefully considered.

*Genital feminizing surgery for patients with CAH is typically performed prior to the age of consent. It is an unanswered question whether most women with CAH would knowingly sacrifice genital function for appearance. The difficult decision of opting for surgery (or not) is often left to parents/caregivers. As such, at the time of consent, they should be armed with clear information relating to the possibility of impairment of genital sensitivity and function. As noted by the authors, "informed consent should be based not just on the technical aspects of surgery and risks, but on a developed understanding and appreciation of potential implications for future sexual lives."*

David E. Sandberg, PhD

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3. Mollard P, Juskiewski S, Sarkissian J. Clitoroplasty in intersex: a new technique. *Br J Urol*. 1981;53:371-3.





## Diagnosis of Congenital Central Hypothyroidism in Infants

In the Netherlands, since 1995, a primary thyroxine ( $T_4$ ) determination with supplemental thyroid-stimulating hormone (TSH) and  $T_4$ -binding globulin (TBG) measurements have been used as a routine screening protocol for congenital hypothyroidism. This screening approach was developed as congenital hypothyroidism of central origin (CH-C)—often complicated by hypoglycemia due to growth hormone deficiency and/or ACTH deficiency—poses an additional threat to the central nervous system development. The authors considered that a rapid diagnosis is critical in this population. The neonatal CH screening program was therefore adapted to improve detection of TSH deficiency. Indeed in a recent evaluation of the nationwide prospective screening program from 1995–2000 an increase of 1/16404 of CH-C was demonstrated with a detection rate of 91.6%. From these data CH-C—formerly considered as a rare entity—would make up 13.5% of all cases of permanent CH detected in a 6-year study period. The results of this showed that the TRH test plays a pivotal role in young infants.

Infants with neonatal screening results indicative of CH-C and subsequent free  $T_4$  <0.93 ng/dL and TSH <15  $\mu$ U/mL were enrolled in the study; 26 out of 385,042 neonates met the criteria and were tested within 3 months of birth. A TRH test was performed on 21 subjects; 6 of these children were found to have false-positive screening results. The remaining 15 infants were found to have CH-C during a 5-year follow-up. In this group cortisol deficiency was present in 9 cases, GHD in 10 cases, and gonadotropins deficiency in 6 subjects. TRH tests were interpreted by plotting results at several times after TRH administration. On the basis of former studies an adequate TSH response to TRH was characterized by a peak concentration greater than 15  $\mu$ U/mL and a return to baseline within 3 hours. In response to TRH, patients showed either diminished increase (type 2 response) or slightly delayed but excessive increase and delayed decrease of plasma TSH (type 3 response). All patients with type 3 TSH response had multiple pituitary hormone deficiencies (MPHD), whereas the majority of patients (67%) with type 2 response—which reflects an impaired TSH secretion—had isolated TSH deficiency. In 12 of 15 infants, the screening test provided the first indication of CH-C. The most frequently encountered problems were pathological neonatal jaundice (40%), hypoglycemia (33%), and persistent vomiting (20%). Fourteen children underwent MRI of the brain, 8 had posterior pituitary ectopia (PPE). All of these patients had MPHD.

The TRH test, in spite of the difficulties in establishing the pattern of a normal response in relation to age, appears to be crucial in the diagnosis of CH-C. It allows

immediate assessment of the hypothalamic-pituitary function, and therefore rapid and appropriate treatment may be given. This appears to be particularly relevant for the group of infants screened in the early neonatal period as presenting at the typical TSH response: first increased and thereafter with a delayed return to normal.

van Tijn DA, de Vijlder JJ, Vulsma T. Role of the thyrotropin-releasing hormone stimulation test in diagnosis of congenital central hypothyroidism in infants. *J Clin Endocrinol Metab*. 2008;93:410–9.

**Editor's Comment:** A frequently used strategy for the diagnosis of CH is to first measure  $T_4$  in all samples, followed by TSH measurement for samples with low  $T_4$  values. Several North American states use this strategy. In the Netherlands, the latter approach was extended with the determination of TBG levels for the lowest 5% of  $T_4$  values. The  $T_4$  /TBG ratio serves as an indirect measure of the free  $T_4$  concentration (which cannot be determined directly in dried blood spots). In contrast to most screening programs, in which TSH levels are determined for the lowest 10% of  $T_4$  readings, TSH levels are measured for the lowest 20% of  $T_4$  values. In this way, the Dutch screening program provides unique information about the prevalence of CH-C.

Such an approach cannot be performed in countries that have based neonatal screening on blood TSH values. However the merit of the Dutch group had been to adapt the neonatal CH screening in order to be able to detect CH-C. They have shown an unexpectedly rather high frequency of central hypothyroidism at birth. From a clinical point of view the issue is important as many of these children are at risk for neuropsychological disorders and appropriate diagnosis would have been missed or delayed.

There are obvious limitations to the use of the TRH-stimulation test in infants: non-availability of the product in many countries, difficulties in establishing the normal pattern of TSH response, and possible variations in relation to age. Importantly, the subset of patients with MPHD and anatomical defects at the MRI have a serum TSH response distinctly different from the control group. The group of patients with type 2 response showing a flat response due to an impaired release most frequently did not show MRI abnormalities, had a lower incidence of MPHD, and a male predominance. Some had isolated deficiency of unknown origin.

Congenital hypothyroidism can often be diagnosed on a set of clinical symptoms without TRH testing; however it is frequently delayed if midline defects are not present. In patients with an abnormal newborn screen suggestive of CH-C, in whom a TRH test cannot be administered, treatment with thyroid supplementation should be considered throughout the infancy until the diagnosis is established later in life.

*In this interesting study the abnormal neonatal screen was the first sign in over 90% of the identified cases with MPH. It appears that the TRH stimulation test may aid in differentiating CH-C from other diseases in a context of newborn screening with low false-positive rates.<sup>2</sup>*

Raphaël Rappaport, MD

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2. Mettall SA, Wondisford FE. TRH testing in its infancy. *J Clin Endocrinol Metab*. 2008;93:378-9.

## Effect of Levo-thyroxine Treatment on Weight and BMI in Children with Acquired Hypothyroidism

Lomenick and colleagues performed a retrospective analysis of children with the diagnosis of hypothyroidism evaluated in their clinics between July 1995 and July 2006. These authors sought to determine short-term and long-term changes in weight with levo-thyroxine treatment of hypothyroidism. Inclusion criteria were met by 68 subjects, ie under 18 years of age at the time of initial assessment, diagnosis of acquired hypothyroidism, initiation of levo-thyroxine treatment at the first clinic visit, and seen at least once in follow-up. History, physical exams, and laboratory data were obtained from the medical records. Subjects were examined for weight to the nearest 0.1 kg and height to the nearest 0.1 cm as well as BMI. Subjects were divided into 2 groups based on their weight at the second clinic visit compared to their weight at the initial visit; those who lost weight (Group 1; n=21) and those who had no change in weight or who gained weight (Group 2; n=47). Variables were assessed at baseline, first follow-up visit after starting treatment, first visit 2 years after starting treatment, and the first visit 4 years after starting treatment.

The degree of hypothyroidism was variable (TSH 5.5 – 1600 µU/mL) and 81% of the subjects were female. There were no differences in mean age, weight, height, or BMI at baseline between Groups 1 and 2. Children in Group 1 had more severe hypothyroidism with an initial mean TSH of 414 vs 41.4 in Group 2. As anticipated, mean TSH decreased (147 – 5.0 µU/mL) from the initial visit to the first follow-up (an average of 4.4 months after starting treatment). The decrease was not associated with a significant change in mean weight, mean weight percentile, weight z-score, BMI, BMI percentile, or BMI z-score. Mean weight loss in Group 1 children was 2.3 kg which was not significant from baseline.

Thirty subjects had at least 2 years of follow-up. During this interval BMI percentile did not change significantly nor did BMI z-score, weight percentile, or weight z-score. Nineteen children had 4 years of follow and again there was no significant change in BMI percentile, BMI z-score, weight percentile, or weight z-score. Thirty-nine of the 68 subjects were classified

as overweight or obese initially (based on BMI). These children exhibited no change in weight or BMI from baseline to the first follow-up. At the second visit (first follow-up) significant correlations were found between initial TSH and change in weight percentile, BMI, BMI z-score, and BMI percentile. After 2 years the initial TSH was negatively correlated with BMI percentile and after 4 years there was a trend toward a correlation between initial TSH and change in BMI percentile.

The authors pointed out that the association between hypothyroidism and weight gain is well described in pediatric textbooks including textbooks on endocrinology and pediatric endocrinology. Indeed practitioners evaluating overweight children often request thyroid tests and prescribe levo-thyroxine for mild hypothyroidism in hope of assisting with weight loss. The current study does not support the notion of hypothyroidism as a cause of obesity and the authors suggested that practitioners should not expect significant changes in weight after treatment in most children with hypothyroidism.

Lomenick JP, El-Sayyid M, Smith WJ. Effect of levo-thyroxine treatment on weight and body mass index in children with acquired hypothyroidism. *J Pediatr*. 2008;152:96-100.

**Editor's Comment:** *Lomenick and colleagues have performed a very valuable study. Pediatric endocrinologists who receive referrals from primary care physicians of overweight children with slight elevations in TSH levels are well aware that treatment of such subclinical hypothyroidism rarely achieves significant weight loss. Despite the retrospective nature of this manuscript, it provides significant and important supporting evidence for discouraging unrealistic expectations in families whose overweight children have mild elevations in TSH. This manuscript should be mandatory reading for all physicians who hold out such hope to children or who make referrals to pediatric endocrinologists of such children. The pediatric endocrinology community should congratulate Lomenick and his colleagues and thank them for such a timely manuscript.*

William L. Clarke, MD

## Geographic Distribution of Childhood Diabetes and Obesity: Workforce of Pediatric Endocrinologists

Lee and associates determined the geographic distribution using the American Board of Pediatrics (ABP) list of pediatric endocrinologists (board certified, less than 65 years of age) by state and data from the National Survey of Children's Health (NSCH). The estimates from the NSCH were obtained by a nationally representative cross-sectional random digit telephone survey of households with children younger than 18 years of age. A single question was asked, "Has a doctor or healthcare professional ever told you your child has diabetes?" The weighted number of children with diabetes was then calculated for geographic divisions in regions of the US (Northeast, Midwest, South, and West). Type 1 and type 2 diabetes prevalence were not separated; the BMI was calculated using CDC growth charts and based on parental reported weight and height, and only obesity (BMI  $\geq$  95<sup>th</sup> percentile) was utilized in this analysis. Separate ratios of children to pediatric endocrinologists for diabetes and obesity were calculated by dividing the estimated number of children with these disorders by the census region and division. In addition, to determine the extent to which variation and disease prevalence versus pediatric endocrinologist supply affected the differences in geographic ratios, the observed ratios were compared under "index" conditions of greater supply and equitable distribution of pediatric endocrinologists. This calculation assumed that the ratio of child population to endocrinologists for each state would be similar to the state with the largest supply, Massachusetts. Then the ratio of obese children to pediatric endocrinologists was recalculated and the proportion of the observed ratio that would have been attributed to differences in supply was determined.

The authors determined there are an estimated 229,240 children with diabetes and 798 board certified pediatric endocrinologists in the US. The ratio of children with diabetes to board certified endocrinologists is therefore 290:1. Considerable variation by region was seen as the ratios in the Midwest, South, and West were more than double that in the Northeast. There are 17,441 obese children for every board certified pediatric endocrinologist and a 19-fold difference between the highest and lowest ratios per state. Overall the difference between index and observed ratios attributable to supply is 57% for children with diabetes and 69% for children with obesity. In order to reach the index ratios for children with diabetes an additional 2,091 pediatric endocrinologists are needed, and an additional 1,518 pediatric endocrinologists are needed to care for the children with obesity in the US.

The authors noted that although there are benchmarks

for the numbers of children in the population per healthcare provider, there are no ideal benchmark ratios for children with chronic diseases to pediatric subspecialists. Given that the average waiting time to see an endocrinologist is approximately 9 weeks, that many board certified pediatric endocrinologists spend only 62% of their time in direct patient care, that annually approximately 76 pediatric endocrinologists have entered the workforce (since 1997), the overall supply will unlikely meet the rising demand due to increasing number of children with diabetes in the US. Suggestions were made for organizing healthcare for diabetes and obesity, including an alternative model of a diabetes team led by a nurse practitioner in consultation with a pediatric endocrinologist may need to substitute for the American Diabetes Association (ADA) recommended diabetes team led by a pediatric endocrinologist. In addition, general pediatricians will need to be taught how to screen, evaluate, and manage obese children while reserving referrals to subspecialists for those for whom specific endocrinological abnormalities are identified.

Lee JM, Davis MM, Menon RK, Freed GL. Geographic distribution of childhood diabetes and obesity relative to the supply of pediatric endocrinologists in the United States. *J Pediatr*. 2008;152:331-6.

**Editor's Comment:** The information presented in this manuscript is not surprising to pediatric endocrinologists who have seen their patient populations grow beyond the level of comfort for providing optimal subspecialty patient care. It is important to note that this particular study was limited to diabetes and obesity and did not include children with other endocrine abnormalities. Thus, the supply of pediatric endocrinologists is much less than that presented for 2 of the most common referrals. It is unfortunate that supply and demand economics are not applied to the care of children with pediatric endocrine disorders. Reimbursement for multidisciplinary diabetes care remains low while that for managing obesity is non-existent in many instances. This manuscript did not address ways in which the supply of pediatric endocrinologists might be augmented, but rather dealt with some suggestions for how a different approach to the care of these children might be entertained. Creative pediatric endocrinologists are called upon to devise creative models for the care of these children which recognize the obvious disparity between index and observed workforce ratios. Such solutions will be mandatory given the rising incidence of diabetes and obesity in our population.

William L. Clarke, MD



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## THE GROWTH HORMONE GENE CLUSTER: PHYSIOLOGICAL ACTIONS AND REGULATION DURING PREGNANCY

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given to the roles for these hormones in the regulation of fetal growth and metabolism and to the hormonal and other factors that regulate their expression. Particular attention is given to studies from the author's laboratory. Several excellent comprehensive reviews have been published over the past few years that focus on the biology of the individual genes of the cluster.<sup>1,2</sup>

### INTRODUCTION

This manuscript reviews selective aspects of the molecular biology and physiology of the human growth hormone cluster genes during pregnancy. Special emphasis is

### THE HUMAN GROWTH HORMONE CLUSTER

The human growth hormone gene cluster consists of the genes that code for placental lactogen (PL; also known as chorionic

### From The Editor's Desk 2009

"You cannot build a house for last year's summer." This Ethiopian proverb may be applicable to the state of GGH on its 25th anniversary. Since 1984, when the journal was conceived, the editorial board has worked tirelessly to produce a journal of high scientific value, publishing original lead articles and reviews of the most important publications in the field with erudite editorial comments. We have pushed against our deadlines and provided our readers a high quality publication—without commercial bias. It has been gratifying, and GGH has become a very well appreciated source of information to pediatric endocrinologists and other specialists interested in the field. We have remained on top of the medical specialty and have been innovative—8 years ago we launched the journal on line. We now reach more than 11,000 subscribers worldwide and almost 500 readers every single day! We have told ourselves, and our readers have acknowledged, it has been GGH at its best; and, since it's inception it has been treasured.

However, in the new world of endless headlines and multiple sources of information an educational journal like ours has become difficult to fund. Scientific breakthroughs are published in *The New York Times* and repeated endlessly on cable news. Most scientific journals now contain editorials and review articles and derive strength from the members of the society that funds the journal. They often publish targeted supplements supported by industry. Pharmaceutical companies utilize multiple means to market their products directly to physicians and have turned away from supporting an educational journal like GGH, or fallen on hard times themselves. We have made major efforts to continue publishing the journal and have sought support from multiple sources including the pediatric endocrine societies, and industry—to no avail.

We believe that while there is no shortage of information, there is a scarcity of objective, unbiased insight into specific issues in pediatric endocrinology. This has been GGH's niche and this is why it should continue informing our very large audience. But our strategy is no longer sustainable as sponsors utilize direct means of reaching and targeting their prospects. Therefore, this GGH issue will be the last one of the series that you have enjoyed for 25 years. You will be hearing from us if we are able to obtain the funding necessary to provide you with a valuable unbiased educational resource.

Your thoughts will be welcome at FimaLifshitz@GGHjournal.com.

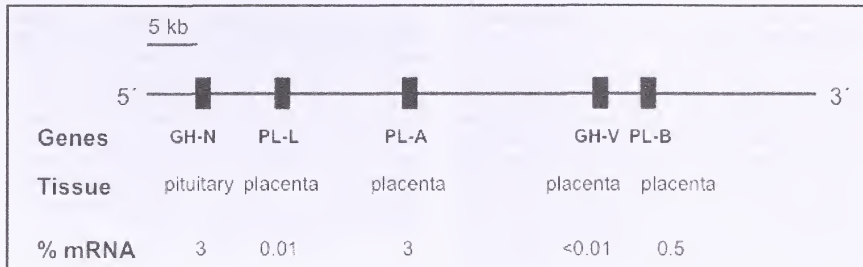
Respectfully,  
Fima Lifshitz, MD  
Editor-in-Chief





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**Figure 1. The human growth hormone gene cluster.**

The orientation and tissue-specific expression of the five genes comprising the cluster are shown from 5' to 3'. Also shown is the percentage of total mRNA for each gene in the placenta or pituitary. The abundance of hPL greatly exceeds that of hGH-V.

somatomammotropin, CS), growth hormone variant (GH-V; also known as placental growth hormone) and growth hormone normal (GH-N; also known as pituitary growth hormone). The cluster contains five genes, three PL and two GH genes that evolved from a common ancestral precursor by recombination events involving moderately repeated sequences. The cluster spans 66 kb on chromosome 17 (q22-q24).<sup>3</sup> The individual genes are organized in the same transcriptional orientation and are each composed of five exons and four introns. The order of the genes from 5' to 3' is GH-N, PL-L, PL-A, GH-V, and PL-B (Figure 1). The PL genes and GH-V are expressed exclusively in the placenta, and GH-N is expressed exclusively in the pituitary. As discussed below, the expression of the GH cluster is controlled by a locus control region (LCR) that is located 14.5 to 32 kb upstream of the GH-N gene.<sup>4,5</sup>

PL and GH-V are synthesized and secreted by the trophoblast layer of the placental villus, and the expression of the genes is tightly coupled to placental differentiation. The trophoblast layer is composed of two cell types—multinucleated syncytiotrophoblast cells and the underlying mononuclear cells that are the precursor cells that proliferate and fuse to form the overlying syncytium.<sup>6</sup> PL and GH-V are not expressed by the cytotrophoblast cells but are expressed as the cells undergo differentiation to a syncytiotrophoblast phenotype. Because of the tight coupling between GH gene cluster expression and villous trophoblast differentiation, the expression of the cluster genes in the placenta is regulated in large part by transcription factors and other signaling molecules that are critical for trophoblast differentiation.

The members of the GH gene cluster share 91% to 99% sequence identities throughout the coding regions and within a 500 bp region immediately upstream of the genes (for review see 7). PL-A and GH-V are alternatively spliced and encode 22 and 26 kD gene products, while PL-B encodes a single 22 kD protein product. The PL-A and PL-B mRNAs are 98% homologous and encode

identical mature proteins that are 85% identical to GH-N. The mRNAs for PL-A and PL-B are among the most abundant mRNAs in the placenta, comprising approximately 3.5% of the total mRNA. The PL-A gene is normally expressed at levels three to six times greater than the PL-B gene, probably due to differences in stability of the two mRNAs. The expression of the mRNA encoding PL-L increases towards term;<sup>8</sup> however, the PL-L protein product(s) is not secreted. The amino acid sequence of GH-V and GH-N differ in fifteen positions, thirteen of which are in the mature protein and are distributed throughout the sequence.<sup>9-11</sup> The GH-N gene encodes two alternatively spliced mRNAs that are translated into 22 and 20 kD GH proteins. Although GH-N and GH-V share striking homologies in structure, immunoassays for GH-N do not detect GH-V and visa versa. Consequently, immunoassays for GH-N cannot be used to measure GH-V.

#### EXPRESSION OF PL AND GH-V IN NORMAL AND PATHOLOGIC PREGNANCIES

The maternal concentrations of PL and GH-V increase markedly during pregnancy. Human PL is first detected in syncytiotrophoblast cells at 5-10 days after implantation and in maternal plasma at about six weeks of pregnancy.<sup>12</sup> Its concentration then increases linearly until weeks 32-35 of gestation when peak concentrations of 5000 to 7000 ng/mL are attained.<sup>13</sup> The secretion rate near term is about 1.0 gm/day, a rate considerably greater than that of any other polypeptide hormone. Throughout pregnancy, the plasma concentration of PL in the mother correlates with placental mass and is greater in multiple than in singleton gestations. In addition, the pattern of PL secretion during pregnancy roughly parallels the marked increase in maternal plasma insulin-like growth factor (IGF)-I concentrations that normally occurs in pregnancy. Direct measurement of the plasma concentrations of PL in the human fetus in vivo reveals a rise in fetal PL levels from a mean of 5 ng/mL at 20 weeks of gestation to a mean of 20-30 ng/mL at birth.<sup>14</sup> Since radiolabeled PL does not cross the placenta from the maternal

to the fetal circulations, PL appears to be secreted directly into fetal blood.

Aberrations of PL secretion have been detected in many common pathologic conditions of pregnancy, including diabetes mellitus, pre-eclampsia and hypertensive vascular disease.<sup>15-18</sup> In one large series of patients, a single PL concentration below 4 mcg/mL in the last five weeks of pregnancy was associated with 30% risk of fetal distress or neonatal asphyxia. Low PL concentrations on two separate occasions during the last five weeks were associated with a fetal risk of 50% and low concentrations on three occasions with a risk of 71%.<sup>19</sup> In another series of patients, PL concentrations below 4 mcg/mL were detected in 47 of 98 pre-eclamptic patients.<sup>20</sup> Perinatal mortality in the neonates born to the mothers with low PL concentrations was 13% and intrauterine growth retardation was noted in 57% of the neonates.

The lower than normal plasma concentrations of PL and other placental hormones in pregnancies complicated by intrauterine growth retardation (IUGR), pre-eclampsia and other pathologic conditions are probably due in large part to the placental hypoxia and decreased placental mass that is usually found in these conditions. In pre-eclampsia, for example, there is shallow invasion of cytotrophoblast cells into the endometrium, myometrium and spiral arteries of the uterus that results in decreased exchange of substrates, oxygen, hormones and other factors across the placenta. Consequently in pre-eclampsia, IUGR and other pathologic conditions of pregnancy associated with decreased PL, there are multiple factors that contribute to the growth failure of the fetus; and it is not possible to determine the relative contribution of decreased PL concentrations to the growth retardation.

GH-V is first detected in the maternal circulation at about 10 weeks of pregnancy, reaching a maximum in the third trimester of approximately 20-60 ng/mL, becoming the predominant form of GH in maternal serum throughout the latter half of pregnancy.<sup>21,22</sup> GH-V is not detected in fetal serum at any time during pregnancy,<sup>21</sup> indicating that the effects of the hormone on fetal metabolism or growth must be mediated indirectly through actions on maternal and possibly uteroplacental tissues. In contrast, the fetal circulation contains abundant amounts of GH-N, the levels of which rise to a maximum at mid gestation ( $33.6 \pm 2.1$  ng/mL by periumbilical blood sampling)<sup>23</sup> with a slow decline to levels approximating 20 ng/mL at term. There is a positive correlation between GH-V concentrations and the birth weight of the fetus; however, GH-V levels in the late second trimester or early third trimester are not predictive of fetal birth weight. Higher GH-V levels have been reported in pregnant women carrying female fetuses, suggesting a gender influence. Both GH-V and PL levels are increased in multiple pregnancies.

Mittal and co-workers have shown that preeclampsia is associated with higher concentrations of placental growth hormone in both the maternal and fetal circulations compared to normal pregnancy.<sup>24</sup> They have also shown that patients with preeclampsia plus small for gestational age (SGA) have lower maternal serum concentrations of GH-V than preeclampsia patients without SGA. Little is known about the regulation of GH-V production in abnormal pregnancies. However, recent studies demonstrate that maternal GH-V levels are reduced in pregnancies associated with IUGR.<sup>22,23,25</sup>

In contrast, GH-N concentrations during gestation remain relatively stable at 4 to 6 ng/mL. Fetal PL concentrations near term are 80 to 125 ng/mL, while GH-V is not detected in fetal plasma. Although GH-N levels remain low in the maternal circulation during pregnancy, GH-N is detected at relatively high concentrations in the fetus. GH-N concentrations in fetal plasma at term are 28 to 38 ng/mL, significantly greater than those detected in maternal plasma.

#### PHYSIOLOGICAL ACTIONS OF PL AND GH-V

Both PL and GH-V bind to sommatotropic and lactogenic receptors on a wide variety of tissues and have biological actions in many tissues, including liver, bone, blood cells and placenta. The potency of GH-V in growth-promoting assays is about 7-fold greater than that of PL; and the lactogenic potencies of the two hormones are comparable to that of prolactin. The rise in IGF-I levels in response to the placental hormones likely induces growth of maternal tissues, including the uterus, breast, and thyroid gland. Actions on the heart and kidney may increase cardiac output and maternal blood volume. Recent studies have shown that hGH-V regulates the invasion of extravillous trophoblast cells in the uterus,<sup>26</sup> but it is not known whether PL acts in an autocrine or paracrine manner to regulate placental development and/or function.

Maternal intermediary metabolism undergoes striking changes during pregnancy. In early and mid-gestation, body fat accumulates, while, in mid- to late gestation, the sensitivity to insulin declines and the mother develops postprandial hyperglycemia, hypertriglyceridemia and hyperinsulinemia. Prolonged fasting in late pregnancy leads to exaggerated production of free fatty acids and ketone bodies. These adaptations are thought to insure the continuous supply of glucose and amino acids to the fetus, thereby promoting fetal growth. Several lines of evidence strongly suggest that PL and GH-V play important roles in the metabolic adaptation to pregnancy. PL increases food intake and stimulates glucose uptake, glucose oxidation and the incorporation of glucose into glycogen, glycerol and fatty acids in isolated rat adipocytes, facilitating lipid and glycogen accumulation in the mother in early and mid-pregnancy pregnancy and during the fed state. PL, in concert with prolactin, progesterone, glucocorticoids

and other hormones, reduces insulin sensitivity and induces carbohydrate intolerance *in vivo*,<sup>27,28</sup> and stimulates <sup>3</sup>H-thymidine incorporation, insulin gene transcription, insulin production and glucose-dependent insulin secretion in pancreatic islet cells.<sup>29,30</sup> These actions of PL therefore contribute to postprandial hyperglycemia and hyperinsulinemia in the pregnant mother in mid to late pregnancy. PL also increases the basal rates of lipolysis in adipocytes and the plasma concentrations of nonesterified fatty acids, ketones and glycerol. The mobilization and utilization of maternal free fatty acids for energy spares maternal glucose for the fetus.

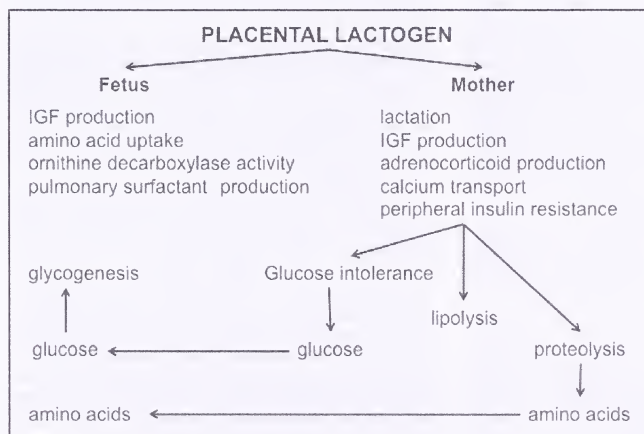
Several lines of evidence suggest that PL also has direct anabolic effects on fetal metabolism that promote fetal growth. PL is present in the fetal circulation in relatively high concentrations, binds to fetal tissues that are critical for fetal growth, and has direct growth-promoting actions on fetal tissues. The administration of PL to hypophysectomized rats increases tibia epiphyseal growth and plasma IGF-I concentrations with a potency approximately 5% to 10% that of GH. Furthermore, the placental hormone stimulates amino acid uptake, DNA synthesis and IGF-I production in cultured human fetal myoblasts, fibroblasts and hepatocytes.<sup>31-33</sup> The effects of PL on <sup>3</sup>H-thymidine incorporation and amino acid transport are blunted, though not abolished, by an antiserum to IGF-I, suggesting that the action of PL is mediated in part through the paracrine release of IGF-I.

PL and prolactin also stimulate DNA synthesis and insulin production in fetal and neonatal pancreatic explants and promote the formation of islet-like cell clusters in cultured pancreas cells.<sup>30,34,35</sup> These findings strongly suggest roles for PL in the induction of islet cell growth and insulin production in the late-gestational fetus. Other possible roles for PL in the fetus include the production of fetal adrenocortical steroid hormones and development of the fetal lung. PL also stimulates DNA synthesis in human mammary epithelial cells and growth of ductal epithelium, suggesting that the hormone may facilitate mammary development prior to delivery. A summary of the biological actions of PL in the mother and fetus is shown in Figure 2.

On the other hand, several lines of evidence indicate that GH-N plays only a limited role in fetal linear growth. Patients with isolated GH deficiency, pituitary aplasia or anencephaly have only minimal or modest (and inconsistent) reductions in birth length.<sup>36</sup> Furthermore, a deficiency of GH in experimental animals has little or no effect on fetal growth. For example, dwarf mice deficient in pituitary GH have normal tail lengths at birth and modest (14%) reductions in birth weight, though serum IGF-I and IGF-II concentrations are reduced significantly.<sup>37</sup> Decapitation, encephalotomy or hypophysectomy of fetal rabbits, rhesus monkeys, rats, mice, or pigs is not accompanied by fetal growth failure or reductions in serum IGF-I concentrations, and electrolytic destruction of the ovine fetal medial-

basal hypothalamus with concomitant GH deficiency has no effect on fetal plasma IGF-I or IGF-II concentrations (reviewed in 38). Conversely, an excess of fetal GH is not accompanied by fetal overgrowth.

Although GH-N may have only a limited effect on the longitudinal growth of the fetus, the hormone appears to have important effects on fetal metabolism and development. For example, clinical experience substantiates a role for GH-N in perinatal carbohydrate metabolism. The neonatal hypoglycemia may result in part from heightened sensitivity to insulin; however, deficient storage of glycogen in fetal liver may also play a role because GH stimulates glycogen synthesis and inhibits glycogenolysis<sup>39</sup> in



**Figure 2. The biologic actions of PL in the mother and fetus.**

PL has direct effects on fetal and maternal tissues that modulate fetal growth and metabolism. The induction of peripheral insulin resistance in the mother leads to glucose intolerance with resulting hyperglycemia as well as to an increase in lipolysis and proteolysis. The net effect of these changes is the transport of glucose and amino acids to the fetus and the stimulation of glycogenesis and protein synthesis in the fetus. PL also induces IGF production in the mother and fetus and stimulates lactation in the mother and pulmonary surfactant production in the fetus. The enzyme ornithine decarboxylase is important for the synthesis of DNA, RNA and protein.



isolated hepatocytes from fetal sheep and fetal rats. GH also stimulates DNA synthesis and IGF-I production in isolated human fetal hepatocytes as well as DNA synthesis, insulin production and glucose-dependent insulin secretion in isolated pancreatic islets from human adults and fetal and neonatal rats and mice. These latter observations implicate a role for GH in perinatal islet development and function. The high prevalence of micropenis in newborn males with GH deficiency or GH resistance<sup>40,41</sup> implicates a role for pituitary GH in the regulation of human phallic growth in utero.

## REGULATION OF THE HUMAN GROWTH HORMONE GENE CLUSTER

As discussed below, the GH cluster is controlled by a remote LCR that is located 14.5 to 32 kb upstream of the GH-N gene.<sup>4,5</sup> The LCR contains five hypersensitive sites (HSI-HSV), which are short regions of chromatin detected by supersensitivity to cleavage by DNase 1. These sites, which are only found in active genes, appear before the initiation of transcription and are generated as a result of the binding of transcription factors that displace histones after binding to DNA within the hypersensitive site. Closely linked HSI and HSII are pituitary specific, HSIV is placental specific, and HSIII and HSV are present in both tissues. HSV and HSIII, at -32 kb and -28 kb, are detected in pituitary somatotrope and placental syncytiotrophoblast cell chromatin; HSIV, at -30 kb, is specific to syncytiotrophoblast cell chromatin; and HSI and HSII, at -14.5 kb to -15.5 kb, are specific to somatotrope chromatin. The GH LCR and the GH-N promoter are encompassed by a continuous 32 kb pituitary-specific domain of acetylated histones H3 and H4 with a central peak located at HSI. Histone acetylation is linked to transcriptional activation; and histone acetyltransferases (HATs) and histone deacetylases (HDACs) are recruited to promoters through physical interaction with sequence-specific transcription factors. Site-specific inactivation of HSI results in loss of acetylation throughout this domain, loss of critical transfactor occupancy at the GH-N promoter, and a 20-fold reduction in GH-N expression. Thus, HSI plays an essential role in the establishment of the acetylated domain and in activation of GH-N transcription in the pituitary.

Histone acetyltransferase (HAT) activity recruited to HSI establishes a continuous 32 kb domain of histone acetylation connecting the LCR and the GH-N promoter. This acetylated domain facilitates transfactor binding at the GH-N promoter and transcriptional activation of GH-N. Activation of the placental genes in the term placental syncytiotrophoblast cells is marked by activating histone modifications that are restricted to HSV-HSIII and to the placental genes; the regions between, which include HSII and the GH-N gene, remain unmodified. Based on the present knowledge of cellular differentiation and epigenetic alterations, it seems reasonable to propose

that chromatin structures in the placenta are altered during the terminal transition from cytotrophoblast cells to syncytiotrophoblast cells to result in robust induction of gene expression from the GH cluster.

Gene activation during cytotrophoblast cells differentiation to a syncytiotrophoblast cell phenotype is initiated by H3K4 methylation of HSIII-HSV of each individual placental gene repeat (PGR) unit.<sup>42</sup> Subsequent transcriptional activation is accompanied by acetylation of histones H3 and H4 encompassing the entire placenta-expressed region of the cluster. The distribution and progression of chromatin modifications suggests that each PGR independently initiates transcription. Initial activating chromatin modifications are nucleated within the individual PGR units; and subsequent transcriptional induction relies on additional determinants and more extended chromatin modifications.

## REGULATION OF PL AND GH-V EXPRESSION

Although *in vivo* studies have provided information about the regulation of PL and GH-V secretion, most information about the expression of these hormones has been obtained using primary cultures of human cytotrophoblast cells or explant cultures. The primary cytotrophoblast cells, which are prepared by enzymatic dispersion of term or pre-term placental tissue, undergo spontaneous aggregation, syncytialization and terminal differentiation and express genes normally expressed by syncytiotrophoblast cells, including PL and GH-V.

Using these *in vitro* model systems, many factors have been shown to induce trophoblast differentiation and the expression of PL and GH-V. These factors include epidermal growth factor,<sup>43</sup> chorionic gonadotropin,<sup>44</sup> leukemia inhibitory factor,<sup>45</sup> colony stimulating factor-1,<sup>46</sup> IGF-I,<sup>47</sup> cyclic AMP,<sup>48</sup> members of the transforming growth factor  $\beta$  superfamily,<sup>49</sup> the Wnt/ $\beta$ -catenin pathway,<sup>50-52</sup> the transcription factors PPAR $\gamma$ ,<sup>53</sup> Ikaros,<sup>54</sup> GATA-2/3,<sup>55</sup> and several other factors in the differentiation process. Oxygen has also been shown to be a critical factor in the differentiation process and the induction of the GH cluster genes (for summary see 56). Low oxygen tension directs placental differentiation along the extravillous trophoblast cell pathway in which cytotrophoblast cells invade the uterus. Greater oxygen tension directs differentiation along the villous trophoblast cell pathway and the formation of the trophoblast layer that lines the placental villus. Recent studies from the author's laboratory have also demonstrated a critical role for the transcription factor TFAP2A (also known as AP2, activator protein 2) in syncytiotrophoblast formation and the induction of PL and GH-V.<sup>57,58</sup>

Knockout experiments in the mouse have identified many transcription factors that are important in the differentiation of the various cell types constituting the murine placenta,<sup>59-61</sup> including HOXB6, HOXC5, HOXC6,

HOX3E, HB24, GCM1, GAX, MSX2, DLX4, Pit-1, HAND1, TF-1, TEF5, c-Ets1 and several other transcription factors, many of which are helix-loop-helix (bHLH) proteins. ID-2, a member of a family of inhibitors of bHLH binding, acts in trophoblast cells as a dominant/negative bHLH transcription factor;<sup>62</sup> and constitutive overexpression prevents differentiation of the cells. However, the roles for homologs of these transcription factors in human placental development are not known.<sup>63,64</sup>

### PL expression

At present, the specific hormonal and metabolic factors that regulate the secretion of PL are incompletely PL understood. Although PL has striking homologies in structure and function to GH-N, the factors that regulate the expression of the two hormones are different. For example, changes in circulating levels of free fatty acid concentrations, amino acids such as arginine, estrogens, oxytocin, prostaglandins, epinephrine, TRH, GnRH, dopamine and glucocorticoids do not effect modulate PL secretion. While changes in blood glucose concentrations modulate GH-N secretion, glucose does not appear to have consistent effects on PL secretion in the mother. Most investigators have failed to demonstrate significant changes in PL concentrations following glucose administration or insulin-induced hypoglycemia. However, a significant decrease in plasma PL concentrations was noted in one study following two intravenous infusions of glucose one hour apart or the continuous infusion of glucose over several hours. Several studies have reported a 30%–40% increase in plasma PL concentrations in women fasted 84–90 hours during weeks 16–22 of gestation (prior to therapeutic abortion<sup>65</sup>). Interestingly, angiotensin II has also been shown to stimulate PL secretion *in vitro*.<sup>66</sup>

Studies of the regulation of PL gene expression suggest a role for autocrine/paracrine factors in the regulation of PL gene expression. 1,25-dihydroxyvitamin D<sub>3</sub>, interleukin (IL)-6 and IL-1, all of which are synthesized and secreted by syncytiotrophoblast cells, stimulate the synthesis and release of PL by trophoblast cells.<sup>67,68</sup> 1,25-dihydroxyvitamin D<sub>3</sub> stimulates PL gene expression via the vitamin D receptor that binds to a composite nuclear hormone receptor site on the PL promoter.<sup>69</sup> Retinoic acid and thyroid hormone also stimulate PL gene expression via the binding of RARA and TRB receptors to the same composite site.<sup>70</sup> The action of IL-6 is mediated, at least in part, by the transcription factor NF-IL6 that binds to three consensus NF-IL6 elements on the distal PL promoter.<sup>71</sup> It is likely that other cytokines and nuclear hormone receptors are also involved in the regulation of PL expression.

Recent studies strongly suggest a novel physiologic role for high density lipoproteins (HDL) in the regulation of PL gene expression during pregnancy.<sup>72</sup> The stimulation appears to be due primarily to pre- $\beta$  HDL, a minor

component of the total HDL in the circulation that is much smaller in size than the major circulating form ( $\alpha$ -HDL) but which contains a much higher apolipoprotein (apo) A-I/lipid ratio. During pregnancy, pre- $\beta$  concentrations in maternal plasma increase markedly with a pattern that parallels that of PL.<sup>54</sup> Pre- $\beta$  HDL concentrations increase from 3% to 4% of the total HDL in the early first trimester to about 20% at term.

The stimulation by HDL is mediated by apoA-I and, to a much lesser extent, apoA-II and apoC. Amphipathic peptides that mimic the tree dimensional structure of apoA-I also stimulate PL promoter activity and the expression of PL from cultured trophoblast cells. The action of apoA-I is due, at least in part, by activation of adenylate cyclase and phospholipase C. ApoA-I stimulates a time- and dose-dependent increase in MAP kinase activity. ApoA-I has also been shown to have other non-lipid-dependent effects, including the stimulation of endothelial cell proliferation, endothelin-1 production by renal cells, and the inhibition of degranulation and superoxide dismutase activity in neutrophils. Plasma apoA-I concentrations have been reported to be significantly lower than normal in several pathologic conditions of pregnancy associated with decreased plasma PL concentrations and IUGR, including pre-eclampsia<sup>73</sup> pregnancy-induced hypertension<sup>74</sup> and insulin-dependent diabetes mellitus.<sup>75</sup> Whether the low apoA-I concentrations contribute to the decrease in PL secretion in these patients is unknown.

### GH-V expression

The secretion of GH-V, like of GH-N, is induced by hypoglycemia and suppressed by glucose and is regulated by cAMP. However, unlike GH-N, GH-V is released tonically and is not regulated by growth hormone releasing hormone, ghrelin and somatostatin. In addition, the GH-V promoter is not regulated by the transcription factor Pit-1. Studies by Lominick and Handwerger<sup>6</sup> have shown that the GH-V promoter is transactivated by the transcription factors MEF2 and FOXF1 but not FOXF2. Since FOXF1 and FOXF2 bind to the same DNA binding site, the difference in the ability of the two FOX proteins to transactivate the GH-V promoter is likely due to differential binding of the proteins to one or more co-activators.

### SUMMARY

The human GH cluster consists of five closely related genes. GH-V, PL-A, PL-V and PL-L are expressed exclusively in the placenta, while GH-N is expressed exclusively in the pituitary. Both PL and GH-V have growth-promoting and lactogenic activities during pregnancy. PL is detected in both the maternal and fetal circulations and has direct growth-promoting actions in both compartments. GH-V, on the other hand, is only detected in the mother. Although PL and GH-V have striking structural and biological homologies to GH-N, the

factors that regulate the expression of placental genes are different from those that regulate pituitary growth hormone. Aberrations in PL and GH-V have been noted in several pathologic conditions of pregnancy, including preeclampsia and IUGR.

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## Letter to the Editor

### Disorders of Sex Development: Nomenclature

The European Society for Paediatric Endocrinology (ESPE) published a classification of pediatric endocrine diagnoses in 2007. Diagnoses made by pediatric endocrinologists were divided into 14 groups, including *Disorders of Sex Development* (DSD). DSD were subdivided into the categories of Sex Chromosome DSD; 46,XY DSD; 46,XX DSD; and Unclassified Forms of Abnormal Sexual Development/Anatomical Disruptions. The impetus for this letter is the exclusion by ESPE of "disorders of gonadal differentiation that do not result in sex reversal/virilised female infant/undervirilised male" from the category of Sex Chromosome DSD. Specific examples of conditions excluded are Klinefelter syndrome and Turner syndrome, both of which are instead classified under the general category of Syndromes with Endocrine Features (subcategory of Chromosomal Abnormalities).

Although a comment on a nomenclature first published in November 2007 may seem overdue, the dilemma that the ESPE DSD classification system has created remains unresolved. To the best of our knowledge, the points we raise have not previously been enunciated, and the issue remains every bit as problematic as when the new nomenclature was first published.

In 2005, working groups, comprised of 50 international experts (including Sandberg and Vilain), members of the Lawson Wilkins Pediatric Endocrine Society (LWPES) and ESPE, assembled in Chicago to formulate a consensus document on the clinical management of individuals born with intersex conditions.<sup>1</sup> One of the working groups focused on nomenclature and several significant changes were adopted by the whole consensus group. The most visible modification to the previous nomenclature recommended was the removal of terms perceived as offensive such as "hermaphrodite" and "pseudohermaphrodite," and the change of "intersex" – a politically charged and somewhat vague term – to DSD. Yet two additional profound changes were implemented. One was to incorporate all aspects of sexual variations under one umbrella term (DSD) defined as "congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical." This allowed doing away with the simplifying notion that gonads are the only parameter defining sex. The other major modification was to remove references to gender in the diagnostic nomenclature in order to avoid gender labeling – often psychologically disturbing to the patient.

ESPE's revised classification of Sex Chromosome DSD contradicts the nosology endorsed just two years earlier at a meeting co-sponsored by ESPE itself. A group of distinguished international clinical and scientific experts from a large variety of fields (genetics, endocrinology, psychology, psychiatry, surgery) and representatives of patient support groups participated in a long and complex process involving preparation of draft documents prior to the consensus meeting, working group, and general discussions during the meeting, group writing of the consensus statement, and multiple post-meeting edits. It is unclear why one party to a consensus agreement, representing only one subspecialty (pediatric endocrinology), from one region of the world (Europe), would unilaterally modify the product of an International Consensus Group which had painstakingly considered the complex issues of nosology for DSD.

The principle guiding exclusion from Sex Chromosome DSD (ie, "disorders of gonadal differentiation that do not result in sex reversal/virilised female infant/undervirilised male") implies that atypical genital appearance is the sine qua non of DSD. If we follow the argument that Turner and Klinefelter syndromes should not be classified as DSD because the external genitalia are normal, we should also exclude from DSD women with XY pure gonadal dysgenesis – who have normal external genitalia, males who are XX caused by a translocation of SRY, who often have normal male genitals, and even Complete Androgen Insensitivity Syndrome (CAIS), who appear at birth with normal female genitalia. One of the reasons why the term "intersex" was set aside was its vague meaning. Intersex implied sexual ambiguity, yet every physician agreed that CAIS was encompassed by the term intersex.

In addition, the nomenclature adopted at the International Consensus Conference was designed to overturn the practice of classifying DSD exclusively based on the characteristics of the gonads, which did not reflect the various parameters influencing sexual development. The definition of DSD now includes not only the gonads and the genitals, but also the sex chromosomes as a parameter.

Furthermore, excluding Klinefelter syndrome from the subcategory of sex chromosome DSD because it does not result in "undervirilised males" is questionable and depends on one's definition of "undervirilised." Suggesting that small, dysgenetic testes, which do not support spermatogenesis – a major male function – are not undervirilised seems to be a subjective interpretation.

Finally, the ESPE document uses the word "sex reversal" that was clearly abandoned in the consensus statement because of its uncertain meaning, but reemerges in the ESPE document.

By using an argument based exclusively on the appearance of the external genitalia to eliminate Klinefelter and Turner from sex chromosome DSD, the ESPE classification implicitly undermines the value of the DSD nomenclature introduced in the consensus statement by weakening the inherent logic behind the classification system, which is about multiple aspects of sexual development, and not exclusively focused on the appearance of the genitals and the issue of gender assignment.

An argument favoring the removal of Klinefelter and Turner syndromes from the category of DSD is articulated by the editors in the foreword of the ESPE classification, where they note that "we have tried to follow the logic of the paediatric endocrine clinician as much as possible, so that it would be as easy as possible to find the diagnosis in the structure of each chapter." However, they also state that the coding system should "follow one general principle (e.g. nosology, aetiology, pathogenesis or symptomatology)." The editors have followed both standards: the latter, principle-driven, by embracing the term DSD and its definition, and the former, practitioner-friendly, by inserting Klinefelter and Turner syndrome in a different section where it has traditionally been found. The classification of DSD could indeed be entirely based on clinical phenotype and clinician observations. Turner syndrome could then be classified with XY gonadal dysgenesis and

CAIS, based on the appearance of the external genitalia. This would discount recent advances in the understanding of DSD, which are crucial in outcome and prognosis studies. Classifications and nomenclatures evolve with science, and the comfort of practicing endocrinologists should be balanced with the realities of biology and the specific needs of our patients. This is why Turner and Klinefelter syndromes, which are clear disorders of sexual development, undoubtedly belong within the DSD classification.

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## REVIEWS & COMMENTS FROM THE LITERATURE

### Another Cause of Primary IGF Deficiency

Primary insulin-like growth-factor deficiency (PIGFD), abnormally low levels of IGF-1 despite normal or elevated levels of growth hormone (GH), has been attributed to mutations in 4 genes to date: *GHR*, *IGF1*, *STAT5b*, and *IGFALS*. *IGFALS* encodes the acid-labile subunit (ALS) of the ternary complex, also under GH control. Fofanova-Gambetti et al reported 2 patients with 3 novel mutations in *IGFALS*, plus another 2 patients in the amendment while the paper was in press, to add to the currently published tally of 5 patients from 3 families harboring 4 different mutations. Of note, in contrast to patients with mutations of the other PIGFD genes, all patients with *IGFALS* mutations presented with modest short stature (height z-scores above -3 SD).

#### Previously published patients:

Case 1: A boy aged 14.6 years from Argentina with a height z-score of -2.05 SD and homozygous *IGFALS* mutation1338delG (E35fsX120), in the amino terminal flanking region.<sup>1</sup>

Case 2: A Turkish boy aged 12.1 years with a height z-score of -2.9 SD and homozygous *IGFALS* D440N missense mutation in the 17<sup>th</sup> leucine-rich repeat (LRR) domain.<sup>2</sup>

Cases 3-5: Three Norwegian/German siblings (2 male, 1 female) aged 15.3 to 19.6 years, with height z-scores of -0.5 to -2.0 SD and compound heterozygous C540R/583\_591dup9 *IGFALS* mutations in the cysteine-rich region of the carboxy terminus and the 7<sup>th</sup> LRR domain, respectively.<sup>3</sup>

**Currently reported patients:**

Case 1: A boy of 6.7 years of Mayan origin with a height z-score of  $-2.91$  SD, delayed bone age (5.5 years) and homozygous *IGFALS* 1308\_1316 dup9 mutation in the 17<sup>th</sup> LRR domain. GH treatment began at the age of 8.5 years and was discontinued 1 year later due to development of nonalcoholic steatotic hepatitis. The patient's transaminase levels continued to climb when he was off treatment, however they subsequently returned to normal. GH therapy was tried again from age 10 years for another 2 years. Despite increasing doses of GH, he failed to improve his growth velocity or normalize his IGF-I and IGF binding protein (IGFBP)-3 levels. During this time, at the chronological age of 10.5 years, he initiated spontaneous puberty and was started on LH-RH analogue therapy to preserve growth potential while on GH. At age 12 years, he was switched from GH to IGF-I therapy.

Case 2: A girl aged 4.1 years of Eastern European Jewish/Icelandic-Western European ethnic origin with a height z-score of  $-2.14$  SD, bone age consistent to her chronologic age, and compound heterozygous *IGFALS* C60S/L244F missense mutations in the 1<sup>st</sup> and 9<sup>th</sup> LRR domains, respectively. She started GH treatment at age 4.4 years, increasing her height z-score in 13 months to  $-1.67$  SD; IGF-I and IGFBP-3 levels nonetheless remained abnormally low, and ALS was undetectable.

**Patients reported in the amendment:**

Case 1: An Indian/Pakistani boy aged 15.2 years with a height z-score of  $-3.17$  SD, delayed bone age (11 years), sexual infantilism and homozygous *IGFALS* L134Q missense mutations in the 4<sup>th</sup> LRR domain. His parents, both heterozygous carriers, had normal heights ( $-0.09$  and  $-1.35$  SDS).

Case 2: An Ashkenazi Jewish boy aged 12.7 years with a height z-score of  $-2.87$  SD, bone age of 11.5 years, sexual infantilism and compound heterozygous *IGFALS* P73L/L241P missense mutations in the 1<sup>st</sup> and 8<sup>th</sup>-9<sup>th</sup> LRR domains, respectively. His parents, both heterozygous carriers of one of the mutations, had normal heights ( $-1.68$  and  $+0.85$  SDS).

ALS protein, a member of the LRR superfamily of proteins involved in protein-protein interactions, contains 20 LRR domains that form a donut shape with a closed structure. The LRRs contain  $\beta$ -strands that form sheets inside the donut, and  $\alpha$ -helices that flank the structure's outer circumference. This paper highlights the ethnic

and genetic heterogeneity of *IGFALS* mutations that are pathogenic in causing PIGFD and modest short stature that responds poorly to GH therapy. Although GH can induce IGF-I and IGFBP-3 production, without ALS, circulating levels of the growth factor are not sustained. This is a nice *in vivo* illustration of the importance of the ternary complex in prolonging the circulating half-life, and hence activity, of IGF-I.

Fofanova-Gambetti OV, Hwa V, Kirsch S, et al. Three novel *IGFALS* gene mutations resulting in total ALS and severe circulating IGF-I/IGFBP-3 deficiency in children of different ethnic origins. *Horm Res.* 2009;71:100-110.

**Editor's Comment:** *Genotyping of the parents of Girl #2 in this paper was not available. The authors hypothesized that her mutations must be in the compound heterozygous state because her ALS protein was undetectable; had her mutations occurred in cis, then her wild-type allele would be expected to produce wild-type ALS that should have been detected, as was the case for the carrier parent of the Turkish boy with a homozygous missense mutation.<sup>2</sup> Another possibility is that the double mutations in cis so altered the ALS protein product that it functioned as a dominant negative, tying up the wild-type ALS in the ER or Golgi and preventing its secretion. This second hypothesis would require that one of the parents similarly carry the dominant negative in cis mutations, have undetectable ALS, and be affected. The father's height z-score was  $+0.30$  SD while the mother's was  $-2.13$  SD. Since one of the main teaching points of this paper is that ALS mutations cause PIGFD with only modest short stature, perhaps the mother is affected like her daughter?*

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## Acute Vascular Effects of GH Appear to be Independent of Both Local and Systemic IGF-I Production

Growth hormone (GH) has been shown to regulate vascular tone and reactivity in humans, but it is unclear whether this action is a result of a direct stimulatory effect of GH or if it is dependent on systemic and local insulin-like growth factor (IGF)-I production. In this study, Li et al

evaluated the mechanisms underlying the acute vascular effects of GH. Ten healthy lean young volunteers (20 to 27 years of age; 7 male and 3 females) were studied after an overnight fast. GH was infused for 6 hours at 0.06 mcg/kg/minute and a biopsy of the vastus lateralis muscle was



obtained in 7 of these subjects before and after infusion for analysis of IGF-I mRNA and Akt phosphorylation. Blood was obtained serially every 10 minutes during the infusion for GH, IGF-I, insulin and glucose assessments. GH infusion increased plasma GH and forearm blood flow by 66% ( $p < 0.001$ ), but did not change plasma IGF-I concentrations, muscle IGF-I mRNA expression, or muscle Akt phosphorylation—therefore suggesting a lack of IGF-I action in muscle. Additionally, human aortic endothelial cells (HAECs) were incubated with GH (30 ng/mL) in vitro for 3 or 6 hours. GH did not alter endothelial nitric oxide synthase (eNOS) protein content, but induced a time-dependent increase of the phosphorylation of eNOS. This study demonstrated that GH exerts an acute vascular effect, independent of both systemic and local IGF-I production and that this effect probably occurs via direct action on GH receptors and eNOS in the vascular endothelium.

Li G, del Rincon P, Jahn LA, et al. Growth hormone exerts acute vascular effects independently of systemic or muscle insulin-like growth factor I. *J Clin Endocrinol Metab.* 2008;93:1379-1385.

**Editor's Comment:** Endothelial dysfunction appears to explain much of the increased cardiovascular risk of GH deficiency. GH seems to play an important role in the regulation of peripheral vascular resistance and vascular reactivity; these effects appear to be mediated

by the activation of the NO pathway. GH deficiency is associated with decreased systemic NO formation and decreased forearm release of nitrite and cyclic GMP during acetylcholine stimulation, as well as a decreased peak hyperemic response to ischemia, which reverts to normal during GH replacement. Significant endothelial dysfunction—as determined by an impaired endothelium-dependent brachial artery dilatatory response to occlusion ischemia and by abnormalities of several biochemical markers of endothelial cell activation—has been reported in adolescents and adults with GH deficiency.<sup>1,2</sup> It is not clear whether these effects are a result of a direct effect of GH on the vascular endothelium or whether they are dependent on systemic and local IGF-I production. This study seems to indicate that the acute vasodilatory effect of GH is exerted independent of IGF-I, very possibly through GH receptor mediated eNOS activation.

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## Nedd4 Controls Animal Growth by Regulating IGF-I Signaling

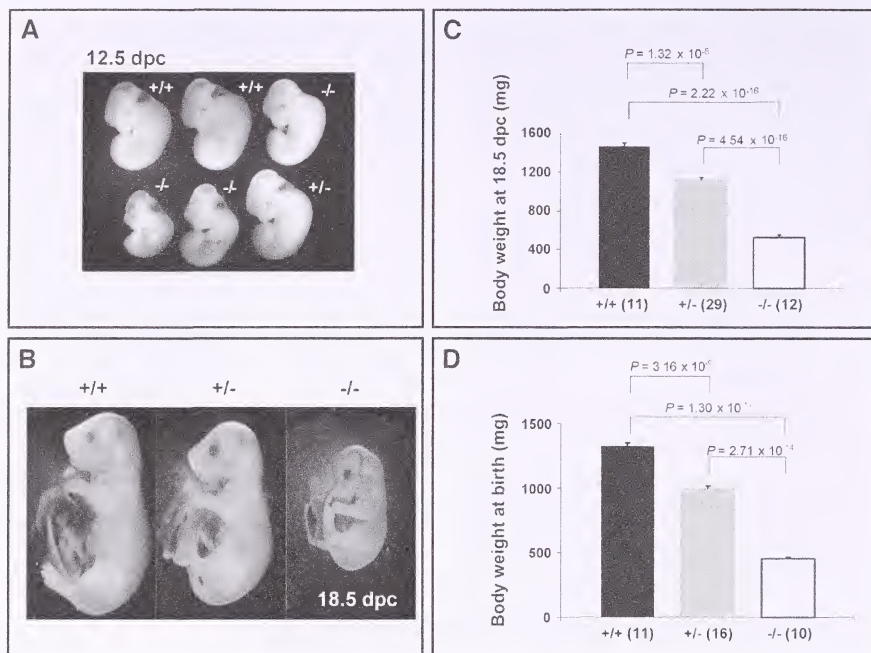
Nedd4 (Neural precursor cell expressed developmentally down regulated 4 - OMIM 602278, chromosome 15q) is a cytoplasmic ubiquitin ligase that regulates protein movement and structure thereby its function or directs a protein into ubiquitin-proteasomal degradative pathway. Cao et al demonstrated that Nedd4 is essential for transduction of intracellular signals initiated by insulin and insulin-like growth factor (IGF)-I and the localization of the insulin receptor (IR, OMIM 147670, chromosome 19p13.2) and the IGF-I receptor (IGF1R, OMIM 147370, chromosome 15q25-q26) to the cell plasma membrane. Nedd4 does not bind to IR or IGF1R directly, but links to an adaptor protein, Grb10 (Growth factor receptor-bound protein10, OMIM 601523, chromosome 7p12-p11.2), which in turn is bound by IR and IGF1R. Grb10 inhibits movement of these receptors to their localization sites in the plasma membrane and thereby impairs function of IR and IGF1R. This effect is opposed by the binding of Nedd4 to Grb10. Cao and colleagues generated Nedd4 knockout (KO) mice. Nedd4<sup>-/-</sup> mice died during gestation or shortly after birth due to immature lung development and aeration (Figure); their linear growth and weight were severely impaired by embryonic day 12.5. Heterozygous Nedd4<sup>+/-</sup> mice were also small at birth and through post-natal age 3 months (the end of the study period). In vitro, the proliferation of Nedd4<sup>-/-</sup> fibroblasts was impaired relative to that of

wild-type fibroblasts due to decreased progression through the cell cycle at phases G<sub>0</sub> and G<sub>1</sub>. IGF-I and insulin mediated intracellular signaling was substantially reduced in Nedd4<sup>-/-</sup> and Nedd4<sup>+/-</sup> fibroblasts and could be restored by expression of Nedd4 in these cells. However, in Nedd4<sup>-/-</sup> fibroblasts, the expression and translation of IR and IGF1R were normal, but the receptors did not reach the cell surface, an abnormality that could also be reversed by expression of Nedd4 in these cells. Further studies demonstrated that the amount of Grb10 was increased in Nedd4<sup>-/-</sup> fibroblasts and that "knockdown" of Grb10 by small interfering RNA (siRNA) restored insulin and IGF-I signaling in Nedd4<sup>-/-</sup> fibroblasts. The investigators concluded that Nedd4 positively regulates IGF-I and insulin signaling by enhancing the movement of their receptors to the cell surface. Nedd4 does so by dis-inhibiting the inhibitory effect of Grb10 on this process—perhaps by controlling the rate of degradation of Grb10 itself through the ubiquitin-proteasomal system.

Cao XR, Lill NL, Boase N, et al. Nedd4 controls animal growth by regulating IGF-1 signaling. *Sci Signal.* 2008;1:ra5. [DOI:10.1126/scisignal.1160940]

**Editor's Comment:** This study has identified another intracellular signal transduction site (Nedd4-Grb10) to examine when a patient with severe growth retardation due





*Nedd4*<sup>-/-</sup> mice die immediately after birth, and *Nedd4*<sup>+/-</sup> and *Nedd4*<sup>-/-</sup> mice exhibit intrauterine growth retardation. No mice homozygous for disruption of the *Nedd4* gene were found 2 or 3 weeks after birth. Ratios of heterozygotes and homozygous mutants were thus assessed at earlier time points: (A) 12.5 dpc, (B) and (C) 18.5 dpc, and (D) immediately after birth. Both heterozygotes and homozygous mutants showed signs of intrauterine growth retardation as early as 12.5 dpc (A) and at late gestation [18.5 dpc (B) and (C)]. At the time of birth [postnatal day 1 (D)], the body weights among three genotypes differed significantly: *Nedd4*<sup>-/-</sup> body weight averaged 64 to 68% lower relative to that of wild-type littermates; heterozygote body weight averaged about 15 to 20% reduction in body weight relative to that of wild-type littermates. In (C) and (D), the numbers of animals used for the analyses are shown in parentheses, the body weight was significantly different between groups of mice, with P values indicated. Reprinted with permission Cao XR, et al. Sci Signal. 2008;1: ra5. Copyright © AAAS 2008. All rights reserved.

to insensitivity to IGF-I and an intact IGF1R is encountered. A polymorphic variant or mutation in either one of these proteins might also account for impaired intrauterine

growth in some small-for-gestational age neonates.

Allen W. Root, MD

## Growth Hormone Deficiency: Transient or Permanent?

In this multicenter study, Berberoglu and colleagues tried to assess the need for continuation of growth hormone (GH) treatment in adulthood after growth is completed and also to evaluate factors that would predict persistent GH deficiency (GHD). A total of 70 (31 female, 39 male) GHD patients were included in the study; 52 patients (74%) had isolated GHD and 18 patients (26%) had multiple pituitary hormone deficiency (MPHD). The initial diagnosis was based on a peak GH level <10 ng/mL in 2 pharmacological tests. GH treatment was discontinued in these patients when growth velocity during the

previous year decreased to less than 2 cm and the bone age had reached greater than 14 years in girls, and greater than 16 years in boys, and after completion of puberty. All patients were re-tested by insulin tolerance test (ITT) at least 6 weeks after discontinuation of the replacement treatment. Serum insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 concentrations were determined at the same time. If GH peak during ITT was <3 ng/mL, the patient was diagnosed to have severe permanent GHD.

Among the patients with isolated GHD, 9 patients

(17.3%) were found to have persistent GHD and 43 (82.7%) to be transiently GH deficient. On the other hand, among patients with MPHD only 2 patients (11.1%) were transiently GH deficient.

None of the parameters differed significantly with respect to gender. There were significant positive correlations between peak GH and IGF-I, and IGFBP-3 levels in all patients (IGF-1  $r=0.297$ ,  $p=0.036$ ; IGFBP-3  $r=0.45$ ,  $p=0.03$ ). The IGF-I and IGFBP-3 SDS values were lower in the group that had peak GH values  $<3$  ng/mL. When the cut-off was taken as  $-2$  SD, specificity and sensitivity of IGF-I in confirming persistency of GHD were 65.7% and 73.3%, respectively. Its positive predictive value and negative predictive value were 33.3% and 85.2%, respectively. For IGFBP-3, specificity and sensitivity were 84%, and 60%, respectively. The positive and negative predictive values were 60%, and 84%, in the same order. Finally, while the negative predictive values were high for both of these parameters, an IGFBP-3 value below  $-2$  SD was found to be more specific than an IGF-I value below  $-2$  SD.

The data in this study confirmed that there were no auxological and clinical signs to predict the transiency or the persistence of GHD except for a history of organic disease and presence of MPHD. The authors concluded that most patients with childhood onset GHD were idiopathic and GHD was frequently transient in this group of patients. In contrast, GHD was persistent in patients with MPHD. They emphasized the high negative predictive values for IGF-I and IGFBP-3 (85.1% and 84%, respectively) suggesting that normal IGF-I and IGFBP-3 levels highly exclude the diagnosis of GHD.

Berberoglu M, Siklar Z, Darendeliler F, et al. Evaluation of permanent growth hormone deficiency (GHD) in young adults with childhood onset GHD: a multicenter study. *J Clin Res Ped Endo*. 2008;1:30-37.

**Editor's Comment:** *The question of how to confirm the diagnosis of adult GHD in an adolescent patient who has completed linear growth is still being debated. The Growth Hormone Research Society guidelines suggest a peak GH response on ITT of  $<3$  ng/mL as being diagnostic*

*of GHD in adulthood.<sup>1</sup> Although patients with MPHD have peak GH levels  $<3$  ng/mL, it is not clear whether this value can confirm adult GHD exactly. In addition, despite the high negative predictive values of IGF-I and IGFBP-3, the use of serum IGF-I and IGFBP-3 alone to predict GHD cannot be recommended. The majority of children with GHD, when retested as adults, do not have the classical severe GHD.<sup>2</sup> This high incidence (70%) of normal GH responses on retesting has been shown in patients with idiopathic and isolated GHD.<sup>3</sup> This finding indicates that the organic etiologies are often severe and can be assumed to be permanent at the beginning of the therapy. Therefore, those patients with organic MPHD could be excluded from retesting.*

*The patients who have peak GH cut-off values between 3-5 ng/mL might be GH deficient as well. In fact, in the transition period in late adolescence a cut-off value of 5 ng/mL is advocated for the diagnosis of persistent GHD and continuation of GH therapy because adolescents have higher GH levels than adults. In this study, there were 3 additional patients with peak GH level between 3-5 ng/mL in the isolated GHD group and none in the MPHD group. Therefore, no suggestion is available for patients in this gray zone. Furthermore, the prognosis of patients with a GH response of 5-10 ng/mL is not known. Therefore, it is important to keep in mind that clinical signs of GHD may occur later in life and the clinician must look for these manifestations in patients with a history of childhood GHD.<sup>3</sup>*

Ömer Tarım, MD

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## IGFBP-3 Promoter Polymorphism Affects Response to GH Treatment for GH Deficiency

Growth responses to growth hormone (GH) therapy vary considerably among children with GH deficiency despite receiving standardized per kg body weight doses. Several clinical factors have been identified in influencing responsiveness to treatment,<sup>1</sup> but about half of the variation remains unexplained. These clinical factors only indirectly consider genetic traits, by including parental target heights.

Thus, Costalonga et al sought to examine the effects of an *insulin-like binding protein (IGFBP)-3* promoter polymorphism on growth velocity during the first year

of GH treatment in prepubertal children with severe GH deficiency. In twin studies, about 60% of the interindividual variability in circulating IGFBP-3 levels was found to be genetically determined.<sup>2</sup> A single nucleotide change 202 bp upstream of the transcription start site was found to affect IGFBP-3 promoter activity in vitro and in vivo; mean circulating IGFBP-3 levels in healthy adults were highest in those with AA genotype at the  $-202$  position, less in AC and lowest in those with CC.

Costalonga et al studied 48 boys and 23 girls with severe GH deficiency (mean height z-score of  $-4.3 \pm 1.4$  SD,

mean bone age delay of  $4.3 \pm 2.7$  years, and peak GH response in 2 stimulation tests ranging from  $<0.1$  to  $3.3$  mcg/L. All children were prepubertal with a mean age of  $8.6 \pm 4.1$  years, and treated exclusively with GH at a mean dose of  $32$  mcg/kg/day adjusted to weight every 3 to 4 months. Seventeen percent of subjects had a defined genetic etiology for GH deficiency, 63% had ectopic posterior pituitary and 25% had interrupted stalk on MRI imaging; only 8% had idiopathic GH deficiency, and patients with central nervous system tumors, meningoencephalocele or previous radiation therapy were excluded from the study.

Among the 71 subjects, 21% had  $-202$  IGFBP3 genotype of AA, 54% had AC, and 25% had CC. The genotype subgroups did not differ clinically at the start of treatment, nor in mean GH treatment doses. Mean circulating IGFBP-3 levels also were not significantly different at baseline, they gained significance with GH treatment; AA subjects had higher IGFBP-3 levels than C allele carriers in codominant ( $P<0.005$ ) and recessive models ( $P<0.001$ ), and developed greater increases in IGFBP-3 z-scores with treatment. The IGFBP3 polymorphism accounted for 19% of variability in circulating IGFBP-3 levels ( $P<0.001$ ) and 54% of variability when combined with age and gender.

The IGFBP3 polymorphism did not associate with IGF-I levels either at baseline or during GH treatment, but it did affect growth response to treatment. Mean first year growth velocity was  $13.0 \pm 2.1$  cm/year in AA subjects,  $11.4 \pm 2.5$  cm/year in AC subjects, and  $10.8 \pm 1.9$  cm/year in CC subjects ( $P<0.05$ ). Single and multiple linear regression analyses found the effect of IGFBP3 polymorphism independent of other variables in associating with growth velocity. It accounted for 10% of variability in growth velocity ( $P<0.005$ ) and 29% of variability when combined with height z-score and age at start of treatment.

This is the first study of the  $-202$  A/C IGFBP3 polymorphism in children. Because the genotype was significantly associated with circulating IGFBP-3 levels in healthy adults and in children with severe GH deficiency only after GH treatment but not at baseline, the authors concluded the effect is at least in part dependent on GH action.

Costalonga EF, Antonini SR, Guerra-Junior G, Mendonca BB, Arnhold IJ, Jorge AA. The  $-202$  A allele of insulin-like growth factor binding protein-3 (IGFBP3) promoter polymorphism is associated with higher IGFBP-3 serum levels and better growth response to growth hormone treatment in patients with severe growth hormone deficiency. *J Clin Endocrinol Metab*. 2009;94:588-595.

**Editor's Comment:** This study conveys 2 important lessons. First, the results may seem counter-intuitive: the genotype associated with the highest IGFBP-3 levels had the greatest growth response to GH treatment. The IGFBPs were defined by their high-affinity IGF binding that renders them competitive inhibitors for IGF binding to the type 1 IGF receptor (IGF1R), and hence inhibitors of IGF action.<sup>3</sup> This is an isolated effect. The situation in vivo and some in vitro cell models is more complex, because the balance of ligand binding protein receptor and post-receptor signaling pathways is modulated by multiple factors. Such factors include, but are not limited to, changes in ligand half-life, local IGFBP proteases that convert the high-affinity IGF binders to lower affinity IGFBP fragments, IGF1R trafficking and down-regulation, and interactions with other cell signaling systems. Plus, we now appreciate that the IGFBPs exert IGF-independent actions of their own.

Secondly, this study highlights yet another factor that influences patient responsiveness to GH treatment. I applaud the authors' focus on clearly defined subjects with severe GH deficiency, rather than opening up their sample size to less severe and thus, heterogeneous, patients who may harbor other alterations in their GH/IGF axis function. The authors concluded their paper with the suggestion that future pharmacogenetic studies may support adjusting GH treatment to genotype in order to individualize and thereby optimize therapy. Before moving to genotyping—which is expensive and not readily available—clinicians already have tools to individualize therapy. For example, titrating GH dose to achieve desired IGF-I z-scores, as the principle mediator and biomarker of GH effects, is akin to titrating l-thyroxine dose to thyroid function tests when treating patients with hypothyroidism.<sup>4</sup> This paper provides additional data supporting the notion that the traditional, cookie cutter, one-size-fits-all, weight-based dosing of GH therapy can be improved by individualized approaches to optimize treatment efficacy and safety.

Adda Grimberg, MD

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## GH Treatment for Growth Failure in Pediatric Patients with Crohn's Disease

Heyman and colleagues studied the effects of growth hormone (GH) treatment ( $0.043$  mg/kg/day;  $0.3$  mg/kg/week) on height velocity, body composition,

and disease activity in a group of children and adolescents (mean age  $12.6 \pm 4.5$  years; 6 males) with Crohn's Disease (CD) and growth failure. All



subjects had a confirmed endoscopic, histological, and/or radiographic diagnosis of CD and height below the 5<sup>th</sup> percentile for age with no evidence of catch-up growth (increase in height z-score of 0.5) for the year prior to GH therapy. Exclusion criteria included hepatic abnormalities, renal disease, history of non-compliance, and pre-existing scoliosis. Subjects were seen at baseline and every 3 months for 12 months for a history, physical assessment of anthropometric measurements, calculations of BMI and body fat mass, as well as laboratory studies to evaluate disease activity. Nutritional state, serum vitamin B12, iron levels, red blood cell folate, plasma insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 were measured at each visit and the CD activity was characterized using the Pediatric CD Activity Index (PCDAI). Bone age was determined by wrist radiography. Bone density and body composition were assessed using DEXA at the lumbar spine (L1 to L4) and hips. Age adjusted values were used for comparison and variation of z-scores. The comparison control group was gathered from the PEDI IBD Consortium Registry which included consecutively enrolled patients from 6 sites with inflammatory bowel disease; 989 children were identified as having CD. For each subject receiving GH, 3 comparison subjects with CD were retrospectively matched by age, sex, race, and height (at baseline). The patients in the control group were receiving standard treatment and nutritional supplementation for CD.

The study group had a mean bone age of 10.7 years with an average diagnosis of CD for 2.7 years, PCDAI of 21.9, a height z-score of -2.48, and a weight z-score of -1.88 with a previous year's growth velocity of 2.8 cm/year. The control group had a similar age, and a mean height z-score of -1.8 with a mean weight z-score of -1.19. Each patient remained on his or her clinically indicated therapy for CD which included temporary total parenteral nutrition (TPN), elemental formula diet, or regular diet. All subjects consumed more than 85% of the RDA of calories for age. BMI did not increase significantly from baseline at 12 months, however DEXA scans at 1 year of GH treatment demonstrated an increase in mean lumbar z-scores and a decrease in mean percent body fat; the bone age increased by 0.97. IGF-I level increased from 249.4  $\pm$  146.8 to 447.1  $\pm$  242.6 at the end of treatment. Mean IGF-BP3 was within the range adjusted normal range. No significant changes in thyroid functions, or electrolytes were observed. Mean height velocity increased from 3  $\pm$  1.39 cm/year at baseline to 8.32  $\pm$  3.2 cm after 1 year of GH. Within the control group the mean height velocity was 3.98  $\pm$  2.32 cm/year at baseline and 4.84  $\pm$  2.85 cm/year after 1 year; this difference was significant. The height z-score increased by 0.76 and the weight z-score increased by 0.81 as compared with increases

of 0.16 and 0 in the control group. The mean PCDAI was 21.9 at baseline and 13.1 after 1 year of treatment. No subject experienced any adverse reaction to GH. Two patients were excluded from the comparison, one of whom had a disease exacerbation requiring 2 hospitalizations during the 12 month study period and the other due to a lack of a matched comparison.

The authors stated that their data suggest that children with CD treated with GH experience increased height velocity and improved bone mineral density. There have been 10 other pediatric inflammatory bowel disease uncontrolled GH trials. Results from these studies have varied, but they have included small numbers of subjects and no disease controls. The authors noted that despite the increase in growth, there was no consistent clinical improvement in CD activity. Thus, it would appear that GH is not a primary treatment strategy for CD. They also noted the limitations of having used a retrospective comparison group and the small size of their study, which prohibited controlling for concomitant medications, including corticosteroids and other supplements. They concluded that a larger randomized trial of GH therapy in CD is needed.

Heyman MB, Garnett EA, Wojcicki J, et al. Growth hormone treatment for growth failure in pediatric patients with Crohn's Disease. *J Pediatr*. 2008;151:651-658.

**Editor's Comment:** The authors reported that growth impairment is seen in about 40% of pediatric patients with CD and that this often leads to short stature in adulthood. The possible etiology of this growth failure may include anorexia, inflammation, direct effects of cytokines on bone, GI nutrient losses, GH resistance with low IGF-I and other medications including corticosteroids. Of note, growth impairment may precede the onset of intestinal symptoms in CD.

Pediatric endocrinologists recognize the importance of looking for inflammatory bowel disease when evaluating children with short stature. Indeed CD is occasionally diagnosed during the evaluation for short stature prior to any GI symptoms. The authors clearly pointed out that other studies have shown variable results when GH is used to treat short stature and growth failure in CD and the limitations of those studies.

Studies of growth impairment in complex disease states such as CD may provide information on the importance of a variety of different disease processes associated with growth failure. In other words, are inflammatory processes critical or is the effect of cytokines on bones critical? Thus a study of a large number of individuals for whom assessments of these factors have been well characterized may lead to an important understanding of growth, not just in CD, but in other chronic disease processes.

William L. Clarke, MD



## Pathogenesis of Hypothalamic Obesity in Children

The pathogenesis of hypothalamic obesity is not clear. In this multicenter study, the investigators studied the role of leptin, soluble leptin receptor (sOb-R), resistin, and insulin secretory dynamics in the development of hypothalamic obesity. Children who had hypothalamopituitary tumors were divided into 2 groups. The first group included obese-overweight (hypothalamic obese [HOB] group,  $n=23$ ) and second group included non-obese children (hypothalamic non-obese [HNOB] group,  $n=16$ ). Exogenously obese-overweight children (OB group,  $n=22$ ) were included as controls. Oral glucose tolerance test (OGTT), basal serum leptin, sOb-R, resistin levels, and homeostasis model assessment (HOMA) indexes were compared between the groups. Age, sex, and pubertal status were similar in study groups. Median and interquartile ranges of BMI z-scores were similar in HOB and OB groups.

The ratio of the patients who received chemotherapy and radiotherapy were similar in the 2 groups. Tumor size, relapse rates, and number of operations were not different between the groups. The number of patients with multiple pituitary hormone deficiency as well as ACTH, TSH, GH, ADH, and gonadotropic hormone deficiencies were also similar in HOB and HNOB groups. Growth hormone (GH) replacement dose was 0.025–0.035 mg/kg/day for the patients with GH deficiency, and hydrocortisone replacement dose was  $<10$  mg/m<sup>2</sup>/day in all patients with central adrenal insufficiency. All patients with central hypothyroidism were receiving adequate replacement dose of L-thyroxine to maintain free T<sub>4</sub> levels in the normal range.

|                    | HOB Group     | HNOB Group    | OB Group      |
|--------------------|---------------|---------------|---------------|
| Leptin/BMI         | 4.0 (1.6–5.2) | 1.5 (0.8–3.1) | 2.5 (1.8–3.5) |
| Leptin/sOb-R (FLI) | 2.0 (0.8–3.5) | 0.6 (0.3–1.2) | 1.5 (1.0–2.3) |

Serum leptin levels corrected for BMI were highest and total leptin/sOb-R ratios (free leptin index [FLI]) tended to be higher in HOB than HNOB and OB groups, indicating leptin resistance (Table). Serum resistin levels were similar in all groups. Basal serum glucose, basal and second-hour insulin levels in OGTT, and HOMA index were higher in OB group than the HOB and HNOB groups, indicating insulin resistance in simple obesity; however, the increment of insulin to the same glycemic load in OGTT was highest in the HOB group indicating insulin dysregulation ( $p<0.05$ ). It was concluded that hypothalamic obesity seemed to be related to both dysregulated afferent (leptin) and efferent (insulin) neural outputs through the autonomic nervous system resulting in energy storage as fat.

Guran T, Turan S, Bereket A, et al. The role of leptin, soluble leptin receptor, resistin, and insulin secretory dynamics in the pathogenesis of hypothalamic obesity in children. *Eur J Pediatr*. 2008; Nov 29 [Epub ahead of print]

**Editor's Comment:** Hypothalamic obesity is a frustrating syndrome which develops following an insult to the hypothalamic area.<sup>1,2</sup> The pathophysiology of this condition is not clear and therefore therapeutic attempts usually fail. There may be many confounding factors which may affect body weight and energy homeostasis all of which have been more or less controlled in this study.

In both HOB and HNOB groups, only the leptin levels were remarkably higher in the tumors with hypothalamic/thalamic involvement ( $p$  values 0.023 and 0.01, respectively). The findings of higher leptin/BMI and higher FLI in hypothalamic patients suggest the contribution of leptin resistance in the pathogenesis of hypothalamic obesity. A recent study by Shaikh et al also confirmed that hyperleptinemia is associated with obesity following hypothalamic damage in children.<sup>3</sup>

The primary defect in patients with hypothalamic obesity is believed to be altered neural regulation of the beta-cell secretion resulting in insulin hypersecretion, in contrast with simple obesity, where peripheral insulin resistance is assumed to be the primary defect driving a compensator beta-cell response. In agreement with this hypothesis, this study shows that HOMA index representing insulin resistance is higher in the common obese groups compared to the patients with brain tumors in the hypothalamo-pituitary region. This finding implies the importance of dysregulated insulin secretion to a glycemic load rather than insulin resistance in the development of hypothalamic obesity differently from exogenous obesity.

In conclusion, compared to simple obese children, HOB patients have lower HOMA and lower basal insulin, but a higher insulin response to a glycemic load and higher leptin/BMI. These findings support that in hypothalamic obesity there are both dysregulated afferent (leptin) and efferent (insulin) neural outputs through the autonomic nervous system resulting in energy storage as fat. Dysregulated insulin secretion, rather than insulin resistance, is characteristic of hypothalamic obesity. Obviously, more studies are needed to further elucidate the mechanisms of hypothalamic obesity in order to offer more rewarding therapies for the patients.

Ömer Tarım, MD

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## Hypovitaminosis D In Obese Children

Recent studies have reported a relation between obesity and vitamin D hypovitaminosis.<sup>1</sup> In this cross-sectional study, Çizmecioglu et al aimed to determine the prevalence of vitamin D hypovitaminosis in a highly industrialized city in the Marmara region of Turkey where obesity is on the rise. At the first stage of the study, anthropometric measurements of 2491 subjects participating in the research were performed in the schools. At the second stage, participants whose BMI was over the 85th centile were invited to the hospital for further investigation. A total of 301 students (177 girls, 124 boys) aged 11 to 19 years were selected by multistage stratified sampling design. Children with any systemic disease or using any medications or supplements known to affect skeletal metabolism were excluded from the study.

Of the 301 children and adolescents who were included in the study, 102 were obese (34%) and 145 were overweight (48%). BMI values were within normal percentile ranges in 54 (18%) who had lost weight and returned to normal BMI when the blood samples were collected for further investigation. Serum 25-hydroxyvitamin D (25-OHD), intact parathyroid hormone (iPTH), and alkaline phosphatase (ALP) were measured in late winter months. Vitamin D deficiency was defined as a 25-OHD <10 ng/mL, insufficiency as 25-OHD 10 to 20 ng/mL, and normal vitamin D level as >20 ng/mL.

The prevalence of hypovitaminosis D was 65% in all students (12% deficiency and 53% insufficiency). Vitamin D deficiency in female students was about 2 times more common than in males. None of the girls were veiled in this study. Although the girls appeared to have higher BMI values than the boys, there was no statistically significant difference between their BMI SDS values. There was also no relation between obesity status and vitamin D categories. However, there was a negative correlation between serum vitamin D level and BMI in obese and overweight subjects whose vitamin D level <20 ng/mL ( $r = -0.186$ ,  $p < 0.01$ ). There were no correlations between serum 25-OHD and ALP and iPTH levels.

The authors concluded that vitamin D deficiency and insufficiency were common in obese and overweight schoolchildren, especially in girls, and obesity could be a risk factor in adolescents.

Çizmecioglu FM, Etiler N, Görmüş U, Hamzaoglu O, Hatun Ş. Hypovitaminosis D in obese and overweight schoolchildren. *J Clin Res Ped Endo*. 2008;1:89-96.

**Editor's Comment:** The same authors previously reported high rates of subclinical vitamin D deficiency (65%) in adolescent girls (from the same region) who wear concealing clothing.<sup>2</sup> This study shows that a veil is not the only factor responsible for hypovitaminosis D. Air pollution that may block ultraviolet light may also contribute to the lack of sun exposure in this highly industrialized city. Indeed,

the rate of vitamin D deficiency was higher in industrialized towns compared to the rural area in this study. However, it was shown that among students who live in the same area, serum 25-OHD levels decreased as BMI increased suggesting a causative role of obesity as well. The authors argued that this inverse relationship was consistent with the hypothesis suggesting that the increased adipose tissue decreases vitamin D bioavailability by sequestration in body fat.<sup>3</sup> Unfortunately, there is no information about the dietary intake of these patients and the effect of reduced ingestion of micronutrients such as iron and vitamin D in obese people is not taken into account. The duration of sun exposure could not be assessed either, but it was assumed that industrialized areas would be exposed to less sunlight because of blockage by air pollution. In this study, the cut-off for vitamin sufficiency was taken as 20 ng/mL, in contrast to many other studies where the cut-off is more appropriately suggested at 30 ng/mL or even 40 ng/mL. Nevertheless, vitamin D levels were studied as a continuous variable and regression analysis revealed the inverse relationship between BMI and serum vitamin D level.

Other studies have also reported that inadequate vitamin D intake was associated with obesity in young adults.<sup>4</sup> Infants with rickets are also known to be chubby. Reverse causality may also be suggested, and whether or not vitamin D deficiency per se causes obesity remains to be investigated. However, the possible role of isolated hypovitaminosis D in causing obesity is a difficult issue to be studied in humans since vitamin D deficiency may be associated with other nutritional deficiencies which may lead to malnutrition rather than obesity.

Whether obese children and adolescents require a higher dose of vitamin D supplementation is controversial. As the authors stated, cross-sectional design and the limited number of subjects were limitations of this study. Further longitudinal studies are necessary to define the role, if any, of hypovitaminosis D in the etiology of obesity and the dose and duration of vitamin D supplementation in childhood, particularly in the obese and overweight population.

Ömer Tarım, MD

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## Pediatric Obesity: Meta-Analysis of Non-Surgical Interventions

In order to inform practice guidelines, the Endocrine Society's Task Force on Pediatric Obesity engaged the Mayo Knowledge and Encounter Research Unit to conduct a meta-analysis of published, randomized trials for pediatric obesity lifestyle and pharmacological interventions.<sup>1</sup> Overweight or obese children aged 2- through 18-years served as participants in the individual studies forming the meta-analysis. Pharmacological interventions included medications aimed at reducing measures of obesity in children (ie, BMI, percent overweight, percent fat-free mass and visceral adiposity). Lifestyle interventions included treatment strategies targeting physical activity and/or dietary changes. Eligible treatments targeted the child, parent, family, school, or community. Interventionists included community agents, school personnel, family members, or healthcare personnel.

Fully published randomized trials were identified through a systematic search of the following databases: MEDLINE, EMBASE, ERIC, CINAHL, Cochrane Central Register of Controlled Trials, PSYCInfo, Dissertation Abstracts International, Science Citation Index, and Social Science Citation Index. Publications through February 2006 were included. Reference sections of reviews and published guidelines were reviewed, and suggestions from experts on The Endocrine Society Pediatric Obesity Task Force were included. From these, 76 articles were considered eligible for the meta-analysis; in all, 61 trials had complete data to include in meta-analyses. Working in pairs, trained reviewers extracted study details and mean or variance data were calculated.

Effect size and 95% confidence interval (CI) for the difference between the intervention and control groups were calculated, as well as standardized mean differences. Subgroup analyses were conducted for degree of parental participation, child age, percent body fat versus BMI, and the combination of reduced sedentary behavior and increased physical activity. Standardized mean differences of about 0.2 or less were considered small, about 0.5 as moderate, and about 0.8 or greater as large effect sizes. The likelihood of between-study variability being attributable to true between-study differences (versus chance) was quantified using the  $I^2$  statistic (inconsistency is considered small when  $I^2 < 25\%$ , moderate 25%-50%, and large  $> 50\%$ ).

A total of 17 trials of pharmacological interventions formed this portion of the meta-analysis: sibutramine (3 trials)—the pooled effect size, favoring treatment, was large ( $-1.01$ ; CI =  $-1.8$  to  $-0.73$ ;  $I^2 = 30\%$ ) and consistent with a loss in BMI of  $2.4 \text{ kg/m}^2$  (CI =  $1.8$  to  $3.1 \text{ kg/m}^2$ ) after 6 months of use (patients taking sibutramine had higher rates of elevated blood pressure and pulse rate than patients taking placebo); orlistat (3 trials)—the pooled effect size was small to moderate ( $-0.29$ ; CI =  $-0.46$  to  $-0.12$ ;  $I^2 = 0\%$ ) and consistent with a loss in BMI of  $0.7 \text{ kg/m}^2$  (CI =  $0.3$  to  $1.2 \text{ kg/m}^2$ ). More patients

taking orlistat reported GI side effects than patients on placebo; metformin monotherapy for hyperinsulinemic nondiabetic obese adolescents lead to a small nonsignificant change in obesity outcome at 6 months ( $-0.17$ ; CI =  $0.62$  to  $0.28$ ). The remaining trials measured the effect of sympathomimetics (ephedrine and caffeine, dexfenfluramine), dehydroepiandrosterone, and fiber supplements (results reported in figure). Trials of rimonabant in children or adolescents were not identified in the literature.

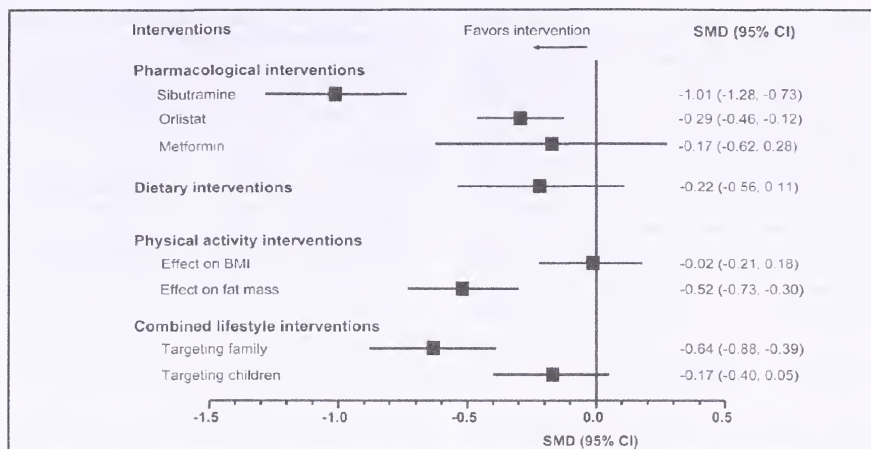
Lifestyle interventions were divided into "dietary interventions only" (ie, reduced-glycemic-load diet, protein-sparing modified diet, low-carbohydrate diet, high-protein diet, and hypocaloric diet versus control;  $n=6$ ), "physical activity interventions only" ( $n=17$ ), and "combined lifestyle interventions" ( $n=23$ ). The pooled effect sizes and between-study inconsistency for dietary interventions were both small ( $-0.22$ ; CI =  $-0.056$  to  $0.11$ ;  $I^2 = 22.5\%$ ). Physical activity interventions yielded inconsistent results: the investigators examined whether the choice of obesity outcome measure accounted for this. Trials that measured effects on adiposity found a moderate treatment effect ( $-0.52$ , CI =  $-0.73$  to  $-0.30$ ;  $I^2 = 0\%$ ), whereas trials measuring the effect of physical activity on BMI found no significant effect ( $-0.02$ , CI =  $-0.21$  to  $0.18$ ;  $I^2 = 0\%$ ).

The pooled estimate across combination lifestyle interventions (physical activity and dietary modification) yielded small to moderate treatment effects. The largest effects were associated with greater parental involvement. There was a nonsignificant interaction between child age and parental involvement, with a trend toward a larger treatment effect for children 8 years or younger ( $-0.70$ ; CI =  $-1.00$  to  $-0.40$ ).

The authors concluded: (1) short-term efficacy of sibutramine and orlistat on BMI; (2) moderate effect of physical activity on adiposity, but not BMI; and (3) small to moderate effect of combined lifestyle interventions on BMI with a nonsignificant trend favoring those interventions with parental involvement, in particular trials involving younger children. The authors discussed research implications related to drawbacks associated with using BMI as an outcome measure (eg, less responsive to change, requires accuracy and reproducibility in measurement, and misinterprets risk in muscular and short children), and suggested using more responsive outcome measures such as fat-free mass or percent body fat in future studies. The authors also suggested that the Endocrine Society's recommendation for a multidisciplinary and multimodal approach to the treatment of pediatric obesity be studied with long-term randomized trials.

McGovern L, Johnson JN, Paulo R, et al. Treatment of pediatric obesity: A systematic review and meta-analysis of randomized trials. *J Clin Endocrinol Metab*. 2008;93:4600-4605.





Overall summary of meta-analyses results of randomized trials of treatments for pediatric obesity.

Plot shows metanalytic estimates (■) and 95% CI (horizontal lines). SMD, standardized mean differences.

Reprinted with permission McGovern L, et al. *J Clin Endocr Metab*. 2008;93:4600-4605. Copyright © Endocrine Society 2008. All rights reserved.

**Editors' Comment:** The generally dim view of the effectiveness of non-surgical approaches to the management of pediatric obesity may be an important contributing factor to the increasing visibility of bariatric surgery programs. The current meta-analysis provides valuable information for the clinician, clinical and public health researcher with an interest in ensuring that management moves forward in an evidence-based manner. Meta-analysis provides the benefit of increasing the statistical power of small or inconclusive studies and can demonstrate how interventions deliver heterogeneous effectiveness in different settings and in different patients. The benefits do not come without drawbacks, however. Meta-analysis cannot improve the quality of the original studies. Further, aggregation of studies without sufficient attention to the heterogeneity of procedures may result in misleading conclusions.

In the case of the current meta-analysis, there was substantial variability in the procedures adopted across lifestyle intervention trials. These studies varied greatly with respect to duration of the intervention, frequency of sessions, content of dietary, behavioral, and exercise components, training of the interventionists, and measurement. For example, in trials categorized as "combined lifestyle intervention category," some used the Traffic Light Diet, whereas others did not state specifically which nutrition recommendations were offered. Duration of exercise also varied; some interventions held exercise sessions 3-times per week, whereas others only provided exercise education. Furthermore, specification of the specific behavioral strategies employed during treatment to promote

behavior change is not consistently described. Given the high degree of unspecified details across studies, it would be premature to adopt generalizations regarding the value of lifestyle interventions, per se.

Effect sizes of the interventions included in this meta-analysis were estimated in reference to control groups. Not only was there great variability across procedures adopted as interventions, there was also great variability with respect to how control groups were defined. Some researchers chose a no-treatment control group, whereas others altered aspects of the intervention delivered to the treatment group and used this modified intervention as the control group. The strength of findings for any single study may be diminished when the intervention group is compared to a control group that is also offering some form of the intervention, albeit modified. The high degree of variability across interventions, together with highly variable control groups, leads us to question whether a meta-analysis is premature.

Another factor to be considered when interpreting the findings of this meta-analysis includes categorization of the lifestyle interventions. Six studies were designated "dietary interventions only." Closer examination of these reveals that 5 of the 6 studies included behavioral and/or physical activity components in addition to the dietary intervention. Only one study was exclusively dietary.<sup>2</sup> Similarly, for the 17 studies designated as "physical activity interventions only,"<sup>8</sup> also included a dietary and/or behavioral component. Although the focus of the studies in these categories may be primarily dietary or physical activity, the addition of other components may weaken the conclusions that can be drawn from a meta-



analysis that is specifically analyzing "dietary interventions only" or "physical activity interventions only."

An additional detail regarding studies classified as "combined lifestyle interventions" is noteworthy. The authors suggested a statistically non-significant trend for a larger treatment effect in interventions involving parental involvement in children 8 years of age or younger. This conclusion was based on only 2 studies in which the majority of participants were under age 8 years. Moreover, one of these studies examines a parent-only approach to weight management.<sup>3</sup>

Given some of our observations, it might be premature to draw firm conclusions about the magnitude of effect sizes of dietary or behavioral interventions and their variability across populations.

*The obvious risk would be to make pronouncements that bias future research agenda.*

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## Prevention of Pediatric Obesity: Meta-Analysis of Behavioral Interventions

The prevalence of overweight (ie, BMI >95th percentile for age) is currently 16% in children of all ages living in the US. The highest rate occurs among African-American youth. The Endocrine Society's Task Force on Pediatric Obesity commissioned a meta-analysis of published, randomized trials for interventions aimed at preventing pediatric obesity.<sup>1</sup> In contrast to previous summaries of the literature that focused on the endpoint of body weight, this study sought to summarize the efficacy of interventions aimed at changing lifestyle behaviors, including increased physical activity (PA), decreased sedentary activity (SA), increased healthy dietary habits (HD), and decreased unhealthy dietary habits (UD) to prevent pediatric obesity. In addition, the investigators sought to assess the effect of these interventions on BMI.

Studies eligible for inclusion in the meta-analysis were randomized controlled trials (RCTs) assessing these lifestyle behavior interventions in children or adolescents 2 to 18 years of age. Participants received the interventions at home, school, clinic, or a community setting and healthcare professionals, community members, or health authorities delivered the interventions. Trials with participants who were all overweight or obese were excluded.

Fully published randomized trials were identified through a systematic search of the following databases: MEDLINE, ERIC, EMBASE, CINHAL, PSYCInfo, DISSERTATION abstracts, Science Citation Index, Social Science Citation Index, and the Cochrane CENTRAL database of Controlled Clinical Trials. Publications through February 2006 were included. Reference sections of reviews and expert suggestions were incorporated; 29 trials were considered eligible for the meta-analysis analyzing at least one behavioral endpoint and 34 trials had complete data for BMI. Working in pairs, trained reviewers extracted study details related to the following intervention components: informational (ie, passive information, education), cognitive (ie, general cognitive strategies, goal setting, problem solving/relapse

prevention), behavioral (ie, reminders and prompts for desired behaviors, skill building, practice and rehearsal, monitoring and feedback, and reinforcement of behavior), environmental (ie, physical changes made to change the environment of the school, home, and community), and parental support (ie, active involvement).

An effect size and 95% confidence interval (CI) for the difference between the intervention and control groups were calculated for each of the 4 behavioral targets (ie, increase physical activity, decrease sedentary activity, increase healthy behavior, and reduce unhealthy dietary behavior) and BMI. Standardized mean differences of about 0.2 or less were considered small, about 0.5 as moderate, and about 0.8 or greater as large effect sizes. The likelihood of between-study variability being attributable to true between-study differences (vs chance) was quantified using the  $I^2$  statistic (inconsistency is considered small when  $I^2$  is >25%, moderate 25%-50%, and large >50%). Several preplanned subgroup analyses of RCTs were performed.

**Interventions to increase physical activity.** Twenty-two randomized trials were included in the meta-analysis to assess the effects of interventions to increase physical activity. Results suggested a small increase in physical activity (effect size = 0.12; CI = 0.4 to 0.20) with moderate inconsistency across trials ( $I^2$  = 63%) which could not be explained by subgroup analyses. There was a trend toward favoring the inclusion of multiple cognitive components (0.15; CI = 0.05 to 0.4; vs 1 or no cognitive components) and reinforcement (0.24; CI = 0.06 to 0.41; vs no reinforcement).

**Interventions to decrease sedentary activity.** Meta-analysis of 14 RCTs yielded a small reduction of sedentary activity (-0.29; CI = -0.35 to -0.22), with high consistency in results across studies ( $I^2$  = 0%). Several significant treatment x subgroup interactions were detected: treatment effects were greater in trials measuring in-treatment outcomes (-0.32; CI = -0.39 to -0.25; vs outcome measured after treatment), treatment duration >6 months

(-0.31; CI = -0.39 to 0.24; vs briefer trials), and when trials involved children were enrolled (-0.31; CI = -0.39 to -0.24; vs adolescents). A trend also emerged toward favoring multiple cognitive components (-0.31; CI = -0.38 to -0.24; vs one or no cognitive components).

**Interventions to increase healthy dietary behavior.** Meta-analysis of 14 RCTs suggested, overall, a small and nonsignificant increase (0.06; CI = -0.09 to 0.21) with considerable heterogeneity ( $I^2 = 83\%$ ) in healthy dietary behavior. The trials showed great effect when reinforcement was included (0.41; CI = -0.05 to 0.76).

**Interventions to reduce unhealthy dietary behavior.** This category included 23 RCTs. Results indicated a small but significant reduction in unhealthy dietary behavior (-0.15; CI = -0.22 to -0.08), with greater treatment effects for trials with briefer training (-0.40; CI = -0.62 to -0.19). Thirty-four trials were examined for effects on BMI and the results were not significant (-0.02; CI = -0.06 to -0.02).

**Interventions to reduce BMI.** A meta-analysis of 34 RCTs of lifestyle interventions (involving 43 comparisons) on BMI failed to reveal a significant benefit (-0.02; CI = -0.06 to 0.02;  $I^2 = 17\%$ ). All modalities of intervention (dietary only, physical activity only, or combined lifestyle interventions) yielded similar trivial to small effects on BMI compared with controls.

Kamath CC, Vickers KS, Ehrlich A, et al. Behavioral interventions to prevent childhood obesity: A systematic review and metaanalyses of randomized trials. *J Clin Endocrinol Metab.* 2008;93:4606-4615.

**Editors' Comment:** The meta-analyses of interventions to prevent childhood obesity described above represent the first attempt to systematically quantify the benefits of cognitive, behavioral, informational, and environmental components of obesity prevention programs. While the authors acknowledged that analyses may be underpowered to detect interactions between the intervention components and the outcomes of interest; this report remains an important first step in the process of determining which intervention strategies are most effective for preventing childhood obesity. The authors discussed that previous attempts to summarize the prevention literature have been limited by the heterogeneity of the interventions and the measurement of obesity outcomes.

Conceptually, this meta-analysis would have benefited from categorizing the prevention programs as primary

(ie, all children in a given population), secondary (ie, only delivered to individuals with risk factors, such as a parent being overweight), or tertiary (ie, targeted to children who are already overweight). For the randomized trials selected for this study, categorization would be limited to primary or secondary prevention programs, as the exclusion criteria included programs with the majority of participants classified as overweight or obese. Categorizing programs in such a way would provide further information about the sample, leading us to better understand which intervention strategies may be most helpful for participants with particular characteristics and risk-profiles. It may also help us to determine if being at greater risk for obesity affects parent and child adherence to obesity prevention activities.

An additional factor to be considered when interpreting this study is the designation of intervention strategies as cognitive or behavioral. Goal-setting is considered by some to represent a behavioral strategy.<sup>2</sup> Generally, goal-setting is not a strategy that is used in isolation; rather, participants usually set a goal and then monitor the behavior targeted to determine how many days the goal was met. By designating goal-setting as cognitive, it may incorrectly overemphasize the importance of cognitive strategies and inadvertently diminish the impact of behavioral skills. Perhaps looking at specific strategies, rather than the classification of strategies (ie, cognitive or behavioral), may provide us with information that is easier to interpret and implement in future research studies.

We wholeheartedly agree with the authors that future publications of prevention and treatment research must detail the specifics of intervention programs. This should become a standard practice that scientific journals require.

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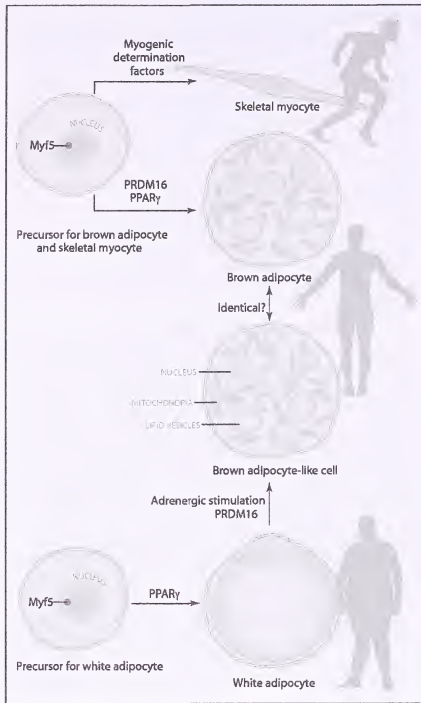
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## Brown Fat Controls – PRDM16 and Bone Morphogenetic Protein 7

Adipocytes are cells that store fats as triglycerides. White fat cells (WFC) store fats within one large, cell-filling lipid droplet. After readily available energy sources have been exhausted, the WFC hydrolyzes triglycerides and exports fatty acids to be utilized as fuel by other cells.<sup>1</sup> Brown fat cells (BFCs) store lipids in multiple small

droplets, have a large number of mitochondria (that stain brown), and actively hydrolyze triglycerides to fatty acids which are then oxidized to produce heat. The BFC is able to oxidize fatty acids, because it expresses uncoupling protein 1 (UCP1, chromosome 4q31, OMIM 113730) that, in association with its co-factor (coenzyme Q),



**Figure 1. Paths to muscle and fat.**

Skeletal myocytes and brown adipocytes derive from a common precursor cell that expresses the transcription factor Myf5. White adipocytes derive from a Myf5-negative precursor, as do brown adipocyte-like cells that appear in white fat depots after adrenergic stimulation. These distinct cell types play very different roles in physiology. Reprinted with permission Lazar MA. *Science*. 2008;321:1048-1049. Copyright © AAAS 2008. All rights reserved.

allows protons to "leak" across the inner mitochondrial membrane thereby diverting energy from ATP synthesis to (non-shivering) thermogenesis. Adipocytes are derived from a mesenchymal precursor stem cell that also gives rise to osteoblasts, chondroblasts, myoblasts, and fibroblasts. An osteoblast can be transformed to an adipocyte if *Pparγ2* (peroxisome proliferator-activated receptor γ2) is expressed, while an adipocyte can be converted to an osteoblast if *Runx2* is expressed.<sup>2</sup> In the presence of a β-adrenergic stimulus, a WFC can be transformed into a BFC—both morphologically and functionally. WFCs are found subcutaneously and intra-abdominally. Foci of BFCs are more abundant in infants but are also present in adults and are distinct from those BFCs that are sparsely interspersed among WFCs. It has long been assumed that the WFC and BFC arise

from the same precursor cell.<sup>3</sup>

Searle et al now demonstrated that the BFC is actually derived from a precursor cell that differentiates into either a skeletal myocyte if it expresses a myogenic determining factor (eg, *Myf5*) or into a BFC if it expresses *Prdm16* (Proline rich domain-containing protein 16, chromosome 1p36.3, OMIM 605557) and *Pparγ2* (Figure 1). Depleting BFC precursor cells of PRDM16 in vitro resulted in their differentiation into myocytes morphologically and functionally, as these cells expressed myogenic genes rather than genes characteristic of BFCs. "Knock-in" of *Prdm16* into committed myoblasts led to their differentiation into BFCs morphologically and by expression of BFC genes. The investigators further demonstrated that the BFC that arose from the WFC in response to β-adrenergic stimulation was not derived from a myogenic precursor cell. Although there are zinc fingers within the structure of PRDM16, it does not bind to DNA but rather to other intracellular proteins. Binding of PRDM16 to PPARγ stimulates the transcriptional activity of PPARγ and BFC differentiation from the myogenic precursor cell. The authors concluded that the primary BFC is derived from a precursor cell that can differentiate either into either a myocyte or a BFC.

Tseng et al complement the findings of Searle et al by demonstrating that bone morphogenetic protein 7 (*BMP7*, chromosome 20, OMIM 112267) can also induce BFC differentiation by directing mesenchymal precursor cells to the BFC differentiation pathway. It does so by inducing expression of PRDM16 and PPARγ-coactivator-1α, a co-factor for PPARγ.

Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008;454:961-967.

Tseng YH, Kokkoto E, Schulz TJ, et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature*. 2008;454:1000-1004.

**First Editor's Comment:** Myocytes and BFCs arise from a common precursor cell, this links the oxidative function of these 2 cell types, and perhaps provides a reason why BFCs primarily catabolize rather than store lipids. Understanding the pathway that leads to BFC development and directed oxidation of fatty acids to thermogenesis rather than to energy generation holds the potential promise for the development of drugs (perhaps agonists of *BMP7*) that may be able to stimulate PRDM16 activity and BFC generation and increase dissipation of fat stores. Might agonists or antagonists of this pathway even be of use in clinical conditions in which control of core body temperature is indicated?

Allen W. Root, MD

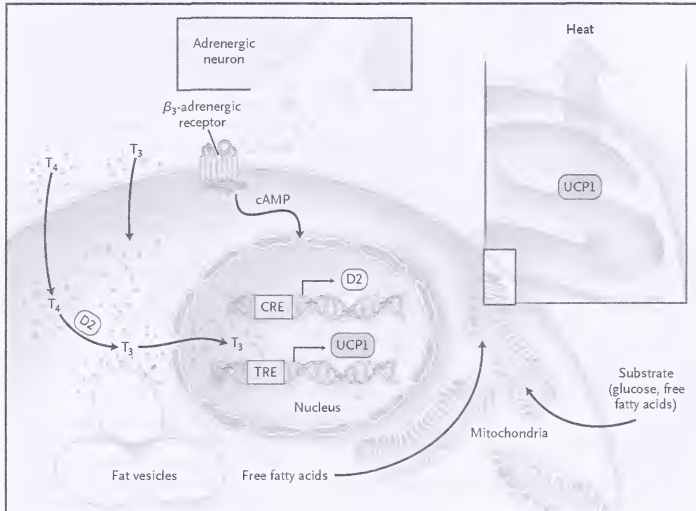
**Second Editor's Comment:** A recent paper published in the *New England Journal of Medicine* highlighted



the cold-activated brown adipose tissue in healthy men.<sup>4</sup> An accompanying editorial highlighted the pathophysiology of this tissue and the potential implication for stimulating energy expenditure (Figure 2).<sup>5</sup> These 2 papers proposed the concept of target

interventions—pharmacological and environmental—aimed at modulating energy metabolism. Wouldn't it be great if lowering the thermostat could help prevent and treat obesity?

Fima Lifshitz, MD



**Figure 2. The Activation of Brown Adipose Tissue.**

Stimulation of  $\beta_3$ -adrenergic receptors leads to the dramatic increase in the intracellular concentration of triiodothyronine ( $T_3$ ) by means of the type 2 5' deiodinase (D2);  $T_3$  in turn stimulates the transcription of uncoupling protein 1 (UCP1), which causes the leakage of protons from the inner membrane of the mitochondria, hence dissipating energy in the form of heat. The abbreviation cAMP denotes cyclic adenosine monophosphate, CRE cAMP response element,  $T_4$  thyroxine, and TRE thyroid hormone response element.

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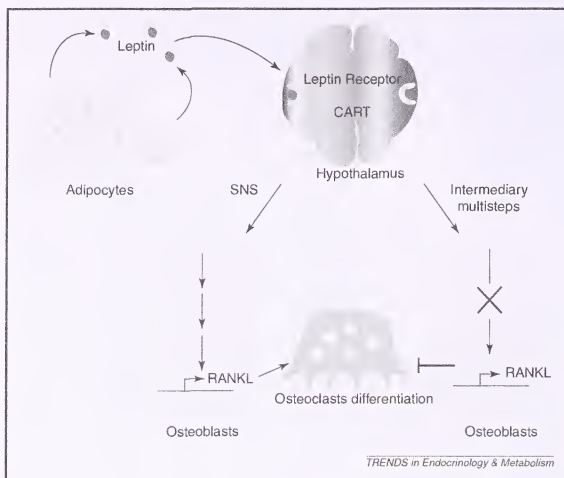
## Reciprocal Regulation of Bone and Energy Metabolism

Remodeling allows bones to renew themselves and this process requires a fair amount of energy. In view of this, Lee and Karsenty hypothesized that there is somewhat of a common control of bone and energy metabolism. This started a search for a bone derived hormone that in turn regulates energy metabolism. The clinical observation that obesity protects from osteoporosis led to the proposal that bone remodeling was dependent, in some way, from an adipose tissue derived hormone, leptin. The researchers showed that leptin regulates bone mass. It binds to its receptor on hypothalamic neurons and then uses 2 pathways: (1) sympathetic signaling in osteoblasts which favors osteoclast differentiation by inducing *RANKL* gene expression, and (2) through CART (cocaine- and amphetamine-regulated transcript) inhibiting the *RANKL* expression in osteoblast (Figure 1). In view of these data they raised

the question: is the skeleton, in turn, regulating any aspect of energy metabolism? This led to the search of a bone derived hormone.

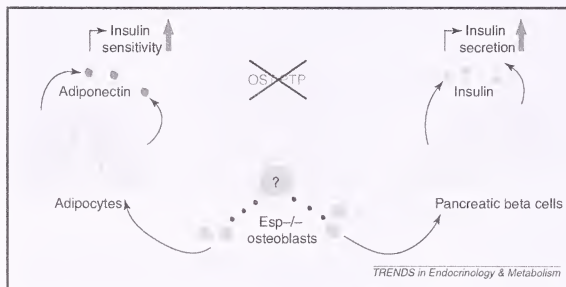
In search of that hormone, an osteocalcin<sup>-/-</sup> mouse was generated. It displayed a high bone mass phenotype and it also had an abnormal amount of visceral fat. This was the first evidence suggesting that skeleton regulates energy metabolism. Thereafter, mutant mice-lacking genes, expressed preferentially in osteoblast, were generated. The first gene of interest was *Esp*; it encodes a receptor like protein tyrosine phosphatase termed OST-PTP4. These mice had a surprising phenotype pointing towards new modes of regulation of glucose metabolism: increased insulin secretion with hypoglycemia, increase in beta cell proliferation, and an increase in insulin sensitivity. Given the increase in insulin sensitivity, the mutants should be fatter. Instead they had





**Figure 1. Schematic representation of bone mass regulation by fat.** The adipocyte-derived hormone leptin binds to its receptor on hypothalamic neurons and then uses the sympathetic tone and cocaine- and amphetamine-regulated transcript (CART) to regulate bone function and RANKL expression in osteoblasts.

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**Figure 2. Osteocalcin in its uncarboxylated form is an osteoblast-derived hormone that improves glucose handling.** OST-PTP, the Esp gene product, favors, through yet unknown mechanisms, the carboxylation of osteocalcin. In the absence of Esp, more osteocalcin is uncarboxylated. This uncarboxylated form of osteocalcin increases expression of the insulin gene in  $\beta$  cells and the expression of the adiponectin gene in adipocytes, resulting in an increase in insulin secretion and in insulin sensitivity, respectively.

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less fat because of an increase in energy expenditure; furthermore appetite was not affected. In addition, mice overexpressing *Esp* only in osteoblasts developed type 2 diabetes phenotype on a normal diet.

Osteocalcin was shown to be the molecule made by osteoblasts that accounts for the osteoblast-mediated regulation of gene expression of insulin

in islets and of adiponectin, an insulin-sensitizing adipokine in adipocytes. It then appears that the *Esp*-deficient mice metabolic phenotype is caused by a gain of osteocalcin bioactivity and that OST-PTP regulates indirectly osteocalcin's post-translational modifications; it favors the carboxylation of osteocalcin to transform it into bone gla protein. A small portion of non carboxylated osteocalcin is circulating and has the ability to improve glucose handling as described (Figure 2).

The authors further evaluated the potential relevance of these findings. They interpreted a series of experiments concluding that the increase in insulin sensitivity might protect from diabetes in a situation in which insulin secretion is low but not absent.

Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. *Trend Endocrinol Metab.* 2008;19:161-166.

**Editor's Comment:** In recent years this provocative work has attracted great attention. It shows that communication from metabolism to bone is not unidirectional and that bone regulates glucose metabolism and fat mass via the uncarboxylated form of osteocalcin in a complex crosstalk.<sup>1</sup> Therefore it is of interest that the association between serum osteocalcin concentration and markers of dysmetabolic phenotype was evaluated in a group of adults.<sup>2</sup> It was shown that serum osteocalcin was inversely associated with blood markers of dysmetabolic condition and measures of obesity. These findings need to be replicated to further test the initial hypothesis and to be extended to further studies in relation to type 2 diabetes. There is presently a major interest in this new presentation of energy metabolism including bone as a major actor.

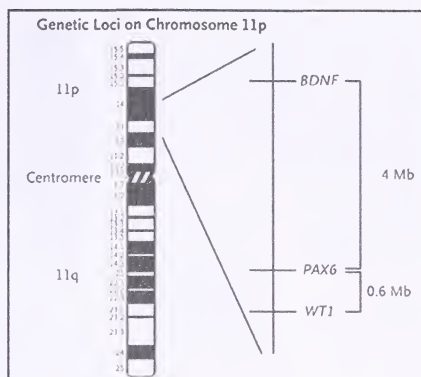
Raphaël Rappaport, MD

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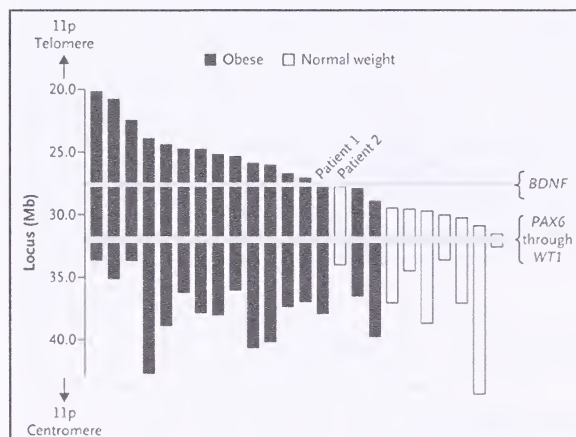
# Brain-Derived Neurotrophic Factors and Obesity in WAGR Syndrome

The syndrome of Wilms' tumor, aniridia, anomalies of the genitourinary tract including ambiguous external genitalia, mental retardation and hemihypertrophy (WAGR - OMIM 194072, chromosome 11p13, Figures 1 and 2) is associated with heterozygous microdeletions of chromosome 11p13 and loss of the contiguous genes *WT1* (OMIM 607102) and *PAX6* (OMIM 607108). Obesity has been found in some subjects with this disorder. Also on chromosome 11p13 is the neuronal survival factor, brain-derived neurotrophic factor (*BDNF*, OMIM 113505), which has been found to affect energy metabolism in rodents. Loss of *Bdnf* in mice results in excessive weight gain in adulthood due to increased caloric intake.<sup>1</sup> Thus, *BDNF* may be an anorexigenic factor. In order to examine the effect of *BDNF* on energy metabolism in humans, the investigators examined the relationship between the presence or absence of *BDNF* and weight in patients with WAGR by examining the extent of the deletion at chromosome 11p13 in 33 patients. Haploinsufficiency of *BDNF* was present in 19 patients—complete in 17 and partial in 2 subjects. By 2 years of age, weight was greater in all WAGR patients with loss of *BDNF* than in those with an intact gene. Serum concentrations of *BDNF* were



**Figure 1. Genetic Loci on Chromosome 11p in Patients with the WAGR Syndrome.**

The WAGR syndrome is caused by deletions on chromosome 11p that result in haploinsufficiency for the *PAX6* and *WT1* genes. *BDNF* is located approximately 4 Mb telomeric to *PAX6*. Reprinted with permission Han JC, et al. *N Engl J Med*. 2008;359:918-927. Copyright © MMS 2008. All rights reserved.



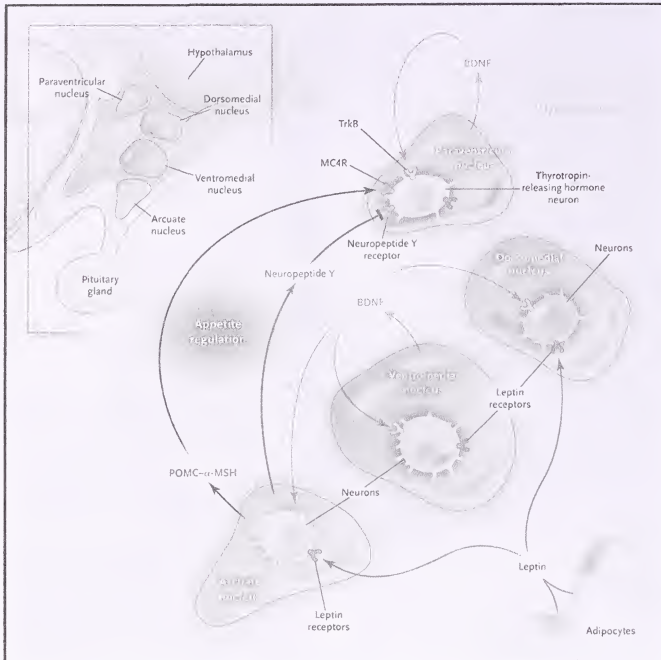
**Figure 2. Regions of Deletion on Chromosome 11p.**

The region of deletion on chromosome 11p is shown for each of the 24 patients in whom the presence or absence of childhood obesity (body-mass index [BMI]  $\geq$  95th percentile by 10 years of age) could be determined. No association between the centromeric deletion boundary and childhood obesity was observed. However, for the telomeric deletion boundary, all patients with heterozygous deletion of all or a portion of *BDNF* had childhood obesity, whereas no deletions involved *BDNF* in the patients who were of normal weight. In Patient 1, who was obese, there was a heterozygous deletion of *BDNF* exons 1 through 3. In Patient 2, who had a normal weight (BMI, approximately 20th percentile at 10 years of age), the deletion region ended 72.5 kb upstream of *BDNF*. Only 20% of the patients without *BDNF* deletions were obese; this rate is similar to the prevalence of childhood obesity in the general U.S. population.<sup>2</sup> Reprinted with permission Han JC, et al. *N Engl J Med*. 2008;359:918-927. Copyright © MMS 2008. All rights reserved.

higher and hyperphagia was more prevalent in the former subjects. Loss of any exon of *BDNF* or of a portion of the 70 to 80-kb region upstream of *BDNF* was associated with obesity. The investigators also demonstrated that heterozygous loss of *BDNF* was associated with decreased pain perception in WAGR subjects. The authors concluded that *BDNF* modulates appetite in humans as well as in experimental animals.

Han JC, Liu QR, Jones MP, et al. Brain-derived neurotrophic factor and obesity in the WAGR syndrome. *N Engl J Med*. 2008;359:918-927.

**Editor's Comment:** These observations suggest that there well may be patients with childhood onset obesity and loss-of-function mutations in *BDNF* itself or in its 5' region and/or that a polymorphic variant(s) of this gene may be related to appetite, caloric intake, and body weight. In the hierarchical mechanisms that regulate appetite, *BDNF* may function downstream of the melanocortin 4 receptor and thus be responsive to the effects of  $\alpha$ MSH and proopiomelanocortin.<sup>2</sup> Other clinical studies have demonstrated a strong association between the



**Figure 3. Model of the Anorectic Action of Brain-Derived Neurotrophic Factor (BDNF) in the Hypothalamus.** BDNF is produced by thyrotropin-releasing hormone neurons in the paraventricular nucleus and acts on neurotrophic tyrosine kinase receptor type 2 (TrkB). BDNF-producing neurons are excited by the release of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) from proopiomelanocortin (POMC) neurons in the arcuate nucleus, which acts on melanocortin 4 receptors (MC4Rs). BDNF-producing neurons in the paraventricular nucleus are inhibited by neuropeptide Y that is released from the arcuate nucleus. Leptin from the periphery can also excite BDNF-producing neurons that express the signaling form of the leptin receptor. Adapted from Levin.<sup>7</sup> Reprinted with permission Forguel P, Blakemore A, *New Engl J Med.* 2008;359:891-893. Copyright © MMS 2008. All rights reserved.

*BDNF Met66 allele of the Val66Met polymorphic variant and anorexia nervosa and other eating disorders.<sup>3</sup> Interestingly, the Val66 allele of BDNF has been associated*

*with child onset bipolar disorder.<sup>4</sup> The model of the anorectic action of BDNF in the hypothalamus<sup>5</sup> is shown in Figure 3.*

Allen W. Root, MD

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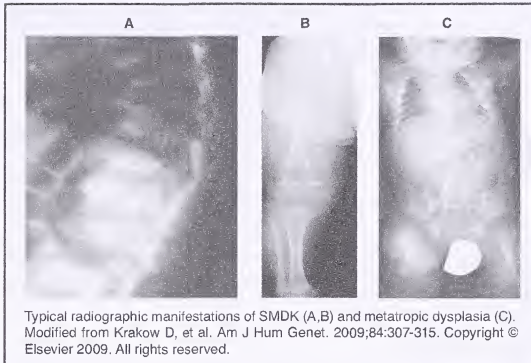
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## Calcium Channel Family of Bone Dysplasias

Brachyolmia is a relatively mild bone dysplasia that primarily affects vertebral body growth leading to mild to moderate short trunk dwarfism. One form of autosomal dominant brachyolmia (MIM 113500) was recently shown to result from activating mutations of TRPV4, a calcium-permeable ion channel protein that has been implicated in skeletal development.<sup>1</sup> Qualitatively similar but more severe radiographic changes are found in 2 other dominantly inherited bone dysplasias. The first, spondylometaphyseal dysplasia

Kozlowski type (SMDK, MIM 1842522), is characterized by postnatal onset of short stature, kyphoscoliosis and progressive deformity. The second, metatropic dysplasia (MIM 156530), presents in newborn infants with short limbs, but evolves to a short trunk clinical phenotype as a result of severe and progressive kyphoscoliosis typically compromising neurologic and respiratory functions. The clinical and radiographic similarities prompted Krakow and colleagues to search for mutations of TRPV4 in the latter disorders, which





Typical radiographic manifestations of SMDK (A,B) and metatropic dysplasia (C). Modified from Krakow D, et al. *Am J Hum Genet.* 2009;84:307-315. Copyright © Elsevier 2009. All rights reserved.

they have now reported.

Heterozygous missense mutations TRPV4 were detected in all 8 patients who were studied, 6 with SMDK and 2 with metatropic dysplasia. One mutation was recurrent in 4 patients with SMDK. It and 2 other mutations mapped to the cytoplasmic domain of the channel protein where the brachyolmia mutations had mapped, but 2 mapped to so-called ankyrin repeats, a common molecular motif thought to be involved in folding and direct interactions between proteins.

Since gain of channel function had been implicated in the brachyolmia-associated mutations of TRPV4, the investigators analyzed basal channel activity and responses to known TRPV4 agonists and antagonists of the 4 SMDK-associated mutations. Three displayed increased basal channel activity. The responses to agonists and antagonists were less clear and the authors concluded that the mutations most likely act

through increasing basal intracellular calcium. They also noted that genetically engineered mice lacking TRPV4 function exhibit a defect in osteoclast function associated with overmineralized bone and suggest that the clinical phenotype in brachyolmia, SMDK and metatropic dysplasia may reflect disturbed TRPV4 function in both chondrocytes and osteoblasts during bone growth.

Krakow D, Vriens J, Camacho N, et al. Mutations in the gene encoding the calcium-permeable ion channel TRPV4 produce spondylometaphyseal dysplasia, Kozłowski type and metatropic dysplasia. *Am J Hum Genet.* 2009;84: 307-315.

**Editor's Comment:** *The family concept that disorders that exhibit qualitatively similar clinical phenotypes result from mutations of the same gene function continues to be borne out, in this case with TRPV4. It will be interesting to watch this story unfold regarding how abnormal calcium channel activity or increased intracellular calcium, as the authors proposed, alters the biology of bone growth. There are a number of drugs used to treat various diseases unrelated to bone growth and there are health food constituents that are thought to affect intracellular calcium concentrations. One wonders if these agents could counter the adverse effects of disturbed TRPV4 channel function in cells that contribute to bone growth.*

William A. Horton, MD

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## Spondyloepimetaphyseal Dysplasia – Aggrecan

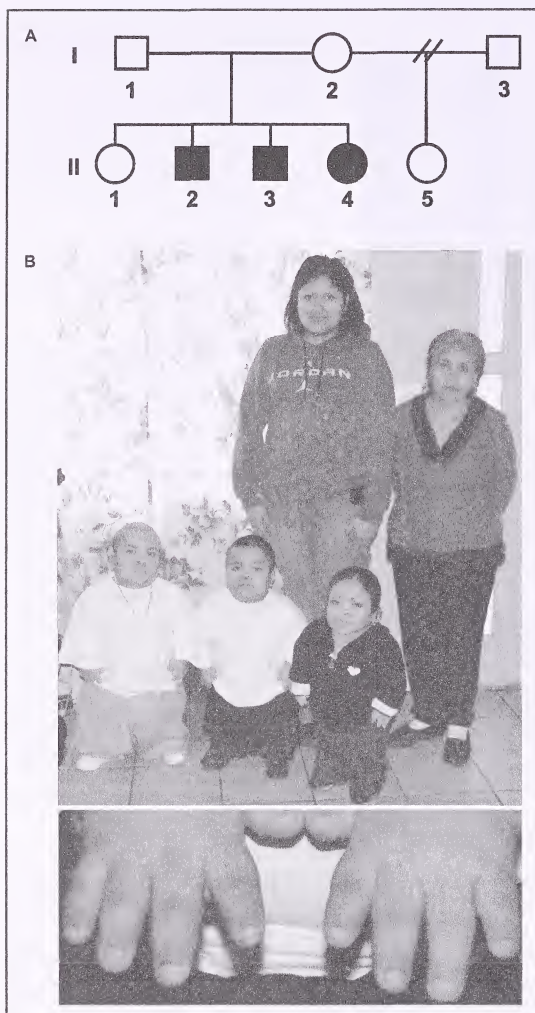
Despite considerable progress in delineating the human chondrodysplasias, there are still many distinctive dwarfing clinical phenotypes for which no mutant gene has been found; conversely, there are genes that encode proteins important for linear bone growth for which few if any chondrodysplasia-causing mutations have been identified. The report by Tompson et al helps to establish a new link in this context.

A family is described with extreme short stature, brachydactyly, distinctive facies and radiographs consistent with spondyloepimetaphyseal dysplasia (SEMD) in 3 of 4 siblings; the parents had average stature suggesting autosomal recessive inheritance (Figure). Consanguinity was denied, but both parents came from a small village in Mexico. Whole-genome single-nucleotide polymorphism (SNP) analysis of the 2 affected siblings

and 1 unaffected sibling revealed several 10-20 cM blocks of shared alleles suggesting a common ancestry and raising the possibility that the affected siblings may have inherited an ancestral mutation that was transmitted through both parents. If so, then they would be expected to be homozygous for the mutation, ie, identity by descent, and the mutation would be expected to map to the genetic region or interval that was shared by the affected siblings but not by the unaffected sibling. A single genetic interval on chromosome 15 met these criteria; it contained 193 annotated or characterized genes and 103 unannotated genes.

When the investigators narrowed down their search to genes expressed only or disproportionately highly in cartilage, because of its essential role in endochondral bone growth, 2 genes emerged: chondroitin sulfate





**Clinical Phenotype.** In the top image, the back row from left to right shows II-1 (23 years old [yo]) and I-1 (58 yo), respectively, and the front row from left to right shows II-2 (19 yo), II-3 (16 yo), and II-4 (26 yo), respectively. Note the telescoping fingers of II-3. Modified from Thompson SW, Merriman N, Funari V, et al. A J Hum Genet. 2009;84:72-79. Copyright © Elsevier 2009. All rights reserved.

proteoglycan 4 (CSPG4) and aggrecan (AGAN). Sequence analysis of AGAN revealed homozygosity in the affected siblings for a missense mutation that

substituted an asparagine for an aspartic acid residue at position 2267 (A2267N) in the C-terminal G3 globular domain of aggrecan. The parents were both heterozygotes for the mutation.

The A2267 aspartic acid is highly conserved across species and even across different proteoglycans that share a similar globular domain; it has been implicated in mediating molecular interactions between aggrecan and other cartilage matrix constituents, such as tenascin. To explore the molecular consequences of the mutation, the authors expressed the normal and mutant G3 domain proteins in cells and then analyzed them biochemically. They observed that the mutant G3 domain was secreted normally, but there was evidence of disturbed binding of the mutant G3 domain to tenascin compared to normal. They also showed that the asparagine residue in the mutant AGAN G3 domain is capable of being glycosylated, which could potentially alter functions as well as biosynthesis and stability of AGAN.

The authors noted that haploinsufficiency due to heterozygous mutations of AGAN has recently been reported in another rare condition, spondyloepiphyseal dysplasia (SED) Kimberley. This is a milder condition that typically presents with mild short stature and precocious osteoarthritis. They also point out that the genomic region to which AGAN has been mapped through genome-wide association studies has been linked to normal height variation.

Thompson SW, Merriman N, Funari V, et al. A recessive skeletal dysplasia, SEMD aggrecan type results from a missense mutation affecting the C-type lectin domain of aggrecan. A J Hum Genet. 2009;84:72-79.

**Editor's Comment:** Given its abundance in growth plate cartilage and presumed importance to endochondral bone growth, the difficulty finding mutations of AGAN in chondrodysplasias is surprising. It would have been interesting to study cartilage tissue, which presumably was not available, since it might have

provided additional clues as to how the mutation disrupts cartilage biology. A factor not discussed is the possibility that alterations in cartilage aggrecan

could alter growth factor signaling within the growth plate, as proteoglycans are thought to influence the mobility and local concentrations of growth factors

in cartilage and possibly their presentation to transmembrane receptors.

William A. Horton, MD

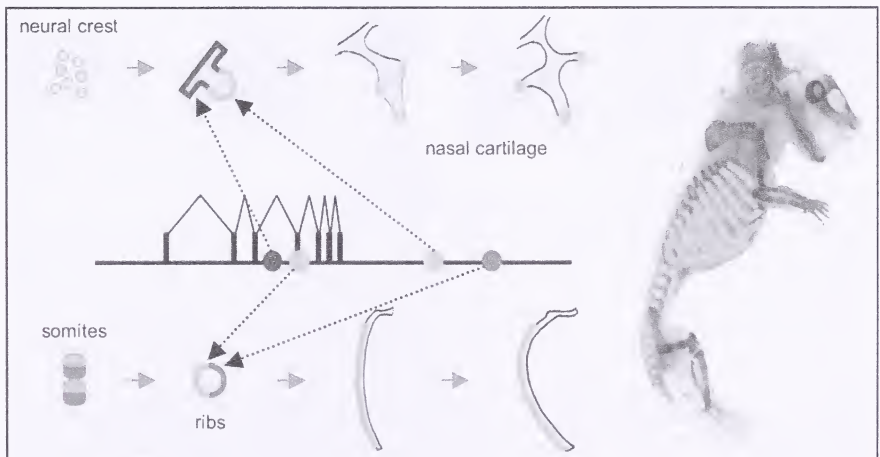
## Anatomy-Specific Enhancers of BMP Genes Fine-Tune Size and Shape of Individual Bones

As the skeleton grows, constituent cartilage and bone tissues are formed into a remarkable range of sizes and shapes. Although the blueprints that sculpt individual bones must be encoded in the genome, little is known about how this occurs. A group headed by David Kingsley at Stanford has recently provided novel insight into how the anatomy of individual bones is regulated. Their story begins with the generally accepted concept that bone shapes are determined by differential growth and erosion along the surfaces of bones. For instance, preferential deposition and erosion on opposite surfaces of a bone would generate lateral displacement or curvature of the bone such as a rib. Localized regions of deposition and erosion would shape ridges, foramina, and other surface structures.

The group focused their attention on the BMP5 gene because it is surrounded by large genomic regions containing regulatory elements required for normal developmental regulation and on rib development because BMP5 is expressed in the perichondrium surrounding

ribs and ribs are suitable for detecting differential growth and erosion. The approach was to generate transgenic mouse embryos harboring both a *lacZ* reporter gene and genomic DNA corresponding to different regions of the BMP5 locus including surrounding genomic DNA.  $\beta$ -galactosidase staining of late-stage transgenic embryos revealed specifically where the regulatory regions, ie, presumed enhancers, were active.

The details of the experiments are beyond the scope of this abstract. However, a regulatory element within the coding region of the gene was found to drive expression of BMP5 in the perichondrium adjacent to the lateral aspect of the ribs, whereas regulatory sequences 100 kb 3' to the coding region drove expression in the perichondrium of the medial aspect of the ribs. A number of confirmatory experiments was done, all of which suggested that BMP5 expression in different domains of the rib perichondrium is controlled by distinct regulatory elements in or near the BMP5 locus. In other words, anatomy-specific enhancers in BMP genes may provide



**Discrete enhancers control growth in distinct anatomical domains of developing bones.** Multiple anatomy-specific enhancers (filled circles) are spread across the *Bmp5* locus. In ribs, 2 enhancers (green and purple circles) may respond to lineage domains established in somites to control growth on opposing sides of the ribs. Local growth on the lateral edge of rib surfaces promotes rib curvature and expansion of the thoracic cavity. Nasal cartilages form from cranial neural crest. Two enhancers (blue and orange circles) in the *Bmp5* gene are expressed in different highly restricted locations, leading to characteristic branching patterns of the nasal turbinates.

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a genomic mechanism for independent developmental control of local growth of individual bones.

During these studies, the investigators also discovered 2 regulatory elements that controlled expression of BMP5 in nasal cartilage. These elements were distinct from those controlling BMP expression in ribs but like them mapped to locations within and 3' from the coding region of the gene as shown in the Figure. The authors suggested that the proposed mechanism may not be limited to regulation of BMP5 but common to other developmentally regulated genes that are involved in fine-tuning morphogenesis.

Guenther C, Pathalena-Filho L, Kingsley DM. Shaping skeletal growth by modular regulatory elements in the *Bmp5* gene. *PLoS Genetics*. 2008;4:1-13.

**Editor's Comment:** This investigation provides novel insight into the fine-tuning of skeletal development. It is interesting to speculate how subtle radiographic findings that allow experts to distinguish between similar bone dysplasias might reflect disturbances in these regulatory mechanisms.

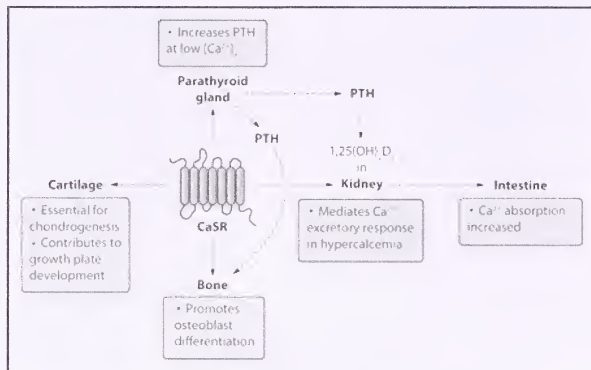
William A. Horton, MD

## Extracellular Calcium-Sensing Receptor: Modulator of Skeletal Development

The roles of the calcium-sensing receptor (CaSR) as a negative regulator of parathyroid hormone (PTH) synthesis and secretion and as an inhibitor of calcium reabsorption by the renal tubule are well documented; its functions in chondrocytes, osteoblasts, and osteoclasts have been less completely documented. In part, this has been due to inability to generate mice in which *CaSR* has been ablated specifically in cartilage and bone cells and to the expression in these cells of an alternatively spliced, functionally active isoform of the CaSR.<sup>1</sup> *CaSR* is expressed in differentiating osteoclasts, chondrocytes, and osteoblasts. Generalized ablation of *CaSR* in mice results in a rachitic phenotype (increased width of the zone of hypertrophic chondrocytes, depressed and disordered calcification of the cartilage growth plate, and decreased rate of cartilage mineralization).<sup>2</sup>

The present investigators have developed strains of mice in which exon 7 of *CaSR* (encoding the 7 transmembrane and 4 intracellular loops of the receptor protein) has been specifically "knocked-out" in parathyroid cells (PTC), growth plate chondrocytes (GPC), and osteoblasts (OB) rendering the *CaSR* functionally inactive. Homozygous loss of *CaSR* in PTC resulted in impaired growth and death within 2 weeks after birth. As anticipated, these mice had increased expression of *Pth* in their PTC. The skeletons of these mice had abundant matrix but were markedly undermineralized, and multiple fractures were present. There was substantially decreased expression of *CaSR* in bone cells

and delayed OB differentiation. In mice in which *CaSR* was specifically ablated in OB, the phenotype of growth retardation, skeletal undermineralization with increased osteoid formation, multiple fractures, and death by 3 weeks of age was observed. OB differentiation was severely impaired as was OB expression of *Igf1*. The rate of apoptosis of OBs was accelerated. The homozygous loss



Classic  $\text{Ca}^{2+}$  homeostasis (dashed arrows) and novel developmental functions (arrows) of CaSR have been revealed by cell type-specific null mutations of *CaSR* in the mouse. CaSR is found in bone, kidney, and gut, which are the three main  $\text{Ca}^{2+}$ -mobilizing organs. The normal homeostatic signaling pathways between these organs and the parathyroid gland have been detailed previously.<sup>3</sup> The functions performed by CaSR in each organ are outlined in boxes. To maintain normal  $\text{Ca}^{2+}$  homeostasis, CaSR in parathyroid cells (PTCs) senses alterations in  $[\text{Ca}^{2+}]_e$ . The release of parathyroid hormone (PTH) enables bone and kidney to respond in a manner to normalize  $[\text{Ca}^{2+}]_e$ , through the activation of key responses in kidney [production of  $1,25(\text{OH})_2\text{D}_3$  and reabsorption of  $\text{Ca}^{2+}$ ], intestine [ $\text{Ca}^{2+}$  absorption through the increased abundance of  $1,25(\text{OH})_2\text{D}_3$ ], and bone matrix resorption through PTH (not shown). The direct role of CaSR in the intestine is questionable because an intestine-specific knockout of CaSR has not been performed. Targeted knockout of *CaSR* through the crossing of *CaSR* floxed mice with mice expressing *Cre* under the control of tissue-specific promoters has identified novel functions for CaSR in skeletal development. Ablation of *CaSR* in the parathyroid gland resulted in the expected phenotypes that occur in patients with inactivating mutations in *CaSR*, such as hyperparathyroidism and hypercalcemia. Deletion of CaSR in chondrocytes demonstrated a requirement for CaSR in early skeletal development, whereas a role for CaSR in promoting bone cell differentiation was determined by deletion of *CaSR* in cells of the osteoblast lineage.

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of expression of *Casr* in GPC was lethal; affected embryos died before day 13 of embryonic life. Development of a mouse model in which "knock-down" of *Casr* expression in GPC at day 16 to 17 of embryonic life after treatment with an estrogen receptor agonist (tamoxifen) resulted in offspring with modestly decreased growth of long bones despite expansion of the hypertrophic zone of the growth plate, decreased differentiation to terminal chondrocytes, and decreased expression of *Igf1* and *Igf1r* by GPC.

The authors concluded that: (1) the elimination of a functional CaSR in PTC also depressed *Casr* expression in osteoblasts (hypothetically through hypercalcemia and increased signaling by the PTH receptor in bone); (2) the CaSR was innately essential for osteoblast differentiation, function, and survival; and (3) that partial and delayed loss of the CaSR in hypertrophic chondrocytes reduced chondrocyte differentiation in part through decreased IGF-1R signaling.

Chang W, Tu C, Chen TH, Bikle D, Shoback D. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. *Sci Signal*. 2008;1:ra11. [DOI:10.1126/scisignal.1159945]

**Editor's Comment:** This research has demonstrated the individual importance of the CaSR in PTCs, OBs, and hypertrophic chondrocytes.<sup>3</sup> Interestingly, "knock-out" of

the CaSR in PTCs secondarily impaired expression of *Casr* in osteoblasts, demonstrating clearly the interdependence of the parathyroid-osteoblast axis. A study of the effect of overexpression of *Casr* in PTCs upon OB expression of *Casr* and bone morphology would be of interest. This manuscript also introduced a new feature of the electronic journal—*Science Signaling*—sponsored by the AAAS, that has until now published review articles and didactic materials on the subjects of intra- and intercellular communications.<sup>4</sup> It will now publish original research articles as well.

Allen W. Root, MD

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## Insulin Analogues...and Cancer?

Insulin analogues with different pharmacokinetics were created by inserting point modifications into the amino acid sequence of human insulin, particularly the C-terminus of its beta-chain which is not involved in binding to the insulin receptor (IR). However, these modifications may alter binding affinity for the closely related type 1 insulin-like growth factor (IGF) receptor (IGF1R). Thus, Weinstein et al asked the important question of how these analogues compare to insulin and IGF-I in eliciting IGF-I activities (namely, proliferation and protection from serum starvation-induced apoptosis) in cultured cancer cells.

They studied 2 long-acting insulin analogues (glargine [Lantus<sup>®</sup>] and detemir [Levemir<sup>®</sup>]) and 2 short-acting analogues (lispro [Humalog<sup>®</sup>] and aspart [Novolog<sup>®</sup>]) in 3 different cell lines: HCT-116 colorectal, PC-3 prostate and MCF-7 breast cancer cells. All experiments were conducted in vitro. Results are summarized in the Table.

HCT-116 cells showed a dose-dependent proliferative response to both glargine and detemir at 72 hours, but not IGF-I (all doses about +21%) nor insulin (all doses negligible effect). The authors then turned to signaling pathways that may underlie the hormonal effects in HCT-116 cells. Basal expression levels of IR and IGF1R were equivalent when measured by Western immunoblotting and immunofluorescent staining. After

Summary of effects on cell behavior in vitro.

| Effect  | Insulin | Glargine | Detemir | Lispro | Aspart | IGF-I |
|---|---------|----------|---------|--------|--------|-------|
| HCT-116 proliferation at 96 hrs (compared to untreated cells) | +0.4%   | +22%     | +17%    | -      | -      | +24%  |
| HCT-116 proliferation at 48 hrs (compared to untreated cells) | +7%     | -        | -       | +20%   | 0      | +22%  |
| PC-3 proliferation at 72 hrs (compared to untreated cells)    | +2%     | +17%     | +15%    | -      | -      | +25%  |
| MCF-7 proliferation at 72 hrs (compared to untreated cells)   | 0       | +14%     | +6%     | -      | -      | +22%  |
| HCT-116 % apoptotic cells at 12 hrs (control = 23%)           | 25%     | 15%      | 18%     | -      | -      | 17%   |
| HCT-116 % apoptotic cells at 24 hrs (control = 30%)           | 30%     | 26%      | 25%     | -      | -      | 24%   |

<sup>1</sup> by MMT assay; all other proliferation experiments were measured by cell counts. Apoptotic cells were quantified via flow cytometry of Annexin V-FITC and Propidium Iodide labeled cells.



10 and 20 minutes of treatment, glargine phosphorylated both IR and IGF1R, and detemir phosphorylated IR but not IGF1R. Glargine further led to increased phosphorylation of both Akt and ERK, representing the 2 major signaling cascades of IR and IGF1R, without changes in the total protein amounts; phosphorylation was maximal at 20 minutes and decreased by 60 minutes. In a test of relative potencies, cells were treated for 30 minutes with each hormone at 50 ng/mL. Glargine and insulin both significantly increased the amount of phosphorylated Akt in comparison to untreated cells, while detemir and IGF-I did not significantly alter Akt phosphorylation. Insulin alone significantly increased ERK phosphorylation.

The authors concluded that at the supra-physiologic doses tested, glargine and detemir have significant IGF-I-like mitogenic activity, which is not shared by insulin. The authors' warning bears repeating: current evidence shows that neither IGF-I nor insulin (and hence, one would expect the insulin analogues as well) can cause malignant transformation. However, IGF-I does increase the aggressivity of already transformed cells. Thus,

the question raised by this paper is whether long-term exposure to the insulin analogues can likewise affect cancer behavior.

Weinstein D, Simon M, Yehzekel E, Laron Z, Werner H. Insulin Analogues Display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. *Diabetes Metab Res Rev*. 2009; 25:41-49.

**Editor's Comment:** *It would take a colossal leap to answer the underlying question based on the data of this pilot study. However, the results are intriguing enough to suggest more rigorous investigations are warranted. The high prevalence of both cancer and diabetes in our society, plus the widespread long-term use of these modified insulin analogues, makes the question an important one to answer. If—and this is a big if—it pans out that one or more of the insulin analogues is more stimulatory for cancer behavior, then cancer risk will become yet another factor clinicians must consider in selecting the particular insulin regimen for an individual patient.*

Adda Grimberg, MD

## Continuous Glucose Monitoring and Intensive Treatment of Type 1 Diabetes

The Juvenile Diabetes Research Foundation (JDRF) Continuous Glucose Monitoring Study Group reported their findings of a multicenter clinical trial which randomly assigned 322 adults and children with type 1 diabetes to continuous glucose monitoring (CGM) or a control group which performed blood glucose monitoring with a glucose meter. All subjects were followed for 6 months to determine whether CGM helped to produce a sustained lowering of HbA1c and a reduction in hypoglycemia. The subjects were stratified by age: 8 to 14 years, 15 to 24 years, and over 25 years of age, and by HbA1c  $\leq 8\%$  and  $>8\%$ . Individuals with HbA1c of  $<7\%$  or  $>10\%$  were excluded. Subjects had to be using an insulin infusion pump, or at least 3 daily insulin injections, to control their diabetes and could not have had experience with CGM for the 6 months prior to the trial. The final study group included subjects who used either the Dexcom 7® (Dexcom™), the Mini-Med Paradigm® Real Time Insulin Pump and Continuous Glucose Monitoring System (Medtronic), or the FreeStyle Navigator® (Abbott Diabetes Care) according to the manufacturer's instructions which included specific calibration procedures and replacement of the sensors every 3 to 7 days.

Subjects were instructed to verify the accuracy of CGM determinations with self blood glucose meters before making treatment decisions. Subjects were also given written instructions on how to use the data generated by the CGM and blood glucose meters to make real-time adjustments in insulin doses. Target pre-meal blood glucose values were identical for the study group and the control group, 70 to 130 mg/dL (3.9

to 7.2 mmol/L); target peak post-prandial values were  $<180$  mg/dL (10 mmol/L), and bedtime overnight values 100 to 150 mg/dL (5.6 to 8.3 mmol/L). Subjects were seen at weeks 1, 4, 8, 13, 19 and 26 with one telephone contact between each visit to review glucose data and adjust diabetes management. After visits at 13 and 26 weeks, the control group used a blinded CGM for one week in order to compare continuous glucose profiles with the treated group. HbA1c was measured at 13 and 26 weeks and adverse events including severe hypoglycemia (defined as requiring assistance from another person and/or the use of glucagon), hyperglycemia with ketoacidosis, or other events were recorded.

The trial included 322 subjects (CGM group n=165; control group n=157); 114 patients were between 8 to 14 years of age (CGM group n=56, control group n=58), 100 subjects between 15 to 24 years of age (CGM group n=57, control group n=53) and 98 participants were over 25 years of age (CGM group n=52, control group n=46). A significant between group difference in the change in HbA1c from baseline to 26 weeks was seen in subjects who were 25 years of age or older, but not in those 15 to 24 years of age, or 8 to 14 years of age. In addition, in the CGM group over 25 years of age there were improvements in all measures of glycemic control including pre-meal and post peak-meal glucose values. The secondary analysis showed more patients in the CGM group had a reduction of 10% or more in mean HbA1c and more patients achieved their target HbA1c of  $<7.0\%$ . Among subjects 15 to 24 years of age, the mean decrease in HbA1c from baseline to

26 weeks was 0.2% in both groups and among those 8 to 14 years of age the mean decrease was 0.37%. There were no statistical differences in the reduction in HbA1c between the CGM group and the control group for both of these ages. There were no significant differences in the incidence of severe hypoglycemic events between the CGM groups according to age; however, severe events were infrequent in both groups. Sensor use was greater among subjects 25 years of age or older with 83% of the subjects using the sensor at least 6 days/week. In the group 15 to 24 years of age 30% used the sensor 6 days/week, and in those 8 to 14 years of age 50% used the sensor 6 days/week. Sensor use was not associated with baseline HbA1c.

The JDRF Continuous Monitoring Study Group concluded that the benefit associated with CGM with regard to improved glycemic control was strongly related to age. Individuals greater than 25 years of age clearly benefitted while those 15 to 24 years of age did not benefit. Those 8 to 14 years of age had greater benefit than those 15 to 24 of age years. The authors further commented that before generalizing these results it is important to remember that all of the subjects in this trial were receiving intensive insulin therapy and that most of them had better than average HbA1c. Of note, the results for subjects using multiple daily injections were similar to the results of those using an insulin pump. The researchers further concluded that CGM may improve HbA1c and enhance the management of type 1 diabetes in adults who have the motivation to use the technology and incorporate it into their daily management.

Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. *N Engl J Med*. 2008;359:1464-1476.

**Editor's Comment:** *Many pediatric endocrinologists have been waiting to see the data in this study regarding CGM. Many may see the results as disappointing, but maybe not surprising. Adults with strong motivation to use the CGM 6 days/week seem more likely to utilize the information to improve their glycemic control. Children 8 to 14 years of age—whose diabetes management is mostly directed by their parents—who also may have great motivation receive a greater benefit than adolescents 15 to 24 years of age, but not as much benefit as the adults. The results from the adolescents (who used CGM the least) is not surprising. CGM provides an incredible amount of real-time information regarding glycemia. For many people this information is overwhelming and of such a magnitude that organizing and responding to it is difficult. The JDRF study does not report any psychological, behavioral, or social information regarding the participants. Indeed such factors may have a great influence on subjects' ability to successfully manage their diabetes. It is hoped that such information was collected and that further reports of this data will include such information. Until such information is reported and correlated with the findings, the study remains incomplete.*

*Pediatric endocrinologists still do not know for whom CGM will provide the greatest benefit and how such information can best be used by their patients. CGM most likely will not be widely used by the majority of persons with type 1 diabetes; but for a subset of individuals the information from CGM may greatly improve their ability to reduce glycemic variability and their risk of long-term complications.*

William L. Clarke, MD

## Primary Thyroid Carcinoma in Childhood Cancer Survivors

With modern therapies and supportive care, the number of the childhood cancer survivors (CCS) has increased considerably. However, these patients suffer from the late-onset complications such as endocrine impairments, neuropsychological problems and second malignancies. These late-onset complications often do not become clinically apparent until decades after therapy. Since the likelihood of follow-up decreases with time, it is important for physicians as well as patients and family members to be aware of the late-onset complications over their lifetime.

Patients who received upper-body radiotherapy for childhood cancer have an increased risk of developing primary thyroid cancer later in life. Brignardello et al set forth the recommendations for monitoring the late-onset complications of thyroid carcinoma by thyroid ultrasound screening into young adulthood, and beyond, in CCS. They observed a very high occurrence of thyroid carcinoma as a second malignant neoplasm in a total of 129 CCS who were previously treated with radiotherapy

involving the head, neck, or upper thorax. The patients had had brain tumors, Hodgkin's disease, acute lymphoblastic leukemia and received preventive brain irradiation or total body irradiation for bone marrow transplantation. Thyroid ultrasound surveillance usually began 5 years after radiotherapy and was repeated every third year, if negative. Median follow-up time since the primary childhood cancer diagnosis was 15.8 years (range 6.1 to 34.8 years). Solid thyroid nodules were found in 35 patients included patients with palpable nodules (n=6) as well as those with solid nodules larger than 0.5 cm detected by thyroid ultrasound. Fourteen patients had nodules over 1 cm, 8 of which were not palpable. Fine-needle aspiration was performed in 19 patients, of which 14 had nodules over 1 cm. Cytological examination of specimens resulted in papillary carcinoma diagnosed in 5 patients and follicular carcinoma in 6 patients. In the remaining 8 patients, 7 had a diagnosis of nodular hyperplasia and one had lymphocytic thyroiditis. The

cytological diagnosis of papillary thyroid carcinoma was confirmed by histological examination in all 5 subjects who underwent surgery. Notably, only 2 of these patients had palpable nodules; the other 3 were smaller than 1 cm and were only detected by ultrasound. However, histological examination showed nodal metastases in 2 of them. In all 6 patients with follicular neoplasms who underwent surgery, the histological examination showed a benign lesion (goiter, n=3; follicular adenoma, n=3). Thyroid function was normal in 87 subjects, whereas 42 had primary hypothyroidism (n=37) or central hypothyroidism (n=5).

Brignardello E, Corrias A, Isolato G, et al. Ultrasound screening for thyroid carcinoma in childhood cancer survivors: A case series. *J Clin Endocrinol Metab.* 2008;93:4840-4843.

**Editor's Comment:** This is a very interesting article; it provides important information for physicians who care for CCS. Because survival rates of childhood cancer patients have improved markedly in recent years, the risk of developing a thyroid neoplasm clearly increases over many years after radiation therapy involving the head, neck, or upper thorax during childhood. Brignardello et al reported the prevalence of thyroid cancer, thyroid nodules and other thyroid alterations increased in the long-term follow-up of CCS.

There are 2 other papers on the subject worthy of discussion. In a 2003 retrospective study of all survivors of childhood and adolescent malignancies treated at Memorial Sloan-Kettering Cancer Center, Acharya et al<sup>1</sup> reported 33 patients who developed a clinically apparent thyroid neoplasm after therapeutic radiation. The median age at the time of diagnosis of the primary malignancy was 12.0 years (range, 3.7 to 18.3 years). The most common primary malignancy seen was Hodgkin's disease (n=18 patients), followed by non-Hodgkin's lymphoma (n=10 patients). The median interval from the time of radiation therapy until the recognition of thyroid disease was 13.0 years (range, 6.2 to 30.1 years). Thirteen of 33 thyroid lesions (39%) were malignant (11 papillary carcinomas and 2 follicular carcinomas). All thyroid abnormalities were detected on routine physical examination. Seventeen patients presented with a single nodule, 7 with multiple nodules, 5 with a multinodular goiter, 2 with lobar enlargement, 1 with a diffuse goiter, and 1 with an enlarged cervical lymph node and a normal thyroid gland. Thyroid ultrasound results were abnormal in 18 of 19 patients. Ultrasound revealed the presence of multiple nodules in 33% of patients, whereas only

15% of those patients had multiple nodules that were appreciated on physical examination.

In 2005, Sigurdson et al reported 72 cases with pathologically confirmed thyroid cancer from 14054 survivors (5 years or longer) of cancer during childhood from the Childhood Cancer Survivor Study cohort.<sup>2</sup> Childhood cancers were diagnosed between 1970 and 1986 with cohort follow-up to 2000. Of the 72 cases with secondary thyroid neoplasms, 56 (78%) were papillary, 11 (15%) follicular, and 5 (7%) of other or unspecified histology; 29 cases had a first diagnosis of Hodgkin's lymphoma and 14 had leukemia. They showed that the risk of subsequent primary thyroid cancer after a first tumor in childhood rose with increasing radiation dose (greatest risk 20–29 Gy), but decreased at doses of more than 30 Gy. Patients younger than 10 years at first cancer diagnosis had a higher risk of thyroid cancer than patients aged 10 years or older.

It is evident from these studies that thyroid nodules, even those greater than 1.5 cm, cannot always be palpated. In the study of Brignardello et al only 2 of the 5 patients with papillary thyroid carcinoma had palpable nodules. In the other 3 cases, the nodules were less than 1 cm, and were only detected by ultrasound. Therefore, the authors recommended monitoring the thyroid cancer by thyroid ultrasound screening in CCS previously treated by radiotherapy involving the head, neck, or upper thorax. Early detection of secondary thyroid cancers could improve the outcome of the patients. However, because thyroid ultrasound also detects many small lesions, the majority of which are benign, a very careful evaluation is needed to ascertain the results of thyroid ultrasound screening.

Brignardello et al emphasized the need for long-term follow-up for all CCS, which clearly must be extended well beyond childhood. Follow-up must

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*address transitional strategies to avoid dropout and improve the overall outcome of childhood cancer treatment and survivors. Also it is necessary for physicians, as well as patients and family members, to know that late-onset complications of a cancer survivor can occur even after many years following cancer treatment.<sup>3</sup>*

Yoshikazu Nishi, MD

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## Renal and Urinary Tract Anomalies in Congenital Hypothyroidism

Newborn screening for congenital hypothyroidism (CH) is one of the major achievements of preventive medicine, as the condition occurs frequently (1/3000–4000 newborns). An early diagnosis and treatment prevents brain damage and the ensuing mental retardation. It is well known that CH has increased incidence of congenital malformations of heart, gastrointestinal, and skeletal systems. However, the prevalence of congenital renal and urologic anomalies on CH has not been well established.

Kumar et al reported that children with CH have significantly increased risk of congenital renal and urological anomalies. They investigated the prevalence of congenital renal and urologic anomalies in children with CH as compared to children without CH. Analysis of Congenital Malformation Registry data showed 980 children with CH and 3,661,585 children without CH born in New York State (1992-2005). Children with CH had a significantly increased risk of congenital renal and urological anomalies with the odds ratio (OR) of 13.2 (10.6-16.5). The other significantly increased defects and prevalence rates in patients with CH were cardiac, gastrointestinal, and skeletal (Table). Analysis of matched data (CH data from New York State newborn screening; 1,538 children with CH and 3,654,033 children without CH) also confirmed an increase of congenital renal and urologic anomalies with an OR of 4.8 (3.7-6.3). There are limitations of their study; the Congenital Malformation Registry is compiled on the basis of hospital-generated data and is limited to children under 2 years of age. Therefore, there may be an underestimating of the true prevalence of congenital renal and urologic anomalies.

Hydronephrosis, UPJ obstruction, hypospadias, renal dysplasia, and renal agenesis were especially significant. Therefore, they suggested that CH children should be evaluated for the presence of congenital renal and urologic anomalies by a renal ultrasound examination.

Kumar J, Gordili R, Kaskel FJ, Druschel CM, Wordnicki RP. Increased prevalence of renal and urinary tract anomalies in children with congenital hypothyroidism. *J Pediatr*. 2009;154:263-266.

**Editor's Comment:** *This is a very interesting article; it provides important information for physicians who care for patients with CH and elucidates the high incidence of*

### Prevalence rates of congenital anomalies in hypothyroidism (CH) and in general population (non CH)

| Congenital anomalies       | CH<br>(RATE/10 000) | Non-CH<br>(RATE/10 000) |
|----------------------------|---------------------|-------------------------|
| <b>Renal</b>               |                     |                         |
| Dysplastic kidney          | 30.6                | 1.7                     |
| Renal agenesis             | 102.0               | 4.3                     |
| Ectopic kidney             | 30.6                | 1.7                     |
| Hydronephrosis             | 346.9               | 21.1                    |
| Hydroureter                | 20.4                | 1.5                     |
| UPJ obstruction            | 30.6                | 1.9                     |
| Reflux                     | 20.4                | 0.4                     |
| Hypospadias                | 275.5               | 39.6                    |
| Obstruction meatus         | 20.4                | 0.3                     |
| Posterior urethral valves  | 10.2                | 0.7                     |
| <b>Cardiovascular</b>      |                     |                         |
| Atrial septal defect       | 622.4               | 29.0                    |
| Ventricular septal defect  | 602.0               | 36.6                    |
| Coartation of aorta        | 81.6                | 4.1                     |
| Tetralogy of Fallot        | 183.7               | 4.6                     |
| Endocardial cushion defect | 275.5               | 3.1                     |
| <b>Gastrointestinal</b>    |                     |                         |
| Duodenal atresia/stenosis  | 51.0                | 1.6                     |
| Gastroschisis              | 10.2                | 1.4                     |
| Omphalocele                | 40.8                | 1.3                     |
| Oral clefts                | 91.3                | 12.9                    |
| Pyloric stenosis           | 40.8                | 17.1                    |
| Tracheoesophageal fistula  | 61.2                | 2.4                     |
| <b>Skeletal</b>            |                     |                         |
| Craniosynostosis           | 50.0                | 4.0                     |
| Congenital hip dysplasia   | 30.6                | 1.7                     |
| Limb reduction             | 40.8                | 3.3                     |

Modified from Kumar J, et al. *J Pediatr*. 2009;154:263-266.  
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congenital renal and urologic anomalies. Early detection of these anomalies may prevent or delay the risk of renal damage and developing end-stage kidney disease. The paper also provides data regarding the prevalence and odd risk ratios of cardiovascular, gastrointestinal, and skeletal anomalies in CH.

The causes of CH are: thyroid agenesis or hypoplasia, which accounts for 20% to 40% of the cases; ectopic thyroid, which accounts for 45% to 60%; and dysmorphogenesis, which accounts for the remaining 10% to 15% of cases. However, Kumar's observation did not discern the association differences of congenital renal and urologic anomalies among these types of CH; they reported that mutations in *PAX8*, *TITF1*, and *FOXE1*

genes have been associated with CH in patients with either isolated thyroid dysplasia or thyroid dysplasia with associated malformations involving kidney, lung, forebrain, and palate.

Hydronephrosis was the major defect in CH while hypospadias was most seen in the general population. The renal and urologic anomalies except hypospadias are not found on a routine physical examination, but can be easily detected by a renal ultrasound examination. Hypospadias can be easily diagnosed on a routine physical examination. Therefore, they recommended a routine renal ultrasound examination in CH.

Yoshikazu Nishi, MD

## Corticotropin-Releasing Hormone Testing in Assessment of Hypothalamic-Pituitary-Adrenal Axis Function in Infants with Congenital Central Hypothyroidism

The ACTH deficiency in neonates with multiple pituitary hormone deficiencies (MPHDs) results in sustained hypoglycemia and neuroglycopenia and is a major cause of morbidity and mortality. Under basal conditions, clinical signs of hypothalamus-pituitary-adrenocortex (HPA) axis dysfunction are usually absent and the HPA axis is probably the most difficult to assess in the neonate. For the assessment of HPA axis function in the neonate the corticotropin-releasing hormone (CRH) test (in which both the ACTH secretion by the pituitary gland and the subsequent cortisol secretion by the adrenal cortex can be evaluated) was considered as the most relevant choice. The overall aim of the study by van Tijn and colleagues was to develop a diagnostic workup for fast and reliable assessment of HPA axis function in neonates with congenital hypothyroidism of central origin (CH-C), detected by neonatal screening.

This was a Dutch nationwide prospective study (enrollment 1994–1996). Patients were included if neonatal CH screening results were indicative of CH-C and HPA axis function could be tested within 6 months of birth. Nine male and 3 female infants with CH-C and 4 infants with false-positive screening results or transient hypothyroidism were included in the study.

The assessment of HPA axis function was based on CRH and ACTH tests, multiple random plasma cortisol samples taken in the 24-hour period between thyrotropin-releasing hormone (TRH) and CRH tests, determination of cortisol excretion in 24-hour urine samples collected during this same interval, and long-term follow-up. For each patient the results of all endocrine examinations, including the other hypothalamic-pituitary axes, in combination with the results of cerebral MRI, added up to profiles on which overall diagnoses of HPA function were based. Diagnoses were reevaluated after 5 and 10 year follow-up (false positives, 3 to 5 year follow-up).

Of the 12 CH-C patients included in the overall

analysis, 3 showed diminished peak responses to CRH of both ACTH and cortisol (subjects 1–3). In addition, their highest measured random plasma cortisol concentrations and 24-hour urine cortisol excretions were below the predefined cutoffs. Another 4 infants (subjects 4–6 and 12) showed adequate ACTH peak response, but diminished cortisol peak response. This discordant response was considered abnormal. All 4 subjects with false-positive screening results included in the overall analysis were diagnosed as having sufficient HPA axis.

The CRH test proved to be a fast and reliable tool in the assessment of HPA axis dysfunction in asymptomatic neonates at risk for serious morbidity and mortality when congenital hypothyroidism had been detected. The discordant response type with normal ACTH, but low cortisol response, which has not been described before, may be an early phase of HPA axis dysfunction. A prolonged follow-up until the age of 10 years in some patients confirmed the neonatal diagnosis and the choice of early hydrocortisone replacement therapy.

van Tijn DA, de Vijlder JJ, Vulsma T. Role of corticotropin-releasing hormone testing in assessment of hypothalamic-pituitary-adrenal axis function in infants with congenital central hypothyroidism. *J Clin Endocrinol Metab*. 2008;93:3794–3803.

**Editor's Comment:** The cortisol peak response to CRH is the most valuable marker of HPA axis function. Ten years of follow-up have shown that it has the highest predictive value of all criteria evaluated in this study. In neonates with hypoglycemia and/or persistent jaundice, HPA deficiency can be suspected. However in the most cases there is no clinical indicator to avoid the high risk of death in early MPH deficiency. With the background provided by neonatal screening for hypothyroidism as suggested by the Dutch set-up<sup>1</sup> the CRH test appears to be the most valuable tool for early diagnosis of HPA axis dysfunction and for hydrocortisone treatment. As already known, hypothalamic-

*pituitary MRI would show in a large proportion of these cases; the most significant developmental abnormalities would be an ectopic posterior pituitary.*

Raphaël Rappaport, MD

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## Predictors of Relapse of Hyperthyroidism

There is debate about how Graves' disease (GD) should be treated in children. Remission is achieved in less than 30% of children treated with antithyroid drugs (ATD) vs 40% – 60% in adult patients. When relapse occurs, thyroidectomy or radioactive iodine treatment is considered, although the use of these therapeutic options in children remains controversial. Reliable predictors of relapse after ATD treatment would greatly improve patient management, by facilitating the identification of children requiring long-term ATD or needing early surgery or radioiodine therapy.

The aim of this study was to identify predictors of relapse after ATD treatment in children with GD. This was a prospective, multicenter cohort study of children (n=154) with GD treated with carbimazole for an intended duration of 24 ± 3 months. Most patients (n=147, 95%) completed 1 course of ATD. After the end of treatment, patients were followed up for at least 2 years. The primary outcome was hyperthyroidism relapse. Cox's regression analysis was used and a prognostic score was constructed.

Hyperthyroidism relapse was frequently observed after ATD treatment was stopped. The overall estimated relapse rate for hyperthyroidism was 59% (95% CI, 52% – 67%) at 1 year and 68% (95% CI, 60% – 76%) at 2 years after the end of ATD treatment. Median time to relapse was 8 months (95% CI, 5.4 to 11.4 months). In total, 87

of the 99 relapses occurred in the first year, principally in the first 6 months (n=64). Five variables were identified as independent predictors of relapse in a multivariate Cox model: age, serum free  $T_4$  and TRAb levels at the time of diagnosis and duration of ATD treatment. Non-Caucasian patients were found to be 2.5 times more likely to suffer a relapse than Caucasian patients. Relapse risk decreased with increasing age at onset (hazard ratio [HR] = 0.74 per 5 year increase in age,  $P = 0.03$ ) and duration of first course of ATD (HR = 0.57 per 12 months,  $P = 0.005$ ). A prognostic score was constructed, allowing the identification of 3 different risk groups, with 2-year relapse rates of 46%, 77%, and 98% (Table). Overall, marked differences in the observed and predicted relapse rates were found among the 3 identified risk groups. The patients in risk group A had a predicted 2-year relapse rate of 46%, whereas those in group C had relapse rates as high as 98% at 2 years after the end of ATD treatment.

In conclusion, this study, which is, to our knowledge, the largest prospective study in children with GD, provided strong evidence that there is an association between ethnicity, age, and disease severity at diagnosis and the risk of relapse 2 years after the end of the initial course of ATD treatment. Results suggested that the use of prolonged courses of ATD treatment is associated with a better outcome. Indeed, the duration of medical treatment seems to be the only variable related to risk of relapse that can be manipulated, as every additional year of treatment was associated with a decrease in relapse rate. The use of a predictive score, with treatment duration adjusted as a function of the patient's characteristics, to improve the prognosis could have important implications in daily practice and should be validated by application to another population of children with GD.

Kaguelidou F, Alberti C, Castanet M, Guittieny M-A, Czernichow P, Léger J for the French Childhood Graves' Disease Study Group. Predictors of autoimmune hyperthyroidism relapse in children after discontinuation of antithyroid drug treatment. *J Clin Endocrinol Metab.* 2008;93:3817-3826.

**First Editor's Comment:** Although radioiodine or surgery have been advocated as the first choice of therapy in children with autoimmune hyperthyroidism, ATD therapy remains the first choice in most clinics. Therefore, this prospective paper deserves much attention. The study was carefully managed and most of its methodological limitations were taken into account. Because it is everyone's experience that the outcome is rather unpredictable, these data with a practical scoring may turn out to be quite

**Prognostic score for relapse in children with GD<sup>1</sup>**

| Weight  | 0          | 1          | 2             | 3          |
|---|------------|------------|---------------|------------|
| Ethnicity   | Caucasian  |            | Non-Caucasian |            |
| Age   | >12 years  | 1-12 years | <5 years      |            |
| Free $T_4$ serum concentration                        | <50 pmol/L |            |               | ≥50 pmol/L |
| Multiple of upper normal limit for TRAb concentration | ≤x4(N)2    | >x4(N)2    |               |            |
| Duration of ATD treatment                             | >24 months |            |               | ≤24 months |

For each patient, score may range from 0 to 11.

<sup>1</sup> The prognostic score was calculated from the data of 138 of 147 patients because of missing data (n=9).

Reprinted with permission Kaguelidou F, et al. *J Clin Endocrinol Metab.* 2008;93:3817-3826. Copyright © The Endocrine Society 2008. All rights reserved.

useful in the management of individual cases and with the difficult task of maintaining compliance. It also made it possible to identify a small group of children at a very high risk of relapse, essentially young (<5 years of age) non-Caucasian children with severe initial hyperthyroidism. In conclusion, a longer initial duration of a euthyroid state with ATD treatment is the most significant prognostic variable. However, the optimal duration remains to be evaluated in further studies.

Raphaël Rappaport, MD

**Second Editor's Comment:** Hyperthyroidism is believed to result from a complex interaction between the autoimmune system, environmental factors, and genetic background; it is mainly due to Graves' disease and is less frequently seen in children than in adults. ATD treatment is the initial form of therapy for all hyperthyroid children in an attempt to normalize thyroid function tests. Whether this form of treatment is continued long-term or whether other therapeutic options, such as surgery or radioactive iodine treatment, are considered is often dependant on the rate of relapse after ATD treatment. Reliable predictors of relapse after ATD treatment would facilitate the management of these children by allowing for the identification of those requiring long-term ATD, or alternatively thyroidectomy or radioiodine therapy.<sup>1</sup> In this study, Kaguelidou et al were able to find the 5 variables most predictive of relapse following ATD. At diagnosis the key factors to consider when evaluating the risk of relapse of a patient are: ethnicity (higher risk for children of Non-Caucasian origin), age (the younger the patients the higher the risk for relapse), severity of the disease as manifested by elevated serum free  $T_4$  and TRAb levels (the higher these concentrations, the higher the risk of relapse) and duration of the disease. It is interesting to note that children receiving longer ATD treatment were less likely to relapse, with a 43% decrease in relapse risk for each additional 12 months of treatment. While it is clear that there is no ideal form of therapy for this disease as the 3 available therapeutical options (ATD, thyroidectomy, and radioactive iodine) are associated with potential complications, drug therapy remains the first line of treatment in many countries. The remission rate after 2 years of ATD treatment (about 30%) observed in this study is in agreement with a 1987 report.<sup>2</sup> This study also demonstrated that the remission rate increases significantly in children and adolescents with every additional year of

treatment. The need for prescribing longer treatment courses in children than in adults is now widely accepted and the duration of medical treatment seems to be the only variable, independent of ethnicity, age and severity of disease, that can be manipulated.

Roberto Lanes, MD

**Third Editor's Comment:** The value of predictors to determine the relapse risk of patients with hyperthyroidism following ATD therapy has long been studied in children and adults. The most controversial factor is the serum TRAb level which may not be sufficiently sensitive to predict a relapse after ATD treatment<sup>3</sup> even though others have considered them useful in children.<sup>4</sup> TRAb data are often lacking at diagnosis and/or during follow-up of these patients with Graves' disease, as was the case in this study of Kaguelidou et al. However, the long-term results of ATD treatment remain generally unsatisfactory in most studies. Poor compliance with medical therapy is often the most important factor that determines the therapeutic outcome, particularly in adolescents. Yet long-term treatment seems to be the only variable that is at the clinician's control to reduce the risk of relapse of the disease. Thus, important arguments have been put forward for considering <sup>131</sup>Iodine therapy or surgical ablation in the treatment of children with hyperthyroidism.<sup>5,6</sup>

Fima Lifshitz, MD

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## Treatment Guidelines for Children with Disorders of Sex Development

Disorders of sex development (DSD) is the umbrella term replacing intersexuality to cover congenital conditions characterized by atypical chromosomal, gonadal, or anatomic sex.<sup>1</sup> This article was published in a special issue of a journal focusing on gender identity disorders (GID). However, Meyer-Bahlburg

sees sufficient differences between gender-variant persons, with and without a DSD, to urge distinct evaluation and treatment approaches.

GID is characterized by discomfort or distress with one's apparent or assigned gender accompanied by a persistent identification with the opposite sex. In



contrast, gender issues may be far less salient for those with DSD. The challenges related to having a chronic (for some, a life-threatening) medical condition, and its associated management, require that the behavioral health professional be competent in the application of psychosocial interventions for problems of medical adherence and in coping with the stigma often associated with congenital or chronic conditions, in general.

Whereas the evaluation and psychosocial treatment of persons with GID can be conducted by a mental health provider alone, the 2005 consensus statement on DSD<sup>1</sup> calls for care to be provided in the context of a multidisciplinary team, including: neonatology, pediatric endocrinology, pediatric urology, gynecology, genetics, genetic counseling, mental health specialists, social work, nursing, and medical ethics. Moreover, communication between the DSD team and the "medical home" (ie, the primary care physician) is strongly recommended. Because of the high stress at the time of ascertainment of the DSD, there is a critical need for good communication among team members and the family to facilitate shared decision making regarding gender assignment and surgical options. Information shared with the family must include the most up-to-date information regarding the patient's specific syndrome, including the range of prognostic outcomes for both physical health and psychological health across the lifespan.

Meyer-Bahlburg describes in considerable detail the complexities of the process of gender assignment, and potential reassignment, for those diagnosed at birth and the long-term risk for the child and family associated with missteps in clinical management. Providing integrated interdisciplinary team care for patients is complex and time-consuming for patients, in general. In the case of DSD, Meyer-Bahlburg notes the importance of sustaining the team approach beyond the period of initial diagnosis and early interventions. Optimal care also requires active outreach by the behavioral health provider of the team to adopt a preventive approach regarding problems with psychosocial adaptation and medical adherence. Children and their families may require assistance in interpreting gender-atypical behavior – a not infrequent occurrence in DSD – as understandable based on what is known of the biology of DSD, rather than as a sign of incorrect gender assignment.

Young children with a DSD, who were misdiagnosed or late diagnosed, create special challenges. Parents may require reassurance and counseling if gender atypical behavior is part of the presentation. Gender reassignment after infancy requires careful psychological evaluation over a prolonged period with particular attention to the child's gender-role behavior and to any symptoms of gender dysphoria.

There is no controversy over performing genital surgery for acute medical reasons. However, medical urgency is the exception rather than the rule. Instead, genital surgery has been performed, typically early in life, to "confirm

the assigned gender by genital appearance." At present, there is no broad consensus regarding the issue of early genital surgery, with the exception of the milder cases of atypical genital development for which deferring surgery is now recommended.<sup>1</sup> Meyer-Bahlburg describes the preparation required for parents and, later, patients regarding genital surgery decisions. The psychological risks for the patient associated with repeated genital examinations, in part to support the training of medical students and residents, requires a rethinking of medical educational models and practices.

This article provides additional guidance regarding androgen treatment in 46,XY children with underdeveloped genitalia and the timing of sex hormone treatment in persons without gonads or with under-functioning gonads. In general, the timing of hormone replacement is best initiated during the period when peers are experiencing endogenous puberty.

The topic of disclosure of medical information to the patient is a crucial component of psychosocial management. Although legal standards generally support the rights of parents to determine what and when details of their child's medical condition is disclosed to them, the majority of clinicians agree that a patient with a DSD be fully informed of all details. Some parents will resist disclosing information to their child or adolescent, in particular in cases in which the assigned gender is at odds with sex chromosomes or gonadal structure. Parents can be reassured by the experience of patients with other medical conditions (eg, pediatric cancer or HIV) that disclosure is associated with enhanced psychosocial adaptation. Meyer-Bahlburg reviews strategies for the disclosure process, including providing a web link to a source for animated visual aids.

Finally, the beneficial role of support groups for persons with medical conditions, in particular for those with rare conditions, is emphasized. Health care providers are encouraged to seek opportunities to dialogue with such groups to ensure that the information disseminated is accurate.

Meyer-Bahlburg HFL. Treatment guidelines for children with disorders of sex development. *Neuropsychiatrie de l'Enfance et de l'Adolescence* 2008; 56:345-349.

**Editor's Comment:** Important distinctions between persons with GID and DSD are often blurred in both the popular and scientific literature. While the entities may share some features (eg, gender concerns), DSD diverge from GID in terms of associated features including prevalence, age of onset, and sex ratio.<sup>2</sup> Failure to differentiate between persons with and without a clearly identifiable DSD may hamper studies of etiology and optimal clinical management.

The recently published consensus on the management of DSD<sup>1</sup> is very clear on the necessity of applying an integrated interdisciplinary team approach to the care of those affected and their families. What



*is not discussed are the barriers that exist to forming and sustaining such teams. The non-reimbursable time required to organize a team is substantial and the essential behavioral health component of service is frequently excluded because clinical services provided by mental health providers are often carved-out by many health insurance plans and require the patient to be seen by an approved insurance panel member who would not be a member of the DSD team. Co-pays for mental health services are characteristically substantially higher than for medical services, forcing families to reject recommended services delivered by the behavioral health member of the team. At present, there is a scarcity of behavioral health experts qualified to immediately join emerging DSD teams. However, the skills set of pediatric psychology makes this subspecialty of child clinical psychology ideally suited to serve as team members at centers of excellence called for in the DSD consensus statement.<sup>1</sup>*

*An evidence-based consensus on best clinical practices regarding gender assignment and genital surgery is only beginning to emerge; in the meantime*

*there is critical need for systematic investigation to understand how parents are counseled and select among treatment options. Clinicians and representatives of patient advocacy organizations voice concerns about the extent and quality of information disclosed to parents during the decision-making process and, importantly, the subsequent validity of parental consent. These factors make this an excellent clinical context in which to study parental medical decision-making.*

David E. Sandberg, PhD

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## GROWTH

### Hepatoblastoma Concerns and Growth Hormone Therapy in Small for Gestational Age Children

YOSHIKAZU NISHI, MD

#### INTRODUCTION

Approximately 5% of children are born small for gestational age (SGA).<sup>1</sup> Most of the SGA children present catch-up growth during their first year with completion of the growth recovery by two years of age.<sup>2</sup> After the initial catch-up, most of the height gain is maintained up to adult height. However, children born SGA usually are shorter during childhood and attain adult heights that on average are approximately 1 SD lower than the mean.<sup>2</sup> Approximately 10% of SGA infants do not experience spontaneous catch-up growth and remain short throughout childhood and adolescence and into adulthood.<sup>1,2</sup> These short adults born SGA comprise up to 20% of the total population of short-statured adults.<sup>3</sup>

Growth hormone (GH) therapy for short children born SGA has been explored for nearly 40 years. Many international studies have shown that most of these children benefit from GH therapy by normalizing height during childhood, maintaining a normal growth velocity during the prepubertal years and through puberty, and attaining an improved adult height. In 2001 GH was approved, by the US Food and Drug Administration (FDA) and in 2003 by the European Agency for Evaluation of Medicinal Products (EMA), for the treatment of short children born SGA who fail to manifest catch-up growth with a height  $<-2.0$  SD by 2 years (FDA) or  $<-2.5$  SD by 4 years (EMA).<sup>2</sup> In 2008, GH treatment was also approved in Japan for short SGA children who fail to manifest catch-up growth with a height  $<-2.5$  SD

#### From The Editor's Desk

The US economy continues to struggle and with it the support for independent educational journals such as GGH. Although this journal has not been published quarterly on a regular basis due to lack of financial support, our readership accessed the GGH website with impressive regularity. Thus, an average of 636 distinct viewers accessed articles from the journal every single day this year. This journal's website serves the needs of those who use it as a resource for their educational activities and reference sources. This continued interest of the subscribers to the journal motivated our editorial board to go forward with the publication of this issue.

A new format was instituted; the editorial board prepared each of their articles from high impact papers published in the literature since last year. Additionally I have extracted papers presented at the ENDO 2010 (The Endocrine Society annual meeting, San Diego, California, USA, June 19-22, 2010) considered to be of interest to GGH readers and not otherwise presented/discussed in other pediatric venues. Also I have added pertinent comments to all the articles.

The publication of this issue marks the 26th anniversary since the inception of GGH; a word of thanks to the editorial board for their voluntary participation. Also, an acknowledgment of the 11,000 subscribers of GGH is in order. Your interest in the journal makes our efforts worthwhile and has kept us going in the hope that when the economic slump can be cured we will be able to elicit appropriate financial support to bring to the readership continuous, unbiased, and regular issues of the journal.

Sincerely,  
Fima Lifshitz, MD

by 3 years. The FDA, EMEA and Japan approved GH doses for SGA treatment (70 µg/kg/day, 35 µg/kg/day and 33–67 µg/kg/day, respectively) are high because of the presumed GH resistance contributing to the lack of catch-up growth in the SGA population and the results of heightened efficacy at high doses.<sup>2</sup> These doses are up to three times greater than the standard replacement doses used to treat children with GH deficiency; furthermore, higher doses are used in children with marked growth retardation.

### GH Therapy for Short SGA Children

The goal of GH therapy in short SGA children is to normalize adult height. To evaluate the impact of GH therapy on adult height in short SGA children, a meta-analysis of randomized controlled trials (RCTs) was performed by Maiorana and Cianfrani.<sup>4</sup> A systematic review of controlled studies was made using as data source the Cochrane Central Register of Controlled Trials, Medline, and the bibliographic references from all retrieved articles describing RCTs up to November 2008. The adult height of the GH-treated group significantly exceeded controls by 0.9 SD. Mean height gain was 1.5 SDS in GH-treated versus 0.25 SD in untreated SGA subjects. No significant difference in adult height was observed between the two GH dose regimens (33 and 67 µg/kg/day). It was concluded that GH therapy seems to be an effective approach to partially reduce the adult height deficit in short SGA children.

The response to GH therapy is highly variable, and therefore additional studies are needed to identify responders versus non-responders. Maiorana and Cianfrani<sup>4</sup> reported that practitioners and policy makers need to address the clinical importance and value of the height gained, including the impact of the height gained on physical and psychosocial well-being, safety and adverse effects, cost of therapy, and patients' expectations.

### Adverse Events in GH treated SGA Children

Simon et al<sup>5</sup> reported that clinical trials and a large post-marketing survey have shown that GH treatment is well tolerated in SGA children. However, two particular issues need to be addressed pertaining to this population of SGA patients: the potential risk of malignancy due to high-dose GH treatment and the effects of GH on glucose metabolism. There is a large body of evidence that suggests that low birth weight (LBW) and very low birth weight (VLBW) are associated with a wide range of metabolic and physiological disorders in later life.<sup>2</sup> It is currently unknown whether GH therapy – with higher doses used for SGA children throughout childhood and adolescence – may be associated with an amplification of risk for metabolic consequences such as glucose metabolism, insulin resistance, metabolic syndrome, coronary heart disease and stroke in adulthood.

GH is a known mitogenic agent and insulin-like growth factor (IGF)-I has antiapoptotic effects; therefore, researchers have expressed concern about the oncogenic potential of GH therapy.<sup>6</sup> It is also known that serum IGF-I levels become high among those receiving the high-dose GH; a dose-dependent increase in the IGF-I level has been observed. High IGF-I levels over a prolonged period of time may increase the risk for malignancies; thus, it is currently recommended that IGF-I levels be monitored closely to maintain them within the normal range during GH treatment in SGA children.<sup>5</sup>

The consensus statement of international societies for the management of children born SGA have not reported that LBW has been shown to be associated with increased risk of cancer in general, with the possible exceptions of testicular, and to a lesser extent renal, cancer.<sup>2</sup> In contrast, there is good evidence that high birth weight is associated with an increased risk of cancer, best documented for breast cancer.<sup>2</sup> To date, no reports (including consensus statements of international societies for management of the child born SGA) have addressed the potential relationship of development of hepatoblastomas (HB) during or after GH therapy. A significantly higher rate of HB has been observed among LBW (<2500 g) and VLBW (<1500 g) children.<sup>7-10</sup>

### Hepatoblastoma in Children with LBW

Hepatoblastoma is the most common liver cancer in children, occurring most frequently in premature infants, particularly those with LBW or VLBW, aged less than 5 years, especially less than 3 years.<sup>7-10</sup> In SGA children treated with GH the occurrence of HB was mostly considered coincidental.<sup>11</sup> Because data on VLBW and other childhood cancers are sparse, Spector et al<sup>7</sup> examined the risk of malignancy with VLBW in a large data set. They combined case-control data sets created by linking the cancer and birth registries of California, Minnesota, New York, Texas, and Washington states, which included 17,672 children diagnosed as having cancer at 0 to 14 years of age and 57,966 randomly selected control subjects. They found that most childhood cancers were not associated with LBWs. However, a birth weight of 350–1499 g was associated with a considerably high risk of HB (odds ratio [OR]: 17.18 and 95% confidence interval [CI]: 7.46–39.54), relative to a weighing ≥2500 g at birth.<sup>7</sup>

Reynolds et al<sup>8</sup> also reported that using California's statewide registry (the California Cancer Registry), a striking elevated risk of HB was found in children from birth to 4 years of age who were born VLBW (OR: 50.57; 95% CI: 6.59–387.97). An analysis of Japanese cancer registry data from 1969–1994 also revealed an increasing trend in HB incidence among children of VLBW.<sup>9</sup> The relative risk of HB among children with birth weights of <1000 to 2499 g compared with children with birth weight ≥2500 g is listed in the Table.<sup>9</sup>



**Table. Relative risk of hepatoblastoma in LBW and VLBW children compared to those >2500 g**

| Birth weight        | <1000 g | 1000-1499 g | 1500-1999 g | 2000-2499 g |
|---------------------|---------|-------------|-------------|-------------|
| Relative risk of HB | 15.64   | 2.53        | 2.71        | 1.21        |
| P                   | <0.001  | =0.129      | =0.001      | =0.381      |

Spector et al<sup>7</sup> also reported that retinoblastoma and glioma (other than astrocytomas and ependymomas) were possibly associated with VLBW. Additionally, VLBW was associated with more than a twofold increased OR for gliomas (birth weight <1500 g, OR: 2.13 [95% CI: 0.71-6.39]; birth weight 1500-1999 g, OR: 3.58 [95% CI: 1.98-6.47]) and retinoblastomas (birth weight <1500 g, OR: 2.43 [95% CI: 1.00-5.89]). There was a significant OR of 1.42 (95% CI: 1.01-1.99) for intracranial embryonal cell tumors associated with birth weights of 2000-2499 g.<sup>7</sup>

### Causes of Hepatoblastoma in LBW Children

The causes of HB development in LBW or VLBW children are not fully understood. Infants born with LBW or VLBW may undergo multiple medical interventions in the NICUs at a time in development when antioxidant capacity is decreased and xenobiotic metabolizing enzyme expression is variable; thus iatrogenic causes of HB, such as prolonged oxygen therapy and furosemide use, are plausible.<sup>7-10</sup> The presence of erythropoietin receptors in HB has been also postulated to potentially contribute to this increased incidence of HB because many premature infants with LBW or VLBW receive erythropoietin during their time in the NICU.<sup>7-10</sup>

Latini et al<sup>12</sup> also proposed that perinatal phthalate exposure may play a role in increasing the risk of HB among children with VLBW. Di(2-ethylhexyl)phthalate (DEHP) is the most commonly used plasticizer in polyvinylchloride (PVC) medical devices. In 2001, the Center for Devices and Radiological Health of the FDA reported that neonates in the NICU constitute a population at a particularly increased risk of toxicity because of multiple medical device-related DEHP exposure. In addition, it is well known that in animal models the liver is the most responsive target of the adverse effects of DEHP and that DEHP is a rodent hepatocarcinogen. As a consequence, prenatal and postnatal exposures to potential carcinogens may have synergistic and cumulative actions in producing adverse neonatal effects, especially for VLBW infants.

The GH-IGF-I axis may also be partially involved in HB development. Gray et al<sup>13</sup> reported that the IGF-I axis plays an important role in many diverse cellular functions including promotion of cell growth and cell survival. The main producer of circulating IGF-I and IGF-II is the liver, and the ability of these peptides to mediate mitogenic, anti-apoptotic and differentiation signals is likely to be primarily via the IGF-I receptor. Using RNAase protein analysis (RPA), Gray et al<sup>13</sup> examined the gene expression for *IGF-1* and *IGF-2*, their receptors (*IGF-1R* and *M6P/IGF-2R*), and two IGF binding

proteins (*IGFBP*); *IGFBP-1* and *IGFBP-2*) in a series of HBs with corresponding normal liver from the same individuals. The results showed that the expression of many of the IGF-axis genes altered between tumor and normal, and indicated that the IGF-axis may be involved in HB development. Gray and

colleagues concluded that the IGF-axis is affected in HB. While there are no definitive explanations on the role of IGF-axis, these alterations may play in the tumorigenesis process. One potential result of these alterations may be local concentrations of IGFs, in combination with reduced levels of IGFBPs, promote clonal expansion of the tumor cells. Further studies are indicated in order to determine the exact importance of the IGF-axis in HB.

### Conclusions

Perinatal medicine has rapidly progressed and its sophisticated services have become standard; the survival of infants with LBW and VLBW has increased in recent decades. Treatment with GH for short children born SGA is also increasing – thereby escalating the risk of developing adverse events during and after GH therapy. Although HB occurs most frequently in infants or very young children before 3 years of age, and the usual start of GH therapy is after 2 years, the occurrence of HB has been mostly considered coincidental. However, an early start of GH therapy in short children born SGA has been encouraged<sup>14</sup>; this may increase the potential risks of developing complications – such as HB – for which these patients may be more susceptible than other types of patients being treated with GH.

The precise diagnosis of malignancies in GH-treated children born SGA has not always been reported; some papers may not classify the malignancies precisely, ie other tumors, and perhaps some patients who developed HB were not necessarily included in those reports. Therefore the prevalence of HB in GH-treated SGA patients is not really known.

Diagnosing HB before clinical signs and symptoms develop is important. HB is usually diagnosed as an asymptomatic abdominal mass. Therefore pediatric endocrinologists who follow short SGA children who are being treated with GH should monitor them carefully and repeatedly. Serum  $\alpha$ -fetoprotein measurements and if possible, abdominal sonography, should be performed before and during GH therapy to assess for HB. Although the occurrence of malignancy is currently considered coincidental, the families of these children should be informed of the possible occurrence of HB. Furthermore, IGF-I levels should be monitored closely to maintain them within the normal range during GH treatment in SGA children.

**Editor's Comment:** The longest post-marketing GH surveillance study has been ongoing in the US for over 25 years – the Genentech National Cooperative Growth



*Study (NCGS). Recently, Bell et al reported more than 20 years of data covering approximately 55,000 patients treated with GH.<sup>15</sup> This is a very valuable analysis of the experience gathered about the use of this drug; the data are reassuring regarding the safety and efficacy of GH.<sup>16</sup> A review of the data by Roberto Lanes was summarized in this issue of GGH. However, the NCGS, as well as other similar studies performed by other companies (eg, KIGS, Pfizer International Growth Database), are not scientific-controlled studies. These post-marketing reporting groups rely on the voluntary reporting by physicians, thus the potential spectrum of potential adverse events may not be comprehensively assessed. The above paper by Yoshikazu Nishi clearly points out this potential weakness; it alerts us to the risk of hepatoblastomas in LBW children who may be treated with GH. The pediatric endocrine community has not hitherto considered this potential risk.*

Fima Lifshitz, MD

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## Practice Guidelines for Pituitary Incidentalomas

*From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010*

Clinical practice guidelines were highlighted at the Endocrine Society meeting and new evidence-based recommendations on the evaluation and treatment of pituitary incidentalomas were presented by Pamela Freda (chair of the task force that developed the guidelines). A pituitary incidentaloma is an incidentally discovered pituitary lesion. The true nature of pituitary incidentaloma usually remains unknown as most do not result in surgery. In the limited surgical cases available, pituitary adenomas are the most common etiology.

The guidelines recommend the initial evaluation of a patient with a pituitary incidentaloma to include laboratory screening for hormone hypersecretion in all incidentaloma patients, including those with and without symptoms. The task force debated the pros and cons of detailed versus limited screening for hormone hypersecretion ie, stimulation tests versus insulin-like growth factor (IGF)-I and midnight salivary cortisol levels. There was agreement on screening for prolactin, which should be measured in all incidentalomas.

The practice guideline also recommends initial routine screening for hypopituitarism in patients with macro-

incidentalomas, both with and without symptoms, but not in all patients with micro-incidentalomas because the incidence of hypopituitarism is not high in these patients. However, the task force suggested screening might be done for patients with large micro-incidentalomas – in the range of 8 to 9 mm. Follow-up testing of pituitary function for hypopituitarism is indicated for patients with macro-incidentalomas 6 months after initial testing and then yearly. However, routine repeated functional testing is not required for patients with micro-incidentalomas when the patient's clinical picture, history and MRIs do not change over time.

Non-surgical follow-up is recommended with clinical assessments and functional testing for patients who do not meet criteria for surgical removal of the pituitary incidentaloma. As for follow-up imaging of non-surgically treated incidentalomas, the guideline recommends MRI of the pituitary in patients with macro-incidentalomas 6 months after the initial scan, and for patients with micro-incidentalomas, one year after the initial assessment.

Finally, the guidelines recommend that patients with pituitary incidentalomas be referred for surgery; if they

have a visual field deficit or signs of compression by the lesion leading to other visual abnormalities; if the lesion abuts the optic nerve or optic chiasm on MRI; if they have pituitary apoplexy with visual disturbance; or if they are found to have a hypersecreting tumor other than a prolactinoma. Other indications for surgery suggested in the guidelines include clinically significant growth of the incidentaloma, loss of endocrine function and unremitting headache, or a lesion that is close to the optic chiasm when the patient is planning pregnancy.

Pamela Freda, MD, Chair of Task Force, Columbia University College of Physicians and Surgeons, New York, USA

**Editor's Comment:** *The prevalence of pituitary incidentalomas will likely increase as there is more frequent use of brain scanning for multiple purposes. CT scanners first began to be installed in 1974 in clinical settings. Currently over 6000 scanners are in use in the US. The CT has become a commonly performed procedure. Scanners are found not only in hospital radiology departments, but also in outpatient offices. Usage of CT has increased dramatically over the last two decades.<sup>1</sup> An estimated 72 million scans<sup>2</sup> were performed in the US in 2007 – it is likely a higher*

*number today. In Calgary Canada 12.1% of people who present to the emergency with an urgent complaint received a CT scan, most commonly either of the head or the abdomen. The percentage of patients who received CT scans, however varied markedly (1.8% to 25%), depending on the emergency physician who saw the patient.<sup>3</sup> Thus, the practice guidelines are very timely. In children careful assessment of growth progression and sexual development should be evaluated, both at the time the incidentaloma is detected and closely monitored thereafter. Patients with pituitary incidentalomas may find it difficult to accept a wait and see plan without neurosurgical and ophthalmological input; these guidelines should help assure the patient and family.*

Fima Lifshitz, MD

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## Inhibitory Role of IGFBP-3 in the Pathogenesis of Asthma

*From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010*

Insulin-like growth factor-binding protein (IGFBP)-3 is a multi-functional protein known for modulating the actions of insulin-like growth factors (IGFs) in somatic growth and a variety of human diseases such as cancer. Despite the critical role of the IGF system in the pathophysiology of many diseases, limited information is available for its role in bronchial asthma. IGFBP-3 fragments have been identified in asthmatic airway tissue extracts. Whether there is any association between IGFBP-3 and asthma remains elusive.

The researchers performed in vitro and in vivo studies to show that IGFBP-3 blocks specific physiological consequences of asthma in an IGF-independent manner. They used a mouse asthma model with normal mice as well as IGFBP-3 transgenic mice challenged to ovalbumin (OVA). The results show IGFBP-3 suppressed in bronchial epithelial cells from normal mice after OVA challenge. Restoration of IGFBP-3 either by recombinant IGFBP-3 treatment or adenoviral IGFBP-3 gene transfer effectively reduced all physiological manifestations of asthma examined in vivo (airway hyperresponsiveness [AHR], cellular and pathological change in bronchoalveolar lavage [BAL] fluid and lung tissue, and expression of numerous proinflammatory molecules). Furthermore, IGFBP-3 treatment restored airway functions as demonstrated by the reduction of OVA-induced AHR. These unique IGFBP-3 effects were IGF/IGF-I receptor

(IGF-IR) independent since IGFBP-3 mutant devoid of IGF binding affinity (IGFBP-3 GGG) had similar effects. The studies using IGFBP-3 transgenic mice further confirmed the effects of IGFBP-3 by demonstrating significant reduction of infiltration of inflammatory cells, cytokine production and OVA-induced AHR compared to that of normal mice after OVA inhalation.

Further in vitro studies using human bronchial epithelial cells demonstrated that IGFBP-3 blocks TNF- $\alpha$ -induced expression of proinflammatory molecules, attenuates the TNF- $\alpha$ -induced migratory response of eosinophils, and negatively regulates TNF- $\alpha$ -induced expression of the key NF- $\kappa$ B regulatory molecules, I $\kappa$ B $\alpha$  and p65-NF- $\kappa$ B, at the post-translational level. Taken together, these results strongly indicated that IGFBP-3 inhibits airway inflammation and airway hyperresponsiveness via an IGF-independent mechanism that involves cross-talk with NF- $\kappa$ B pathway. IGFBP-3 therefore plays a pivotal role in the pathogenesis of asthma, and thus can serve as a potential therapeutic for prevention/treatment of asthma.

Lee Y, Jogle-Brahim S, Harada A, et al. Chonbuk National University, Jeonju, Republic of Korea; University of Manitoba Winnipeg, Canada; and Virginia Commonwealth University, Richmond, Virginia, USA

**Editor's Comment:** *This is a new and interesting view into the pathogenesis of this common disease. IGF-I is*

known to be involved in airway remodeling in bronchial epithelial cells; interleukin (IL)-17F is able to induce the expression of IGF-I via the Raf1-MEK1/2-ERK1/2-MSK1/p90RSK-CREB pathway *in vitro*.<sup>1</sup> Another mechanism of allergic airway remodeling may also be via the secretion of the profibrotic IGFBP-3 from IGF-I-stimulated airway epithelial cells during allergic inflammation.<sup>2</sup> Of interest may be the potential role of the IGF system alterations in allergic disease and asthma in growth retardation. The prevalence of short stature (< 3rd percentile NCHS) among children with respiratory allergy (asthma and/or rhinitis) varies from 2–10%. Hauache et al<sup>3</sup> studied IGF-I, IGFBP-3, and growth hormone (GH) serum levels after stimulation tests in prepubertal allergic boys who had not received steroids. All children were short and had delayed skeletal age in relation to chronological

age, but bone age was normal for height. The serum levels of IGF-I, IGFBP-3, and GH after stimulation tests were normal and they concluded that in these children a deficiency of GH did not seem to be responsible for short stature.

Fima Lifshitz, MD

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## Long-term Growth Hormone Use: Safety Profile and Adverse Events

Roberto Lanes, MD

Bell and colleagues recently reported on the safety profile and adverse events detected with the use of recombinant human growth hormone (rhGH) during 20 years of post-marketing surveillance by the National Cooperative Growth Study (NCGS) of Genentech.<sup>1</sup> Additionally an editorial on the subject by Allen was simultaneously published.<sup>2</sup> These two articles are important and should be carefully studied. They describe and interpret the data on the cumulative enrollment of patients treated with rhGH followed by the NCGS. There were 54,996 patients (65% males, 35% females) treated from December 1985 to January 2006. This included 195,419 patient-years of treatment with Genentech's rhGH products. While the overall safety profile of rhGH continues to be favorable, this analysis highlights new areas of concern, while it tends to discard other safety issues.

There were 1559 serious adverse events, including 174 deaths, most of which were unrelated to rhGH. The most common cause of death in the 19 cases believed to be rhGH related were central nervous system (CNS) tumors, particularly occurring in patients with organic GH deficiency (GHD). There were also 5 deaths due to aortic dissection/rupture in patients with Turner Syndrome (TS) and 2 deaths probably due to respiratory/cardiac problems in patients with Prader Willi Syndrome (PWS). There were 11 events consistent with acute adrenal insufficiency (AI), leading to 4 deaths. Of the 4 fatalities, 3 appeared to be associated with infection as were 5 of the nonfatal cases of serious AI. GH is known to affect the metabolism of glucocorticoids and it has a modulating effect on hepatic 11 $\beta$ -hydroxysteroid dehydrogenase decreasing the conversion of cortisone to cortisol. Therefore, endogenous cortisol secretion may

decrease after rhGH is initiated in hypopituitary patients and previously unsuspected central hypoadrenalism may become apparent during rhGH treatment. Patients who are begun on rhGH may need to consider glucocorticoid replacement, particularly during stress with increased doses above physiologic maintenance. Patients with hypopituitarism are at a lifelong risk of developing AI, regardless of rhGH use, and need to be counseled and to receive appropriate medical attention during illness as these patients have an increased risk of sudden death.

In the past, leukemia was believed to be a major safety issue associated with rhGH administration. However, there were very few patients with new-onset leukemia in the series. Thus, the data confirmed other reports that therapy with rhGH does not appear to increase the incidence of this cancer in children who do not have any other risk factors that are known to be associated with leukemia.<sup>3</sup>

However, there was an increased risk of second malignancies detected by the NCGS. The patients who received irradiation were at a higher risk of developing second malignancies. Second tumors were seen in 49 of 2500 patients with a prior history of malignancy (excluding craniopharyngioma), or 4.6 cases per 1000 patient years of rhGH treatment. The most commonly detected secondary neoplasms were CNS tumors followed by osteogenic sarcoma. There were 4 malignancies and one meningioma that developed in 16 patients with retinoblastoma. Although the risk of developing a new tumor is increased in any patient with a prior malignancy, regardless of rhGH treatment, this risk seems to be further increased by rhGH. Thus, patients and families need to be made aware of this risk.

There are theoretical risks that may account for the



increased risk of post-treatment tumor development in patients who have received rhGH. The mitogenic and anti-apoptotic actions of GH and insulin-like growth factor (IGF)-I suggest that high-normal levels of free IGF-I may increase the rate of cancers of the breast and prostate. IGF-I concentrations in the high-normal range are often detected in children and adolescents treated with rhGH, particularly for non-classical indications in which supra-physiological rhGH doses are often administered. The potential relationship between neoplasms, GH use and increased serum IGF-I levels clearly needs to be considered.

Targeted events reported with an infrequent incidence of <1% included scoliosis and slipped capital femoral epiphysis (SCFE), probably associated with rapid growth. New onset cases of scoliosis were not serious and were detected mainly in patients with TS, known to have an increased incidence, independent of rhGH treatment. SCFE was found in PWS associated with obesity, untreated endocrine conditions (hypothyroidism and GHD), trauma, radiation and growth during puberty.

Also occurring in the population of rhGH treated patients was intracranial hypertension (IH), diabetes mellitus (DM), AI and pancreatitis. IH has been previously documented with rhGH treatment, but its mechanism is not clear. It seems to be more frequent in distinct groups of patients who were at a higher risk for this complication, ie, those with chronic renal insufficiency and TS. These patients are known to have a higher risk of IH independent of rhGH therapy.

The incidence of type 1 DM was not increased with rhGH administration, while type 2 DM and insulin resistance seemed to be associated with rhGH use. These alterations appeared to be transient and reversible when GH was discontinued. Pancreatitis was detected in 3 patients with TS and in 7 other patients treated with rhGH; the mechanism linking pancreatitis to rhGH administration is unknown.

There are potential pitfalls on relying on data obtained from post-marketing surveillance studies. Enrollment of treated patients is incomplete, drug exposure is variable, inconsistent compliance may lead to underreporting of adverse events by physicians, and finally reporting of adverse events in these surveillance studies is limited to the period of rhGH treatment, while detection and reporting of subsequent adverse effects depends on reports to monitoring agencies by the physician.

**Editor's Comment:** *The post-marketing surveillance study reported by Bell et al<sup>1</sup> and reviewed in this issue of GGH by Roberto Lanes, was established, managed and supported by Genentech Inc, manufactures of the first rhGH that was approved for clinical use by the FDA in 1985. This very large and comprehensive project, carried out under the NCGS, is not the only project of this nature. The Pfizer International Growth*

*Study database (KIGS) has also been ongoing and collected data in over 58,000 patients treated with their rhGH product. The post-marketing efforts of the manufactures of rhGH are very important, but they are not scientifically designed studies. Therefore, these data need to be carefully interpreted. There may also be differences in the results between the two large post-marketing studies that should be considered and evaluated to properly understand the differences. For example the KIGS database did not find that rhGH treatment was associated with an increase in the incidence of malignancies<sup>4</sup>; patients with no medical history of risks known to increase the risk of cancer were not at a higher cancer risk with rhGH treatment. However, how long are they planning to look for secondary malignancies later in life? In this issue of GGH Yoshikazu Nishi also points out the potential weakness of such surveillance studies; he calls our attention to the possibility of hepatoblastomas in low birth weight children treated with rhGH.*

*More recently, there have been a number of publications that denote interest of the manufactures of rhGH to address the challenges of adherence to the medication regimen in patients receiving rhGH. Non-compliance with rhGH therapy is high<sup>5</sup> and this must be considered in the interpretation of data regarding growth response and/or adverse events and complications of rhGH. For example, it is known that there is lower concordance of height velocity with the duration of rhGH therapy, choice of delivery device and short prescription durations.<sup>6</sup> Adherence to medication administration has been difficult to assess and often determined indirectly by clinical subjective assessments, although newer electronic devices are being used to improve adherence.<sup>7</sup> It may be expected that with improved adherence to rhGH treatment there may be a better growth response – but there may also be more adverse events.*

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## OBSESITY

### Fat Mass and Obesity Associated Gene (*FTO*)

Allen W. Root, MD

Several genome wide association (GWA) studies have linked *FTO* (Fat mass and obesity-associated gene - OMIM 610966, chromosome 16q12.2) to weight and obesity risk in children and adults of diverse ethnic origin.<sup>1</sup> Several single nucleotide polymorphisms (SNPs) in intron 1 of *FTO* predispose to obesity while others seem to protect the carrier from this trait. *FTO* encodes a nuclear non-heme iron- and 2-oxoglutarate-dependent dioxygenase that catalyzes the conversion of 2-oxoglutarate to succinate and the demethylation of 3-methylthymine and 3-methyluracil in DNA and RNA, respectively.<sup>2,3</sup> Oxidative demethylation of alkylated nucleic acids is essential for maintenance of an intact genome. *FTO* is expressed ubiquitously in all fetal and adult tissues – particularly in the hypothalamic arcuate nucleus, pituitary, heart, and liver. The arcuate nucleus is the site of synthesis of proopiomelanocortin (POMC) and its anorexigenic product  $\alpha$ -melanocyte stimulating hormone and of orexigenic agouti-related peptide (AGRP) and neuropeptide Y (NPY) – essential components of the appetite regulating system. Within the arcuate nucleus, *Fto* is expressed in Pomc synthesizing as well as other neurons. Arcuate nucleus expression of *Fto* is attenuated by fasting and amplified by feeding – particularly of a high fat diet.<sup>2,4</sup>

Boissel et al have identified a consanguineous Palestinian family in which many third generation members displayed impairment of postnatal growth, developmental delay, and death within the first three years of life.<sup>5</sup> They presented malformations involving the CNS (microcephaly, lissencephaly, brain atrophy, neurosensory deafness), heart (ventricular septal and atrioventricular defects, hypertrophic cardiomyopathy), face (anteverted nostrils, thin vermilion borders, retrognathia, cleft palate) and other regions (short neck, brachydactyly, hypoplasia of toenails, ambiguous genitalia). The investigators linked this malformative syndrome to an autosomal recessive, homozygous, loss-of-function mutation in *FTO*. A homozygous guanine to adenine transition at nucleotide position 947 (c.947G→A) resulted in substitution of glutamine for arginine at codon 316 (Arg316Gln = p.R316Q), an absolutely conserved position in related orthologous genes in many species. The p.R316Q substitution significantly impaired the function of the enzyme. In addition, in vitro the rate of proliferation and the life span of cultured skin fibroblasts from one of these patients were significantly reduced indicating that these cells aged quickly.

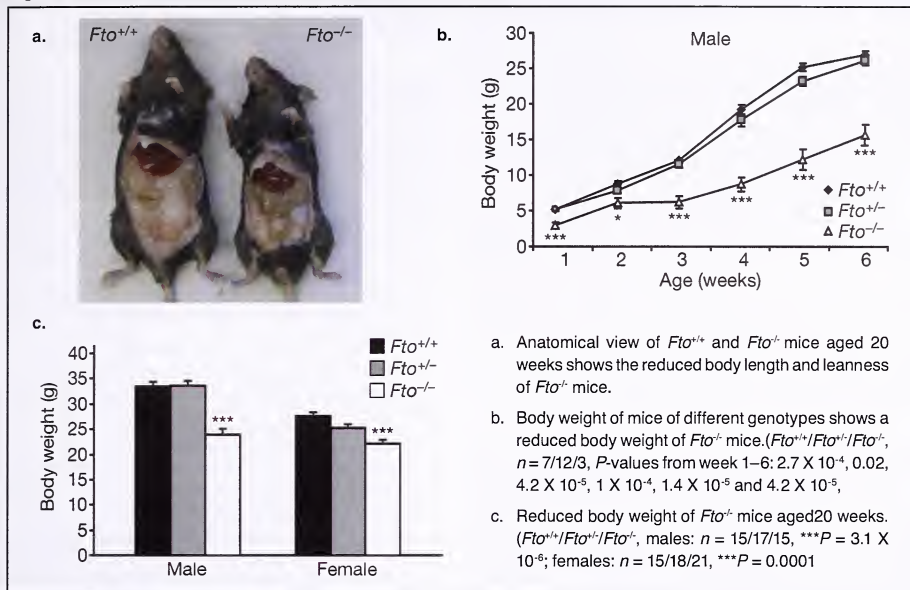
#### *FTO* in Experimental Animals

While loss of *FTO* in humans results in a devastating and lethal complex of anomalies, “knock out” of the murine

homolog *Fto* leads to a less severe outcome. Fischer et al developed *Fto*<sup>-/-</sup> mice by replacing exons 2 and 3 with a neomycin resistant STOP cassette leading to diffuse, germline loss of expression of *Fto*.<sup>6</sup> “Knock out” of *Fto* did not increase fetal wastage; *Fto*<sup>-/-</sup> fetuses had normal embryogenesis and organogenesis. Although of normal size at birth, weight gain and linear growth of male and female *Fto*<sup>-/-</sup> neonates faltered within the first week after birth. The growth of heterozygous *Fto*<sup>+/-</sup> mice was similar to that of wild-type (WT) animals (Figure).

Decreased weight of *Fto*<sup>-/-</sup> mice was due primarily to lower white fat mass compared to WT animals. Interestingly, brown fat mass was similar in WT and *Fto*<sup>-/-</sup> mice. White fat accumulates and stores fat and energy, while brown fat metabolizes and expends energy by uncoupling the processes of heat production and ATP generation by generation of uncoupling proteins encoded by *Ucp1*, *Ucp2*, and *Ucp3*. Further studies revealed that the food intake of the WT and *Fto*<sup>-/-</sup> mice was comparable indicating that relative to body weight the *Fto*<sup>-/-</sup> animals were actually hyperphagic. The expression of *Pomc* and *Npy* in the arcuate nucleus was similar in *Fto*<sup>-/-</sup> and WT mice. Energy expenditure in *Fto*<sup>-/-</sup> mice as assessed by oxygen consumption, carbon dioxide production, and heat generation was significantly greater than in WT mice despite their relative physical inactivity as assessed by determination of spontaneous locomotion. However, increased energy expenditure was not due to greater expression of mitochondrial *Ucp1* in brown adipose tissue or to increased thyroid hormone generation but rather to enhanced sympathetic activity as suggested by higher plasma concentrations of norepinephrine and epinephrine in *Fto*<sup>-/-</sup> than WT mice. The authors concluded that, in mice, loss of *Fto* increases energy expenditure by enhancing sympathetic activity resulting in futile (ie, non-energy producing) metabolism of triglycerides and fatty acids perhaps in skeletal or cardiac muscle or liver.<sup>7</sup> It is also possible that the expression of *Ucp2* and/or *Ucp3* was increased in brown adipose tissue thus dissipating energy through non-shivering thermogenesis.

Church et al developed a mouse model with a missense mutation (A→T) in exon 6 of *Fto* that resulted in replacement of isoleucine by phenylalanine in codon 367 (Ile367Phe = I367F) in the carboxyl terminal region of *Fto*.<sup>8</sup> This site is not within the catalytic core of *Fto* but rather in a highly conserved sequence of ~20 amino acids that is required for dimerization of *Fto* protein and for its optimal catalytic activity. Although *Fto*<sup>367F</sup> localized to the cell nucleus, its expression was reduced and its catalytic activity attenuated but not

**Figure. Phenotypic characteristics of *Fto*-negative mice**

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completely absent. Normal at birth, *both* homozygous *Fto*<sup>1367F</sup> and heterozygous *Fto*<sup>1367F/1367F</sup> male (but not female) mice gained fat mass less rapidly than WT mice after 12 weeks of age; nevertheless, linear growth of mutant mice was comparable to that of WT animals. (The heterozygous *Fto*<sup>1367F/1367F</sup> mutation may exert a dominant-negative effect on the WT protein.) Relative to WT animals, metabolic rate was higher in both homozygous and heterozygous *Fto*<sup>1367F</sup> male mice as estimated by oxygen consumption and carbon dioxide production despite similar levels of physical exertion and brown adipose tissue thermogenic activity. Urinary excretion of catecholamines was greater in mutant than WT animals. In skeletal muscle, expression of the genes encoding the  $\beta$ 3-adrenergic receptor, uncoupling protein-2, and catechol-O-methyl transferase was increased. Microarray analyses in white adipose tissue, skeletal muscle, and liver revealed that in *Fto*<sup>1367F</sup> mice expression of genes associated with inflammation were decreased and those related to both fatty acid catabolism and synthesis were increased. Hypothalamic expression of *Pomc*, *Agrp*, and *Npy* was not altered in *Fto*<sup>1367F</sup> mice. Thus, in a mouse model with less complete loss of *Fto* activity than in the *Fto* “knock-out” model, similar manifestations of *Fto* deficiency were noted but to a lesser extent. Interestingly, the effect of attenuation of *Fto* activity was not observed in

female mice; a somewhat similar observation has been made in humans with a common variant of *FTO*.<sup>9</sup>

The impairment in weight gain and linear growth due to inactivating mutations of *Fto* in mice as demonstrated by Fischer et al<sup>6</sup> and Church et al<sup>8</sup> is primarily due to increased energy expenditure possibly due to augmented adrenergic activity. The more extensive is the loss of *Fto* function in mice, the more dramatic is the effect. The mechanisms by which *FTO* regulates energy intake and utilization are unknown. Inasmuch as *FTO* is a nucleotide demethylase, it is likely that its effects are mediated by differential expression of target genes that are beginning to be identified. Utilizing the male rat as a model, Tung and colleagues<sup>4</sup> stereotactically injected *Fto* cDNA into the arcuate or paraventricular nuclei in order to increase *Fto* expression or shRNA in order to decrease synthesis of endogenous *Fto*. Increased expression of arcuate nucleus *Fto* lowered spontaneous food intake while impaired generation of *Fto* enhanced caloric ingestion. Overexpression of *Fto* in the paraventricular nucleus also impaired food intake in the rat model. They further demonstrated (as did Church et al<sup>8</sup>) that alteration in *Fto* expression did not affect arcuate nucleus expression of *Agrp*, *Npy*, and *Pomc* but enhanced *Fto* expression increased that of *Stat3* and lowered that of *Th* (encoding tyrosine hydroxylase).

Tyrosine hydroxylase is necessary for catecholamine synthesis. Decline in adrenergic hormone synthesis might substantially reduce catecholamine mediated-energy expenditure and thus contribute to obesity in subjects carrying the intron 1 polymorphic variant of *FTO* associated with obesity. Church et al extended these studies to identify possible target genes of *FTO* that regulate fatty acid synthesis and degradation and energy metabolism.<sup>8</sup> Future studies will be directed to deciphering the cellular mechanisms by which *FTO* regulates energy metabolism and body weight.

In humans, the increased adiposity of patients with polymorphic variants in intron 1 of *FTO* associated with obesity has been ascribed to increased appetite (decreased satiety) and caloric intake rather than to reduced energy utilization.<sup>10</sup> The experimental studies demonstrate that polymorphic variants of *FTO* associated with obesity likely reflect increased *FTO* activity, while those linked to resistance to weight gain probably attenuate *FTO* expression.

**Editor's Comment:** The *FTO* was identified as a new obesity candidate by a GWA study by Frayling et al<sup>11</sup> in 2007. They found a strong association between SNPs (eg, rs9939609) and adiposity in the first intron of *FTO*. The predisposition to obesity conferred by this gene was not related to the regulation of energy expenditure, but was mainly accounted for the control of intake of food of high caloric density.<sup>12</sup> The *FTO* gene rs9939609 obesity-risk allele has also been found to be associated with the loss of control over eating.<sup>13</sup> Given the findings of these and other studies of the molecular physiology of weight regulation (some described by Allen Root above), excess food intake (rather than reduced basal energy expenditure) seems to be the major mechanism for obesity in humans. However, reduced energy expenditure in the pathogenesis of obesity should not be underestimated. In an experimental setup we showed that non-human primates (Bonnet Macaque) who spontaneously developed obesity had reduced energy expenditure compared with their non-obese controls.<sup>14</sup>

GWA studies, in which hundreds of thousands of SNPs are tested for association with a disease in hundreds or thousands of persons, have revolutionized the search for genetic influences on complex traits.<sup>15,16</sup> The importance for medicine of GWAs were highlighted in the paper by Christensen and Murray.<sup>17</sup> In the past 5 years GWA studies have identified SNPs implicating hundreds of robustly replicated loci (ie, specific genomic locations) for common traits. Nearly 600 GWA studies covering 150 distinct diseases and traits have been published, with nearly 800 SNP-trait associations reported as significant. The GWAs reported through March 2010 are available within the full text of the article by Manolio and colleagues.<sup>18</sup> The reader is encouraged to review the paper in relation to the

assessment of risk of disease<sup>19</sup> as well as the series of 3 articles by Attia and colleagues regarding the basic concepts of genetic associations.<sup>20-22</sup>

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## The Metabolically Healthy Obese: A Prospective Study on Risk of Development of Cardiovascular Events

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Obesity is a major health problem with its associated elevated risk of cardiovascular disease (CVD). However, some obese subjects do not have concomitant impaired glucose tolerance, hypertension and dyslipidemia. There are no prospective data whether these metabolically healthy obese subjects are protected against CVD. In the ongoing prospective Dutch PREVEND study (n=7356) normal weight (body mass index [BMI] <25 kg/m<sup>2</sup>) at baseline was recorded in 43.2% of participants (3612), while 40.9% (3419) were overweight (BMI 25-29.9 kg/m<sup>2</sup>) and 15.9% (1325) obese (BMI >30 kg/m<sup>2</sup>). In the group with normal weight 39.1% were metabolically healthy (defined as no history of CVD, the absence of diabetes [ADA criteria] and hypertension [JNC 7 criteria] and dyslipidemia [LDL cholesterol >3.50 mmol/L or HDL cholesterol <1.03 mmol/L for men and <1.29 mmol/L for women or triglycerides >1.7 mmol/L or the use of lipid lowering drugs.]) In the overweight or obese groups 13.3% and 6.8%, respectively, were metabolically healthy. During a median follow-up of 7.5 years CVD events occurred in 0.6% of participants with metabolically healthy normal weight, in 1.3% of healthy overweight subjects, and in 1.1% of the healthy obese (P=NS). In metabolically unhealthy participants these percentages were 6.3%, 9.4% and 10.6% for subjects with normal weight, overweight and obesity, respectively

(Table). In addition, Cox regression analysis revealed that BMI was not associated with an elevated CVD risk (HR 1.09, p=0.473), when corrected for gender, year of birth, previous CVD and metabolic parameters.

Metabolically healthy obesity represents only a small subset of the total obese population. Metabolically healthy obese persons do not have an elevated CVD risk when compared to normal weight or overweight subjects with a similar metabolic profile.

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**Editor's Comment:** Improved fitness may be the factor that determines metabolic health, in both normal weight individuals as well as in those with obesity. Exercise capacity is an independent predictor of all-cause mortality. The relationship is inverse and graded, with most survival benefits achieved in those individuals with an exercise capacity >5 METs. Survival improves significantly when unfit individuals became fit.<sup>1</sup> During a 34-year follow-up, leisure-time physical activity in initially healthy middle-aged men had a graded association with reduced mortality that was independent of BMI, CVD risk, and glucose tolerance.<sup>2</sup>

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**Table. Cardiovascular Events in Metabolically Healthy & Unhealthy Normal, Overweight & Obese Subjects**

| BMI        | Baseline | Metabolically Healthy | CVD Events Metabolically Healthy | CVD Events Metabolically Unhealthy |
|------------|----------|-----------------------|----------------------------------|------------------------------------|
| Normal     | 43.2%    | 39.10%                | 0.6%                             | 6.3%                               |
| Overweight | 40.9%    | 13.13%                | 1.3%                             | 9.4%                               |
| Obese      | 15.9%    | 06.80%                | 1.1%                             | 10.6%                              |

BMI: Normal = <25 kg/m<sup>2</sup>; Overweight = 25-29 kg/m<sup>2</sup>; Obese = >30 kg/m<sup>2</sup>.

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## DIABETES

### Maternal Gestational Glucose Concentration Is Associated with Offspring Insulin Sensitivity and $\beta$ -Cell Function in Children Aged 5-10 Years

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Evidence suggests that intrauterine exposure to elevated glucose concentrations may be a mediating factor in prenatal programming of offspring disease risk. However, studies examining the effects of maternal glucose concentration on robust measures of insulin sensitivity and  $\beta$ -cell response in prepubertal children are limited. Therefore, the objective of this study was to determine the associations of maternal glucose concentration with robust and physiologic measures of insulin sensitivity and  $\beta$ -cell response. Participants were

21 children aged 5-10 years. Children's insulin sensitivity index (SI) and measures of basal, static, dynamic, and total  $\beta$ -cell response were determined by mathematical modeling using insulin, glucose, and c-peptide values following a liquid meal tolerance test. Dual-energy X-ray absorptiometry (DEXA) was used for the determination of children's percent total body fat (%BF).

Maternal glucose concentration was determined following a 50-gram, 1-hour oral glucose challenge test at 24-28 weeks of gestation and ranged from 75-229



mg/dL. Independent associations of maternal glucose with SI and  $\beta$ -cell response indices were determined by multiple linear regression analyses. Maternal glucose concentration was significantly, inversely associated with SI, independent of %BF (Parameter Estimate  $\pm$  SE:  $-0.88 \pm 0.27$ ,  $P < 0.01$ ). A significant, positive association was observed for maternal glucose concentration with static  $\beta$ -cell response, independent of %BF and SI (Parameter Estimate  $\pm$  SE:  $1.12 \pm 0.41$ ,  $P < 0.05$ ). Maternal glucose concentration significantly impacted insulin sensitivity and  $\beta$ -cell response, independent of adiposity, in offspring at 5-10 years of age. These results suggest that fetal programming occurs both at the pancreas and at the level of insulin target tissues such as skeletal muscle and liver.

Bush NC, Chandler-Laney PC, Granger WM, Rouse DJ, Gower BA. University of Alabama at Birmingham, Birmingham, Alabama, USA and Brown University, Providence, Rhode Island, USA

**Editor's Comment:** *Maternal glucose concentration in pregnancy appears to be a strong epigenetic factor for fetal programming which impacts insulin sensitivity in offspring during childhood. Perhaps this effect may be imprinted for life! There is growing evidence that even mild gestational diabetes mellitus (GDM) significantly increases the risk of a number of short- and long-term*

*adverse consequences<sup>2</sup> for the fetus and mother, including a predisposition to the development of metabolic syndrome and type 2 diabetes. Maternal and childhood obesity, as well as cardiovascular disease, are also potential long-term consequences of GDM. On the other hand, there is a growing body of evidence suggesting that the risk of many of these consequences can be significantly reduced or eliminated by aggressive treatment of all types of diabetes – including mild GDM.<sup>3</sup> However, there remains, a great deal of controversy over when to begin screening for hyperglycemia in pregnancy and at what level of hyperglycemia aggressive intervention should be initiated.<sup>4-5</sup>*

Fima Lifshitz, MD

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## Erythropoietin Provides Diabetes Protection through Direct Effects on Pancreatic $\beta$ Cells

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Diabetes mellitus is a chronic disorder of insulin insufficiency, resulting in poor glycemic control and vascular complications. The feature common to all forms of diabetes is the insufficient functional pancreatic  $\beta$ -cell mass that is required to maintain euglycemia. Emerging evidence has suggested that erythropoietin (EPO) may exert cytoprotective effects on non-erythroid cells. Interestingly, the EPO receptor (EPO-R) has been found on the pancreatic  $\beta$  cells; however, the biological effects of EPO on the  $\beta$  cells are not well understood.

The effect of recombinant human erythropoietin (rHuEPO) administration was assessed on models of type 1 and type 2 diabetes, using multiple low doses (MLDS) of streptozotocin (STZ) and db/db mice. Mice were given i.p. injections of rHuEPO (50  $\mu$ g/kg) or saline 3 times per week for 4 weeks. In both diabetes models, it was observed that the rHuEPO-treated mice had reduced blood glucose levels compared to controls. The improved glycemic control in the rHuEPO-treated groups was not due to enhanced peripheral insulin sensitivity, but rather enhanced  $\beta$ -cell mass, which was attributed to increased islet proliferation and decreased apoptosis. Treatment with rHuEPO also resulted in enhanced islet

angiogenesis. Western blots of isolated islets from rHuEPO-treated C57BL/6 mice demonstrated activation of the JAK2/STAT5 pathway. Bcl-xL, c-Myc, c-kit, and vegf expression levels were upregulated in the rHuEPO-treated mice. To test for the direct biological effects of EPO on the  $\beta$  cells,  $\beta$  cell-specific EPO-R knockout mice were generated. Treatment with rHuEPO failed to provide diabetes protection in these mutant mice following STZ; this supports the direct role of EPO in pancreatic  $\beta$  cells. To assess for essential downstream signaling,  $\beta$  cell-specific JAK2 knockout mice were also tested. These mice also failed to be protected from STZ-induced diabetes development following rHuEPO treatment. Furthermore, enhancement of  $\beta$ -cell mass and angiogenesis were also abolished in rHuEPO-treated knockout mice. These results show that rHuEPO directly inhibits apoptosis, and enhances proliferation and angiogenesis by activating EPO-R and JAK2 specifically in the  $\beta$  cells.

This study demonstrated that rHuEPO can exert beneficial effects directly on the pancreatic  $\beta$  cells. These results may lead to further elucidation of mechanisms of EPO biology relevant to  $\beta$  cells, which may result in novel therapeutic strategies for diabetes.

Choi D, Schroer SA, Wang L, Wu X, Woo M. University of Toronto, Toronto, Canada; St Michael's Hospital Toronto, Canada

**Editor's Comment:** *The role of rHuEPO in pancreatic cells may bear potential benefits to diabetic patients. Other investigators also found that rHuEPO had no effect on cell apoptosis but it significantly inhibited apoptosis induced by cytokines. It also had no effect on cell insulin secretion, but significantly improved insulin secretion inhibited by cytokines. From these findings,*

*it was concluded that EPO was expressed in NIT-1 cells and EPO could protect NIT-1 cells from apoptosis induced by cytokines.<sup>1</sup> More research is needed before a therapeutic role is considered.*

Fima Lifshitz, MD

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## Prevalence of Vitamin D Deficiency and Association with Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Retrospective Analysis

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Hypovitaminosis D has long been suspected to be a risk factor for glucose intolerance. Several reports have suggested an active role of vitamin D (Vit D) in the functional regulation of pancreatic beta cells. Hypovitaminosis D may be an independent risk factor for type 2 diabetes mellitus (T2DM) and metabolic syndrome. The authors estimated the prevalence of 25 (OH) Vit D deficiency in T2DM and the association of Vit D level with HbA1c. They performed a retrospective continuous chart review of 124 patients with T2DM seen at the endocrine outpatient clinic from 2003 to 2008. The data included: age, race, HbA1c, Vit D, PTH level, family history of T2DM, and calcium intake. Vit D levels were divided into 4 quartiles: normal (Vit D >32 ng/dL), mild deficiency (Vit D >25-32 ng/dL), moderate deficiency (Vit D 14-25 ng/dL), and severe deficiency (Vit D <14 ng/dL). SPSS software was used to apply T-test, ANOVA and Chi-square tests for analysis of data. A total of 113 T2DM patients (91.1%) were found to be Vit D deficient (35.5% severe, 38.7% moderate, 16.9% mild). Serum Vit D level was inversely related to HbA1c (Pearson correlation -0.208, P=0.029). Mean HbA1c was higher in patients with severe Vit D deficiency when compared with patients with normal Vit D (7.1% vs 8.18%, P=0.065). Only 90 of 124 patients had their race documented in the medical record (54 White, 33 Black, 3 Asian). The mean HbA1c was higher in Blacks than in Whites (8.59% vs 7.0%, P <0.05), but the mean Vit D level was lower (15.3% vs 23.4 ng/dL, P <0.05). At the time of presentation 8 of 124 patients (6.4%) were receiving Vit D supplementation (2 with normal Vit D levels, 4 with moderate Vit D deficiency, 2 with severe Vit D deficiency).

The results showed a high prevalence (91.1%) of Vit D

deficiency in T2DM. Only 6.4% of patients were taking Vit D supplements when first seen at the endocrine clinic, despite regular primary care visits. The inverse relationship between Vit D level and glycemic control in this sample supports an active role of Vit D in the pathogenesis of T2DM. The finding of lower Vit D and higher HbA1c levels in Black patients underscores the importance of aggressive screening and supplementation in the population. Since a majority of T2DM patients are diagnosed and treated by primary care providers, screening and Vit D supplementation as part of routine primary care may improve health outcomes in this highly prevalent condition.

Kant R, Chandra R, Arzumanyan H, Krug E. Sinai Hospital of Baltimore, Baltimore, Maryland, USA

**Editor's Comment:** *T2DM is associated with obesity that is often the result of increased energy intake, but not necessarily with an appropriate Vit D intake for the calories consumed. These studies were done in Maryland where sunlight exposure may also be lacking, particularly during the winter months. Vit D is*

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known to regulate the expression of over 200 different genes – including the ones related to apoptosis and immune modulation. There has been an important shift in the views about the actions of Vit D during the past decade. In addition to its well-established role in the regulation of calcium metabolism, Vit D deficiency has been associated with the risk of several extraskeletal diseases. It has been suggested that changes in Vit D intake and sun exposure during the past few decades have contributed to the recent increased prevalence

of diabetes, including T1DM, as well as other chronic conditions.<sup>1</sup> Is the higher prevalence of T1DM and T2DM and of Vit D deficiency casually related? Well-designed, randomized, controlled trials are needed to determine whether the observed associations are indeed causal.

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## Diabetes in the Desert: What Do Patients Know about the Heat?

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Living with diabetes in hot climates poses unique care challenges. Increasing awareness about the interaction between heat and diabetes should be a priority as more patients are living in regions with high temperatures. Data are sparse on what diabetes patients understand concerning heat or what precautions they should take under extreme heat conditions. A survey of patients attending a Southwestern US diabetes clinic was conducted to gauge types of personal protective measures taken against the heat, knowledge of safe temperatures and exposure times, comprehension of weather data and sources of weather information. From November 30 to December 31, 2009 data were collected in 169 completed patient questionnaires. The mean patient age was 66 years, diabetes duration 15 years, 52% were men, 85% had type 2 diabetes, 62% were non-Hispanic white, 67% took insulin by injection, and 6% were on insulin pumps. Mean HgA1c was 7.9%, 38% had a hemoglobin A1c value  $\geq 8.0\%$ , and nearly 40% had values  $\geq 8.0\%$  during the hottest summer months (July and August). Patients employed a variety of personal protective measures, and 68% limited heat exposure to less than one hour. While respondents typically took steps to protect their diabetes equipment and medication (eg, carrying items in a cooler), 36% simply left medications or supplies at home. Although 72% of respondents indicated they had received information regarding the effects of heat on insulin, a minority of patients acknowledged having received information about the effect of heat on oral medications (40%), on glucose monitors (41%), and on glucose monitoring strips (38%). There was considerable variability in temperatures at which patients would consider taking protective measures. Even though 82% knew the correct definition of humidity, only 55% knew the definition of the heat index. Overall, television was the primary source for weather information (89%).

Many patients had suboptimal glycemic control that placed them at risk for dehydration during the hottest months; as well, they used a medication (insulin) particularly susceptible to heat damage. Most respondents had awareness as to the importance of heat in relation to

their diabetes, although knowledge gaps were evident. Increased public awareness of this important topic is needed, and diabetes education should include information about the heat, where regionally appropriate.

Nassar AA, Childs RD, Boyle ME, et al. Mayo Clinic Arizona, Scottsdale, Arizona, USA and National Weather Service, Silver Spring, Maryland, USA

**Editor's Comment:** In a recent paper, Westphal and colleagues<sup>1</sup> reviewed MEDLINE publications from 1966 to 2009 that cross-referenced diabetes mellitus, hot temperature, heat, desert, and insulin. It was found that persons with diabetes might have greater susceptibility to adverse effects from heat (ie, increased number of emergency department visits and hospitalizations, increased occurrence of dehydration and electrolyte abnormalities, and higher death rate) than persons without diabetes. Alterations in glucose homeostasis could also occur, and changes in insulin kinetics and stability were possible. The impact of heat exposure on equipment performance (eg, glucose meters) must be considered. The authors concluded that having diabetes places a person at risk for heat-related health problems. Physicians must be aware of possible complications that diabetic patients may encounter in summer heat to prevent problems. Adolescents with type 1 diabetes mellitus may spend the summer at the beach, and they should be aware of the increased risk, particularly those who are not well controlled. Patient educational materials should be developed relating to self-management skills in the heat, and the topic should be included in standard diabetes education programs when applicable.

As the climate changes, many more people are being subjected to increasing extremes in weather, thus additional education on the health effects of heat on disease and treatment regimens is important. Reid and colleagues have studied the community determinants of heat vulnerability.<sup>2</sup> Four factors explained over 75% of the potential vulnerability variables: a) social/environmental vulnerability (combined education/poverty/race/green space), b) social isolation, c) air conditioning prevalence, and d) proportion of elderly people, and those with



*diabetes. In the US, a higher vulnerability was found in individuals residing in the Northeast and Pacific Coast and the lowest in the Southeast. Urban areas and inner cities showed the highest vulnerability to heat.*

Fima Lifshitz, MD

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## GONADS

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### SF-1 Mutations Cause Isolated Gonadal Dysgenesis and Insufficiency

*Raphaël Rappaport, MD*

#### Introduction

Steroidogenic factor-1 (SF-1; also called Ad4BP, encoded by *NR5A1* gene) was a concept proposed in the early 1990s. It was conceived to be an activator of multiple steps in steroidogenesis (a common protein acting as a regulatory element in the proximal region of the cytochrome P450 steroid hydroxylase genes). The corresponding gene orphan nuclear receptor factor-1 (now termed *NR5A1*) was mapped to the long arm of the chromosome 9 in humans. The expression pattern of this gene plays a central role in regulating the transcription of multiple genes involved in adrenal development, gonadal determination and differentiation, and hypothalamic-pituitary control of reproduction and metabolism.

In 1999 Achermann et al<sup>1</sup> identified the first human SF-1 mutation in a patient with the full phenotype (previously observed in *NR5A1* knock-out mice); the patient had primary adrenal failure and sex reversal with 46,XY gonadal dysgenesis with Mullerian structures present. This patient had a de novo heterozygous loss of function SF-1 mutation that was shown to impair the SF-1 ability to activate the promoters of several target genes. The clinical picture was primary adrenal failure that developed after birth, small intra-abdominal gonads with immature seminiferous tubules accounting for the disorder of sex differentiation (DSD) aspect. A second patient with a similar phenotype was reported shortly thereafter.<sup>2</sup> The parents were first cousins and the patient had a homozygous mutation of SF-1.

The possibility that milder or variant changes in *NR5A1* could be associated with different phenotypes was discussed at length in a paper of Lin and Achermann that focused on testis development.<sup>3</sup> In a recent review, Schimmer and White summarized most data on disease and developmental defects.<sup>4</sup> It is now recognized that changes in *NR5A1* can cause developmental and functional disorders of the gonads in 46,XY and 46,XX individuals, without adrenal insufficiency. This is a new and important consideration in the clinical diagnosis of gonadal dysgenesis. Search for *NR5A1* mutations has become

part of the genetic work-up in intersex patients even in the absence of adrenal failure, when other known causes have been ruled out.

#### *NR5A1* Mutations and 46,XY DSD

Heterozygous loss of function mutations in *NR5A1* have been found in children and adults with 46,XY and apparently normal adrenal function. The first case was diagnosed in an adult patient with clitoromegaly and primary amenorrhea. She had an absent uterus and impaired breast development. In two further cases ambiguous genitalia were observed with dysgenetic testes and the presence of a uterus (in one case). More recently within two cohorts of 46,XY DSD, *NR5A1* changes could be identified in approximately 15% of the patients. Interestingly, the external genitalia were female in three cases (uterus present in one case, remnants or absence in the other two cases), and ambiguous in 12 cases, most of them lacking a uterus.<sup>5,6</sup>

Most of the *NR5A1* mutations appear to arise de novo. However, in one-third of the heterozygous patients mutations were inherited from the mother in a sex-limited dominant fashion; the mother carried the heterozygous change without presenting ovarian dysfunction and she passed on the gene to her affected sons. This condition may be falsely diagnosed as partial androgen insensitivity syndrome. This sex-limited dominant inheritance can mimic an X-linked disorder. This mode of inheritance is important for the strategy of molecular investigation in these patients.

#### SF-1 (*NR5A1*) Gene Mutation as a Frequent Cause of Primary Amenorrhea in 46,XY Female Adolescents

In a recent paper, Philibert et al<sup>7</sup> turned to a selected population of female adolescents with 46,XY and primary amenorrhea, normal female external genitalia, and clitoromegaly. Subjects were separated into two groups according to their plasma testosterone values. Normal or high values suggested androgen insensitivity or 5- $\alpha$  reductase type 2 deficiency. A group of 15 of 31 patients had testosterone levels that

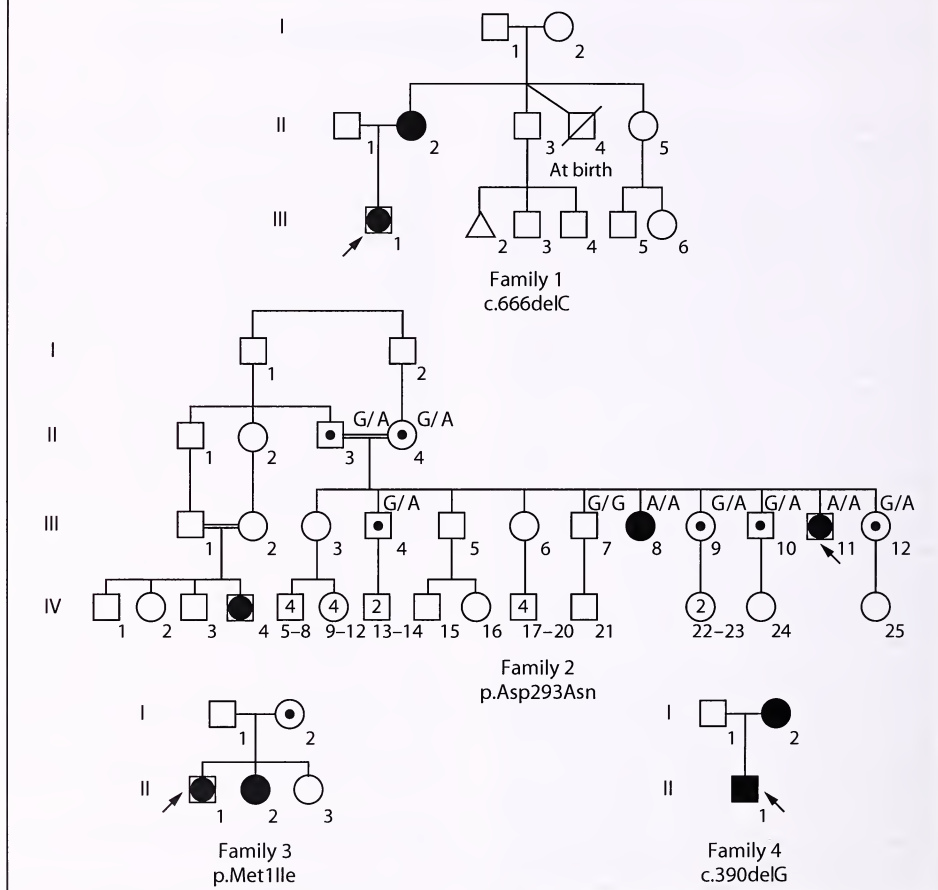


were low for age with elevated gonadotropins. Direct sequencing identified two new *SRY* mutations and one new LH receptor mutation. Five patients had *NR5A1* mutations, two patients had normal external genitalia, and clitoromegaly was present in the other three cases. However, in vitro studies to demonstrate the impact of the mutations were not performed.

It is known that patients with 46,XY DSD include a

large phenotypic range, from complete sex reversal (and absence of a uterus) to those much less affected. In an earlier study, the same group reported 24 patients with bilateral anorchidia (vanishing testes syndrome) with or without micropenis.<sup>8</sup> In one patient they found a variant in *NR5A1* reducing to one-half the SF-1 dependant transcriptional activation. Very rarely, a link between changes in *NR5A1* and late and less severe clinical

**Figure. Pedigree of four families with DSD and POI**



Squares represent male family members and circles represent female family members. Solid squares represent affected 46,XY subjects who were raised as boys, and solid circles represent affected 46,XX subjects. Squares containing solid circles represent affected 46,XY subjects who were raised as girls. Symbols containing a black dot represent apparently unaffected carriers of the mutation. The triangle in Family 1 represents miscarriage, and the symbol with a slash represents a deceased twin. Numbers within symbols indicate multiple siblings. The index patient is indicated with an arrow in each family. Genotyping information is provided for Family 2. The genotypes of the parents of the proband are inferred, whereas all others have been determined by molecular analysis.

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changes (such as cryptorchidism and/or, vanishing testis) could be investigated. This should be looked for in at least the familial cases.

### SF-1 (NR5A1) Gene Mutation and Primary Ovarian Insufficiency in 46,XX Females

Primary ovarian insufficiency (POI) is characterized by primary or secondary amenorrhea, estrogen deficiency and elevated gonadotropins in women younger than 40 years of age. Several genetic causes of syndromic and non-syndromic forms of POI have been identified in recent years. Syndromic forms include monosomy X and the fragile X mental retardation syndrome 1 (FMR 1 gene). In this group other gene mutations include autosomal recessive mutations in the *APECD*, *EIF2B*, and *GALT* genes. POI can also be associated with the blepharophimosis-ptosis-epicanthus inversus syndrome caused by mutations in the *FOXL2* gene.

A key role for *NR5A1* in ovarian development and function has been observed in mice. It is expressed in multiple cell types in the fetal, postnatal, prepubertal, and mature ovary. There is also evidence of a role at the terminal stages of follicle differentiation and/or ovulation with reduced levels of AMH and aromatase expression in granulosa cells.

Lourenço et al<sup>9</sup> showed, for the first time, that *NR5A1* mutations are associated in 46,XX females with primary ovarian insufficiency and that they may combine with 46,XY DSD in some families, without adrenal insufficiency. They identified new mutations in four families and in two of 25 subjects with sporadic POI. The mode of inheritance of the phenotype in the families is consistent with either autosomal recessive or autosomal dominant transmission. The familial cases are shown in the Figure with associated description in the Table.

*NR5A1* (SF-1) mutations may be a significant cause of non-syndromic human ovarian failure. However it remains to be shown if there is a progressive loss of ovarian function in mutation carriers. In addition

the incomplete penetrance and variable expressivity as seen in these families may be explained by other endogenous or environmental factors leading to a more complex picture.

**Editor's Comment:** The reader is referred to a review on SF-1 Mutations in Humans by Tomonobu Hasegawa<sup>10</sup> published in GGH (May 2008 Vol. 24, No. 1) and a minireview on the subject by Schimmer and White.<sup>4</sup> However, the assessment of SF-1 and NR5A1 mutations requires specialized laboratory tests not generally available to endocrinologists who are not practicing in academic medical centers. Furthermore, in the US the medical insurance payer for these patients may not approve the reimbursement for such tests. (In the US, the test is available at Boston University School of Medicine, Center for Human Genetics. They recommend that an insured patient have the tests authorized in advance by the insurance company because payment is quite often denied and patients are left with a bill of \$1395 for the SF-1 and NR5A1 gene mutation tests.) However, these tests seem to be necessary for an accurate diagnosis of patients with amenorrhea, signs of virilization, and any other developmental and functional disorders of the gonads in 46,XY and 46,XX individuals.

Fima Lifshitz, MD

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**Table. Clinical data (from reference 9)**

#### Family 1

- II 2 Premature Ovarian Failure, 36yrs, 46,XX POI
- III 1 Primary amenorrhea, absence of SSC activity, 17 yrs, 46,XY DSD

#### Family 2

- III 8 Primary amenorrhea, 19 yrs, 46,XX POI
- III 11 Primary amenorrhea, signs of virilization, 18 yrs, 46,XY DSD
- IV 3 Complete gonadal dysgenesis, 46,XY DSD

#### Family 3

- II 1 Signs of virilization, partial gonadal dysgenesis, 12 yrs, 46,XY DSD
- II 2 Secondary amenorrhea, 16 yrs, 46,XX POI

#### Family 3

- II 1 Signs of virilization, partial gonadal dysgenesis, 12 yrs, 46,XY DSD
- II 2 Secondary amenorrhea, 16 yrs, 46,XX POI

## Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Blood Levels of Bisphenol A in PCOS Women

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Bisphenol A (BPA) is used primarily in the synthesis of polycarbonate plastics and is a key monomer in production of epoxy resins. It has been shown that the BPA blood levels are higher in men than in women, a finding that is attributed to androgen and BPA interactions on clearance and sex-hormone binding protein (SHBG) binding properties. Additionally, it has been found that the exposure of experimental animals to BPA adversely influences oocyte development and results in ovarian cystic morphology. The aim of the present study was the determination of BPA levels in women with polycystic ovary syndrome (PCOS) compared to controls, age and body mass index (BMI) matched, as well as the investigation of the association between BPA levels and hormonal and metabolic parameters of studied subjects. Subjects included 100 normal and 71 PCOS women (NIH criteria). Anthropometric, hormonal and metabolic parameters, as well as, BPA blood levels were determined in all subjects. Patients and controls were subdivided and matched respectively in two groups, according to BMI, a lean subgroup and an obese subgroup (Table). Compared to controls, the BPA levels were significantly higher in the lean ( $1.12 \pm 0.10$  vs  $0.70 \pm 0.05$ ,  $p < 0.0007$ ) and obese PCOS women ( $0.97 \pm 0.08$  vs  $0.74 \pm 0.07$ ,  $p < 0.044$ ). Additionally, significantly higher insulin and androgen levels were found between PCOS and control subgroups. A significant correlation was found between testosterone ( $r = 0.188$ ,  $p = 0.03$ ),  $\Delta^4$ -androstenedione ( $r = 0.258$ ,  $p = 0.003$ ) and BPA serum levels.

The findings demonstrate that, PCOS women have higher BPA blood levels compared to controls –

independent of BMI – and the demonstrated positive correlations between BPA levels and androgens imply that this endocrine disruptor may play a role in the pathophysiology of this syndrome.

Kandaraki E, Chatzigeorgiou A, Palioura E, et al. Huddersfield Royal Infirmary Hosp West Yorkshire, UK ; Kapodistrian University of Athens, Athens, Greece; University of Athens; and Aristotle University of Thessaloniki, Thessaloniki, Greece

**Editor's Comment:** Human exposure to BPA is nearly universal and recent studies involving this chemical in humans are resulting in growing concerns. Animal studies have documented a variety of endocrine effects of BPA; it acts as an endocrine disruptor. BPA and other endocrine disruptors are finally being considered to play an important role in clinical entities – including PCOS. The association of urinary BPA concentration with medical disorders and laboratory abnormalities was reviewed by Lang et al.<sup>1</sup> Endocrine disruptors have also been shown to alter genital development and puberty, among other clinical conditions. Based on the metabolism of BPA and its endocrine effects, scientists hypothesize that the impact on children will be magnified. Although the Endocrine Society has issued a report expressing serious concerns about endocrine-disrupting compounds, including BPA, the US government health officials still cannot decide whether BPA is safe.<sup>2</sup> The production of plastics<sup>3</sup> will surpass 300 million tons in 2010, therefore we should aim to implement the 5Rs: reduce, reuse, recycle, rethink, and restrain! These actions may benefit all.

Fima Lifshitz, MD

**Table. BPA Blood Levels in Lean and Obese women with PCOS**

|               |                   |
|---------------|-------------------|
| Lean PCOS     | $1.12 \pm 0.10^*$ |
| Lean Control  | $0.70 \pm 0.05$   |
| Obese PCOS    | $0.97 \pm 0.08^*$ |
| Obese Control | $0.74 \pm 0.07$   |

Data are ng/mL. \*P < 0.005 vs controls.

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## THYROID

### Is Thyroid Hormone Therapy Indicated for Euthyroid Sick Syndrome?

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Greet Van de Berghe (pro side, Catholic University of Leuven, Belgium) and Elaine Kaptein (con side, University of Southern California, Los Angeles, USA) debated the use of thyroid hormone therapy for patients with euthyroid sick syndrome (ESS). The debaters

discussed four randomized-controlled trials in ICU patients with prolonged illness treated with thyroid hormone replacement (a total of 190 patients with ESS). Two of the trials used  $T_3$  and two trials used  $T_4$  for the treatment. The  $T_3$  trials showed no change in mortality,



while the  $T_4$  trials showed no change or an increase in mortality. Additionally, in an extensive literature review there were 35 non-randomized controlled studies of thyroid hormone therapy in ESS. For the most part, the results of all 35 trials were inconclusive. All used  $T_3$  and/or  $T_4$  as the active therapy agent. However, the sample size was much too small; for example an ICU study with 23 patients would have needed 142 patients to show statistically significant results. Also the doses of thyroid hormone used in the studies were high, and the wrong hormone may have been utilized. In addition, the effects of malnutrition, medications and other therapies may have played an important role in the outcome of ESS. Sick patients often fast and their nutrition is poor, this depresses serum  $T_3$  levels; proper nutrition quickly corrects the circulating thyroid hormone balance.

It was suggested that a combination of thyrotropin-releasing hormone (TRH) plus growth-hormone releasing peptide (GHRP) may provide benefits in prolonged, critically ill patients. The combination of TRH plus GHRP treatment to correct thyroid hormone levels seemed the most successful in the patients who were receiving adequate nutrition.

There were 14 studies in ESS patients with obesity and calorie restriction, but there were no definitive beneficial effects demonstrated in any of them. Seven other studies of patients with abnormal thyroid findings suggestive of ESS, in various clinical conditions that were treated with thyroid hormone, showed inconsistent results, though one study in patients with coronary artery disease showed decreased systemic vascular resistance. Another study showed an increase in mortality from acute renal failure.

Of the 14 studies performed in postoperative ESS patients who received thyroid hormone therapy, 13 were inconclusive and one showed an increase in cardiac index. A small study in burn patients (14 patients in each group) showed no therapeutic effect, however it would have needed 313 patients in each arm to detect significance.

The evidence in favor of thyroid hormone treatment for ESS is equivocal at best and may increase mortality. Thus, in order to determine the therapy effectiveness and safety in ESS, randomized controlled trials with adequate sample size and appropriate endpoints are needed.

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**Editor's Comment:** *Euthyroid sick syndrome, also known as low  $FT_3$  syndrome, has a high prevalence in hospitalized patients. In a recent study Iglesias et al described the alterations in thyroid hormone levels in up to 85% of patients.<sup>1</sup> In obese patients, alterations in thyroid hormone levels are often detected. These may reflect ESS related to dietary intake or other factors, not the cause of weight gain or obesity. In premature infants and infants in the NICU, these circulating thyroid hormone alterations are also prevalent; although the debate did not address this issue, it is one of great interest to pediatric endocrinologists. The experimental treatment with TRH and GHRP appeared to improve the circulating thyroid hormones in some patients without other measurable benefits. ESS may be a defense against oxidative stress leading to lower energy expenditures and calorie sparing. This results from a number of homeostatic adaptations in sick patients ie, an increase in glutathione peroxidase, selenium, deiodinase activity type 3, and cytokine interleukin (IL)-6. These lead to decreasing the activation of  $T_4$  and the lowering of  $T_3$  levels. Thus, it may be inappropriate to alter the homeostatic process in sick patients with thyroid hormone treatment. The data suggest that there may be no measurable benefit and there may be increased risks – so, why treat?*

Fima Lifshitz, MD

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## Increased Miscarriage Rate in Thyroid Antibody-negative Women with TSH Levels between 2.5-5.0 in the First Trimester of Pregnancy

From ENDO 2010 The Endocrine Society Annual Meeting, San Diego, June 19-22, 2010

Studies over the last two decades have demonstrated an increased miscarriage rate in euthyroid women who are thyroid antibody positive. Similarly, women with overt hypothyroidism have an increased rate of spontaneous pregnancy loss. The impact on pregnancy loss with thyroid-stimulating hormone (TSH) levels between 2.5-5.0 in thyroid antibody negative women is unknown. The present abstract is a component of a larger study in southern Italy in which 4562 women were screened for TSH and thyroid peroxidase (TPO) in the first trimester of pregnancy. Women were randomly assigned to a universal screening (US) group or a case

finding (CF) group and stratified as high risk or low risk for thyroid disease. All women in the US group and high-risk women in the CF group had TSH and thyroid peroxidase antibody performed immediately. Women in the CF low-risk group had their sera assayed postpartum. Antibody-positive women with a TSH >2.5 were treated with levothyroxine. The results on pregnancy outcome are in press.<sup>1</sup> The present study evaluated the miscarriage rate in thyroid antibody-negative pregnant women with TSH levels between 2.5-5.0 as compared to thyroid antibody-negative women with TSH levels <2.5. None of these women were treated with levothyroxine. In the first



trimester of pregnancy 4123 women were TPO negative with a TSH of  $\leq 5.0$  (mean time of screening was 8.8 weeks). The rate of spontaneous pregnancy loss was 6.1% (39/642) in women with a TSH between 2.5–5.0 and 3.6% (127/3481) in women with a TSH  $< 2.5$  ( $p=0.006$ ).

This study demonstrated a significant increase in the rate of spontaneous pregnancy loss in antibody-negative women who have first trimester TSH levels between 2.5–5.0 as compared to antibody-negative women with first trimester TSH  $< 2.5$ . These data provide further evidence that the normal range for TSH in women in the first trimester of pregnancy is  $\leq 2.5$ . Future studies are needed to evaluate the impact on the miscarriage rate of levothyroxine treatment in antibody negative women with TSH between 2.5–5.0 in the first trimester of pregnancy.

Negro R, Schwartz A, Gismondi R, et al. George Washington University, Washington, DC, USA; University of Illinois-Chicago, Chicago, Illinois, USA; Casa di Cura "Salus" Brindisi, Italy; "V Fazzi" Hospital, Lecce, Italy

**Editor's Comment:** These data implied that in pregnancy "compensated hypothyroidism" in thyroid antibody-negative euthyroid women may not be well compensated. Approximately 1–2% of pregnant women receive levothyroxine treatment for overt hypothyroidism. This condition, which commonly has an autoimmune cause, is defined as a low plasma free thyroxine ( $T_4$ ) concentration and a raised plasma TSH

concentration. Another 2.5% of pregnant women have subclinical (compensated) hypothyroidism, which is defined as a raised plasma TSH concentration with a normal free  $T_4$  concentration.<sup>2</sup> It has been suggested that in hypothyroid women anticipating pregnancy (with serum TSH in the lower quartile of normal range) pre-conception adjustment of levothyroxine doses may result in adequate maternal thyroid function.<sup>3</sup> This procedure seems safe and inexpensive; it may be a worthwhile treatment, not only to prevent miscarriage but also in view of the well-known potential effects of even marginal maternal hypothyroid function on the subsequent IQ of the progeny. The data also suggest a role for universal screening in all newly pregnant women with testing for serum TPO antibodies and TSH levels.<sup>4</sup>

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## BONE

### Lethal Skeletal Dysplasia Due to Lack of the Golgin GMAP-210

Allen W. Root, MD

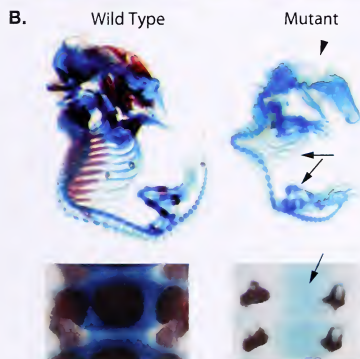
Achondrogenesis is a lethal form of chondrodysplasia. There are 3 types of achondrogenesis - types IA, IB, and II, all of which are phenotypically similar and lethal in utero or in the early postpartum period. Achondrogenesis type IB is due to a biallelic inactivating mutations in *SLC26A2* (OMIM 606718, chromosome 5q32–q33.1) encoding a sulfate transporter. Achondrogenesis type II is due to monoallelic mutations in *COL2A1* (OMIM 120140, chromosome 12q13.1–q13.2) and is characterized by absence of mineralization of the vertebral bodies, sacrum, and pubic bones, a short trunk, and micromelia. In each instance the formation of normal bone is markedly impaired either due to absent synthesis of *COL2A1* (the primary collagen of cartilage) or to an abnormality of post-translational modification of cartilage matrix components as the result of structurally and functionally abnormal Golgi apparatus, or disordered sulfation of essential cartilage matrix proteoglycans.

Smits et al<sup>1</sup> have identified the genetic cause of achondrogenesis type IA (OMIM 200600) as biallelic loss-of-function mutations in *TRIP11* (Thyroid hormone receptor interactor 11; OMIM 604505, chromosome 14q31–q32). The protein encoded by *TRIP11* is not only a

co-factor for transcriptional signaling by triiodothyronine-thyroid hormone receptor (T3-TR) interaction, but is also essential for structural integrity of the Golgi apparatus. The Golgi apparatus is an intracellular organelle that is indispensable for post-translational modification (glycosylation, phosphorylation, sulfation, proteoglycan formation) of proteins and their sorting, packaging, and directing to sites of action within the cell or for extracellular release. They are comprised of stacks of cisternae into which the basic form of the translated protein enters from the endoplasmic reticulum and is modified as it progresses through the apparatus. The method by which the association between achondrogenesis type IA and inactivating mutations in *TRIP11* was recognized is an example of the experimental induction of random mutations of genes that lead to development of a phenotype of interest in a panel of mice and the subsequent identification of the gene(s) responsible for that phenotype – "forward" mutagenesis.<sup>2</sup> Beier and Herron generated the phenotype by treatment of pregnant mice with N-ethyl-N-nitrosourea (ENU), a teratogen that induces single nucleotide (monogenic) mutations, and then selected for genetic characterization

**Figure 1. Effects of *Trip11* mutation in mice**

Panel A shows a wild-type fetal mouse and a fetal mouse with an induced nonsense mutation in *Trip11*, the gene encoding GMAP-210, on embryonic day 18.5. The mouse with the mutation has a domed skull, short snout, short trunk, short limbs, and omphalocele (arrowhead).



In Panel B (top), the staining of cartilage with Alcian blue and bone with alizarin red on embryonic day 17.5 in a wildtype mouse and a mouse with the *Trip11* mutation reveals the absence of mineralization in the skull (arrowhead), rib cage, and limbs (arrows) in the mutant. In Panel B, bottom, staining of cartilage and bone in the vertebral columns of newborn wild-type and mutant mice reveals the absence of mineralization in the vertebral body of the mutant (arrow).

Reprinted with permission from Smits P, et al. *N Engl J Med*. 2010;362:206-16. Copyright © Massachusetts Medical Society 2010. All rights reserved.

the mutated phenotype of interest.<sup>3</sup>

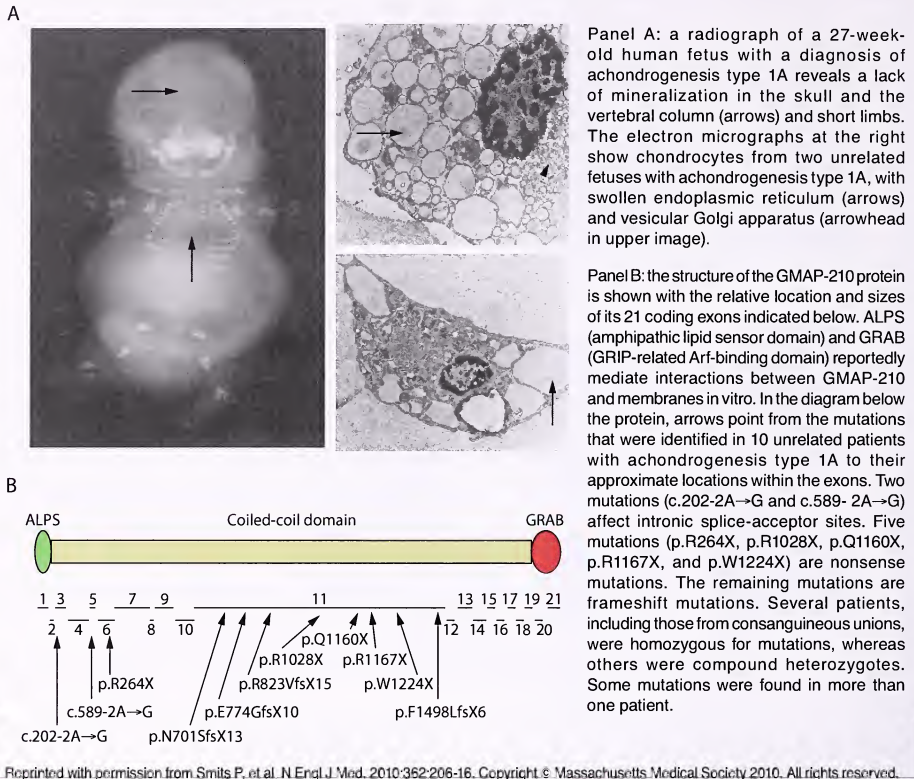
In the Smits et al<sup>3</sup> report, the investigators studied mice with an autosomal recessive phenotype that was lethal in the postpartum period (Figure 1); it was characterized by small thoraces, short limbs and snouts, domed skulls, non-ossified vertebral bodies, delayed mineralization of both endochondral and intramembranous bone, omphalocele, and decreased formation of pulmonary alveoli (likely secondary to impaired thoracic movement). DNA screening revealed a homozygous single nucleotide mutation (c.5003T→A) that generated a stop codon (Leu1668X) in *Trip11* and absence of the intact protein product of this gene in the mutagenized mice. Further studies revealed that in the affected mice cartilage formation was markedly askew without columnar formation, marked slowing of progression of proliferating to hypertrophic chondrocytes, impairment of their terminal differentiation, and early apoptosis of chondrocytes. The organization of the Golgi apparatus was disrupted and appeared as a collection of vesicle-like structures rather than an organized cisternal stack. The product of *TRIP11*

is also termed Golgi-microtubule-associated protein, 210 KD (GMAP210) and is vital for the structural and functional integrity of the Golgi apparatus. GMAP-210 (and other golgins) direct the fusion of vesicles with Golgi membranes and the transport of selected proteins through the endoplasmic reticulum and Golgi apparatus. In mice with the mutation in *Trip11*, there was impaired glycosylation of proteins and delayed transport of the extremely large heparan sulfate proteoglycan - perlecan - through the endoplasmic reticulum and its intracellular accumulation. Noting that the phenotype of the mutant mice resembled that of neonates with achondrogenesis type IA (Figure 2), the investigators then genotyped *TRIP11* in 10 unrelated patients and identified biallelic frame shift, nonsense, and intronic splice-acceptor mutations in all. The authors concluded that inactivating mutations in *TRIP11* interfered with normal cartilage growth and development by impairing structure and function of the Golgi apparatus of chondrocytes.

It is of interest that *TRIP11* also binds to TRβ and enhances T3 dependent transcriptional activity; thus, *TRIP11* is a co-activator for

T3-TRβ. Assessment of thyroid function in subjects with achondrogenesis type IA or other variants of *TRIP11* would be of interest. Inasmuch as thyroid hormone stimulates chondrocyte proliferation and maturation, one might speculate that one mechanism for regulating the effects of T3 on cartilage might be through alteration in intracellular/intranuclear levels of *TRIP11*.

**Editor's Comment:** The osteochondrodysplasias or skeletal dysplasias are a heterogeneous group of over 350 distinct disorders of skeletogenesis. A retrospective analysis evaluated 1500 cases referred to the International Skeletal Dysplasia Registry (ISDR) to determine the relative frequency of specific osteochondrodysplasias and correlation of ultrasound versus radiographic diagnoses for these disorders.<sup>4</sup> Within the retrospective cohort of 1500 cases, 85% of the referred cases represented well-defined skeletal dysplasias, and the other 15% of cases were a mixture of genetic syndromes and probable early-onset intrauterine growth restriction. The three most common prenatal-onset skeletal dysplasias were osteogenesis

**Figure 2. Mutations in *TRIP11* and human achondrogenesis type 1A.**

*imperfecta* type 2, thanatophoric dysplasia, and achondrogenesis 2, accounting for almost 40% of the cases. The lethal osteochondrodysplasias were rare; their prevalence is estimated at 1:10,000 births. Achondrogenesis type 1A (Houston-Harris) is an extremely rare lethal chondrodysplasia with a characteristic severe derangement of endochondral ossification. Molecular analysis in the presented case of achondrogenesis type 1A did not reveal mutations in the *COL2A1* and *SLC26A2* genes, which are known to cause achondrogenesis types 1B and type II. The genetic alteration of Achondrogenesis type 1A (Houston-

Harris) is now elucidated as reviewed by Allen Root in the above paper.

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## Lysosomal Pathology and Osteopetrosis

Allen W. Root, MD

Osteopetrosis is a generic term applied to several clinical disorders of varying severity associated with pathologic

high bone mass resulting in obliteration of bone marrow which causes pancytopenia and extramedullary



hematopoiesis in the spleen and liver, narrowing of cranial foramina leading to loss of cranial nerve function (sight, hearing), paradoxical osseous fragility, and other manifestations.<sup>1,2</sup> It is due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted by autosomal recessive or dominant inheritance patterns. Bone resorption takes place in subosteoclast resorptive pits or lacunae into which are secreted acid ( $H^+$  as hydrochloric acid) that solubilizes the mineral phase of bone and metalloproteases (cathepsin B) that dissolve the protein matrix of bone. The chloride channel in osteoclast lysosomes, which secretes  $H^+$  into the subosteoclast resorptive pit, is encoded by *CLCN7* (OMIM 602727, chromosome 16p13), a protein that is expressed in many tissues including brain (Figure). Inactivating mutations of *CLCN7* result not only in severe to moderate osteopetrosis (depending on the site of the mutation) but also in lysosomal storage, retinal atrophy, and neurodegeneration (OMIM 611490). The *CLCN7* lysosomal chloride channel is primarily a chloride-proton ( $H^+$ ) exchanger—ie,  $H^+$  exits the lysosome through *CLCN7* as  $Cl^-$  enters and accumulates within this organelle. In the osteoclast's ruffled border, the *CLCN7* channel exchanges  $Cl^-$  for  $H^+$  in the resorptive pit while a second channel driven by the conversion of ADP to ATP ( $H^+$  - transporting ATPase) secretes  $H^+$  into the resorptive lacuna.<sup>3</sup>

In order to determine whether the  $H^+$  -  $Cl^-$  exchange

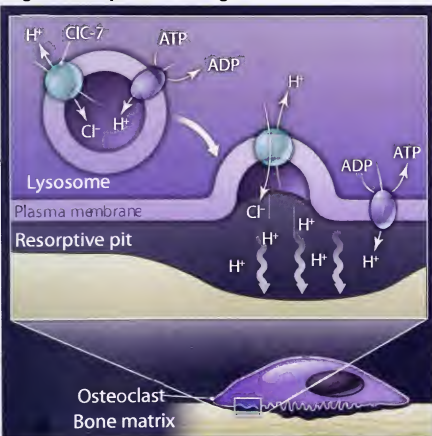
function of *CLCN7* was essential or whether *CLCN7* might function simply as a passive  $Cl^-$  conductor, Weinert and co-workers<sup>4</sup> generated mice in which the  $H^+$  -  $Cl^-$  exchange function was abolished leaving the residual protein to function as an uncoupled  $Cl^-$  conductor. They did so by mutating glutamate (E) to alanine (A) in codon 245 of *Clcn7*, a site essential for  $H^+$  transport by *Clcn7*. Mice homozygous for uncoupled *Clcn7* (*Clcn7<sup>unc/unc</sup>*) developed osteopetrosis and associated neural and retinal abnormalities similar to mice with complete loss of *Clcn7* (*Clcn7<sup>-/-</sup>*) but of somewhat less severity. Thus, *Clcn7<sup>unc/unc</sup>* mice were retarded in growth and died at or before 5 weeks of postpartum age as did *Clcn7<sup>-/-</sup>* mice. However, compared to *Clcn7<sup>-/-</sup>* mice there was a more developed ruffled border, the volume of subosteoclast resorptive pits was larger, and bone mass was less in *Clcn7<sup>unc/unc</sup>* animals. Although initially phenotypically normal, heterozygous mice (*Clcn7<sup>unc/+</sup>*) developed slowly progressive hippocampal neurodegeneration at 5 months of age. The investigators concluded that both the conductance of  $Cl^-$  and the exchange of  $H^+$  and  $Cl^-$  are essential for normal lysosomal function not only in osteoclasts but in other tissues as well.

The present data are important because they further unravel the pathophysiology of loss of *CLCN7*. Such data may ultimately permit more physiologically appropriate therapy of neonates and children with inactivating mutations of *CLCN7*. *OSTM1* (OMIM 607649, chromosome 6q21) and *CLCN7* form a molecular complex that is localized to endosomes, lysosomes, and to the ruffled membrane that caps the subosteoclast resorptive pit, a complex that stabilizes *CLCN7*. In humans, loss of function mutations in *OSTM1* produce a clinical picture that is similar to that of loss of *CLCN7*.

In man, inactivating mutations of *CLCN5* (OMIM 300008, chromosome Xp11.22) are associated with Dent's disease 1 (OMIM 300009)—X-linked hypercalcaemic, hyperphosphaturic nephrolithiasis with microglobulinuria, a phenotype that is mirrored in the *Clcn5<sup>-/-</sup>* knock-out mouse. In the same issue of *Science*, Novarino and Weinert et al<sup>5</sup> reported the effects of separating renal  $H^+$  -  $Cl^-$  exchange from  $Cl^-$  conductance by substituting glutamate for alanine in codon 211 (E211A) in *Clcn5* in mice. In *Clcn5<sup>unc/unc</sup>* mice, clinical and pathophysiological findings were similar to those in *Clcn5<sup>-/-</sup>* animals indicating the critical importance of  $H^+$  -  $Cl^-$  exchange for normal renal tubular function.

**Editor's Comment:** Osteopetrosis is a rare human genetic disorder due to markedly decreased bone resorption. In the past, the only gene whose inactivation was known to be responsible for human osteopetrosis<sup>6</sup> was that encoding carbonic anhydrase type II. Now it is known that osteopetrosis may be due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted

**Figure. Coupled exchange.**



Transporters that import chloride ions in exchange for the export of protons control the function of intracellular vesicles in mammalian cells.

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*by autosomal recessive or dominant inheritance patterns as reviewed by Allen Root above. Sclerosing bone disorders are usually due to mutations in genes required for osteoclast function that can be subdivided according to their clinical presentation, the primarily affected cell type, and the cellular pathways.<sup>1</sup> Clinical aspects of osteopetrosis and the consequences for our understanding of bone biology are discussed by de Vernejoul and Kornak.*

Fima Lifshitz, MD

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